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Microbial Dextran-Hydrolyzing Enzymes: Fundamentals and Applications

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INTRODUCTION

Dextran is a homoglycan of α-D-glucopyranose molecules coupled primarily with α-1,6 linkages. Due to diverse branching of the glucose backbone chain, dextran polymers have a remarkable diversity in chain length and in physicochemical properties. The degradation of dextrans entails a number of glycosyl hydrolases with different specificities and modes of action. Initially, these enzymes were called endo- and exodextranases. However, the divergence has obliged us to specify them in more detail, taking into account the structures of substrates and reaction products. A novel classification system based on amino acid sequence similarities links dextranases to other families of glycoside hydrolases.

Initial interest in the enzymes hydrolyzing dextran arose from studies that aimed to elucidate the structure of dextran and to obtain partially hydrolyzed dextran polymers produced by *Leuconostoc mesenteroides* for infusion purposes (80). Dextranases also have other important industrial applications since these enzymes can depolymerize various troublesome microbial dextran deposits. The presence of dextran in harvested sugar canes and dextran formation by microbes in sugar factories lead to lowered sucrose yield. The fact that dextran is a component of dental plaque, which is considered to contribute to the development of dental caries, has been one of the main driving forces to investigate dextran-hydrolyzing enzymes. Dextran can be modified by dextranases to be used in many biotechnological applications.

Since the first reports on *Cellvibrio fulva* dextranase in the 1940s, more than 1,500 scientific papers and more than 100 patents have been issued on dextran-hydrolyzing enzymes found in a number of microbial groups, fungi being the most important commercial source of dextranase. Higher organisms also possess dextran-hydrolyzing activities, but relatively few studies focusing on such enzymes have been published. The present paper aims to present relevant data on microbial dextranases published thus far. Since this is the first larger overview into the field, earlier literature is also cited rather widely. The enzymatic properties of dextran-hydrolyzing enzymes from different microbial sources, existing nomenclature, cloning and sequence analysis of dextranase genes, methods for measuring dextran-hydrolyzing activity, and potential applications of dextranases are discussed. Because of the increasing

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importance of glycobiology in biosciences, it is possible to predict that dextran and the enzymes involved in its synthesis, modification (e.g., through transglycosylation), and hydrolysis will have increasing significance in the future. To comprehend the special nature of dextran-degrading enzymes, a brief outline of the structure and properties of dextran polymer and dextran-synthesizing enzymes is also presented.

STRUCTURE AND PROPERTIES OF DEXTRAN

Dextran is a collective name for high-molecular-weight polymers composed of D-glucose units connected with α-1,6 linkages and various amounts of side branches linked with α-1,2, α-1,3, or α-1,4 to the main chains. The enzymes that synthesize these glucans from sucrose are known by the generic term dextransucrase (1,6-α-D-glucan-6-α-glucosyltransferase, EC2.4.1.5.). They are glucansucrases produced by various *Leuconostoc* and *Streptococcus* species (135, 136, 191) and by the mold *Rhizopus* spp. (175). Other dextran-producing bacteria, *Acetobacter capsulatus* (renamed *Gluconobacter oxydans*) and *Acetobacter viscous*, produce dextrin dextranase (EC2.4.1.2) that converts dextrins to dextran (192, 229). The *Leuconostoc mesenteroides* strains are inducible and require sucrose in the medium for the biosynthesis of dextrans with the exception of recently isolated constitutive enzyme mutants, e.g., strains B-512 FMC, B-742, B-1142, B-1299, and B-1355 (28, 91, 92, 98, 136, 168). *Streptococcus* species are generally constitutive and do not require sucrose in the growth media for enzyme expression (43, 55, 191).

Dextransucrase catalyzes the synthesis of glucan, which contains 50% or more α-1,6 glucosidic bonds within the main chain (136). The structures and properties of bacterial dextrans vary between microbial strains and according to growth rate and reaction conditions (27, 84, 91, 96, 156, 191, 221). The position of the branch linkages, the degree of branching, the length of branch chains, and molecular weight distribution affect the physicochemical properties of dextrans (1, 2, 127, 187). For example, the degree of solubility in water decreases when the degree of branching is increased. In fact, dextrans with >43% branching through 1,3-α linkages have been considered water insoluble (127).

The most extensively studied dextransucrase from the commercially important strains NRRL B-512 and B-512F of *L. mesenteroides* synthesizes a soluble linear α-1,6-linked dextran with about 5% randomly distributed α-1,3-branched linkages of up to 50 to 100 residues (168, 176, 191). The long branches are important factors for properties of the dextran (209). On the other hand, other strains of *Leuconostoc* are known to produce several dextransucrases resulting in synthesis of glucans composed of water-soluble fraction and water-insoluble or less-soluble fraction (14, 27).

Leuconostoc mesenteroides strain B-1299 produces both extracellular and intracellular dextransucrases that synthesize two kinds of dextrans: fraction L, which precipitates in 38% ethanol, has 27% of α-1,2-, and 1% of α-1,3-branch linkages; and fraction S, which precipitates in 40% ethanol, has 35% of α-1,2-branch linkages (14, 15, 38, 92). *L. mesenteroides* B-742 also expresses two kinds of dextransucrases, producing fraction S with 50% α-1,6-, 50% α-1,3-, and no α-1,4-branch linkages, and fraction L, which contains, in addition to α-1,6 linkages, 14% α-1,4- and about 1% of α-1,3-branch linkages (91).

Dextrans with low degree of branching can be hydrolyzed by *Penicillium* endodextranase, but highly branched dextrans produced by B-1142(S), B-742(S), and B-1299(S) are resistant to endodextranase hydrolysis (92). *L. mesenteroides* NRRL B-1355 produces two distinct glucans. Fraction L is similar to B-512 dextran, but fraction S has an altered sequence of α-1,6 (53%) and α-1,3 linkages and has been named alternan (27, 130, 136).

Detailed chemical structures and properties of glucans produced by streptococci have been reported in a number of publications (23, 43, 55, 84, 126, 191, 203). In earlier investigations water-insoluble polysaccharides, synthesized by streptococcal mutansucrases (EC2.4.1.5), were described as typical dextrans (55, 84, 191). Several *Streptococcus* strains form two groups of glucosyltransferases: those that synthesize water-soluble glucans, primarily consisting of α-1,6 bonds, which classifies them as dextrans, and those that synthesize water-insoluble glucans, primarily having more than 50% of α-1,3-bonds with small proportions of α-1,6 and α-1,4 linkages (52, 136, 223). Water-insoluble glucan from *Streptococcus mutans* strain OMZ176 contains up to 90% of α-1,3-glucosidic linkages. In contrast, gelatinous glucan from *Streptococcus sanguis* strain 804 has equal amounts of α-1,3 and α-1,6-glucosidic linkages (54, 58).

The degree of polydispersity of dextrans significantly affects their in vivo behavior (127). Native dextrans isolated from culture filtrates of *L. mesenteroides* strain B-512F are often extremely polydisperse and contain molecules of all sizes from oligomers to molecular weights of several hundreds of millions. The range of molecular sizes may be reduced (degree of polydispersity ≤2) by either partial acid or enzymatic hydrolysis. Dextrans with average mass of 4 to 2,000 kDa and polydispersity of ≈1.5 are commercially available for research purposes from Amersham, Sweden. Solutions containing small (40 kDa) or large (70 kDa) preparations are called clinical dextrans (127).

CLASSIFICATION OF DEXTRAN-HYDROLYZING ENZYMES

Dextran-degrading enzymes form a diverse group of different carbohydrases and transferases. These enzymes have often been classified as endo- and exodextranases based on the mode of action and commonly called dextranases (49, 50, 231). According to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUB-MB) and the types of reactions catalyzed and product specificity, these enzymes were classified as dextranases (EC3.2.1.11), glucan-1,6-α-D-glucosidases (EC3.2.1.70), glucan-1,6-α-isomaltosidases (EC3.2.1.94), dextran 1,6-α-isomaltotriosidases (EC3.2.1.95), and branched-dextran exo-1,2-α-glucosidases (EC3.2.1.115) (45). Cycloisomaltooligosaccharide glucanotransferase (CITase) also produces hydrolyzed dextran as one of its reaction products (148) (Table 1). Moreover, an unrelated enzyme, α-glucosidase (EC3.2.1.20), catalyzes reactions similar to those of exodextranases (EC3.2.1.70) (85).

In another classification system glycosylhydrolases and glycosyltransferases have been divided into families on the basis of the similarities in the amino acid sequences (61, 62, 63, 64;

TABLE 1. Characteristics of microbial dextran-hydrolyzing enzymes[a]

Enzyme family and source(s)	Enzyme source and localization	Mass (kDa)	pI	pH optimum	Temp optimum (°C)	pH stability; thermal stability (°C), chemical effectors	Enzymatic digestion products	Purity (fold)	Substrate specificity	Reference(s)
Endodextranases (EC3.2.1.11) (molds)	*Penicillium luteum* ATCC 9644 extracellular	44	4.1	4.0–6.0	50	Stable at pH 3.5–7.0 on 24 h incubation at T = 30°C and at T < 50°C at pH 5.0 on 15 min incubation; stable to Ca^{2+}, Mn^{2+}, Fe^{3+}, Ni^{2+}, Co^{2+}, Sr^{2+}, Cu^{2+}, EDTA	D-GL (55%), IM_2, IM_3, IM_N from dextran T-2000	4	Dextran derivatives, dextran IAM (66% of α/-1,6), a series of isomaltodextrins	47, 68
	Penicillium funiculosum	41				I and II are stable over the range			Sephadex G-25, G-50, G-75, G-200	198
	I-extracellular		3.98	6.0	n.d.	pH 5.0–7.5 on 60 min incubation	n.d.	29		
	II-extracellular		4.19	6.0	n.d.	at T = 37°C and stable up to T = 40°C on 30 min incubation at pH 6.0; stable to Ca^{2+} and Fe^{3+}	n.d.	29		
	Penicillium funiculosum NRRL 1768 extracellular	26.5	4.6	4.5–5.5	n.d.	Stable at pH 5.0–7.0 at T = 4°C for at least 1 year rapidly inactivated above 55°C; stable to methanol, acetone; inhibitors 2-mercaptoethanol, mercuric salts	IM_2 from dextran 100	1,000	Clinical-grade dextran 100–200 kDa, Sephadex G-25, *L. mesenteroides* branched dextrans	20, 53
	Penicillium lilacinum NRRL 895 extracellular	26.5	n.d.	5.0–5.8	40–45	Stable at T = 0–37°C for 24 h at pH 4.8–9.0; stable to iodoacetate, PCMB; EDTA; partial inhibition with Cu^{2+} and Ba^{2+}	D-GL, IM_2, IM_3, IM_4	13	Sephadex G-25, G-50, G-100, G-200 branched dextrans of *L. mesenteroides*	35, 53
	Penicillium notatum Westling 1						D-GL, IM_2, IM_3, IM_4 from dextran 110		Dextrans from 5 to 500 kDa, Sephadex G-25-G-200	157, 158
	I-extracellular	55.8	4.9	4.8	50	n.d.		96		
	II-extracellular	50.1	4.75	4.8	50	n.d.		112		
	Penicillium acualeatum extracellular	n.d.	n.d.	5.0–6.0	50–60	Stable up to T = 50°C for 60 min and at pH <6.0; stable at pH 4.5–5.6 for about 2 months; inhibitors Hg^{2+}, Cu^{2+}, Fe^{3+}, Ag^{2+}, Zn^{2+}, Mn^{2+}, PCMB	IM_2, IM_3, IM_N from dextran 200–275 kDa	2	Sephadex G-50 and G-200, dextrins	117
	Aspergillus carneus					I and II are stable at pH 4.5–9.0,	IM_2, IM_3, D-GL, traces IM_N		Dextran IAM (66% of α-1,6), a series of isomaltodextrins (IM_3-IM_8), Sephadex G-50, G-200	67, 68, 213
	I-extracellular	71	4.12	5.0–5.5	60	24 h incubation at 30°C and at T	from dextran 2,000 kDa	66		
	II-extracellular	n.d.	4.35	5.0–5.5	60	< 50°C, 15 min, pH 5.5; stable to Zn^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, EDTA, PCMB		66		
	Chatomium gracile					Stable at pH 5.5–11.0 and T < 55°C;	55% D-GL, IM_2, IM_3 from		*S. sobirnus* K1-R glucans	59, 60, 208
	I-extracellular	77	6.2	5.5	65	inhibitors Hg^{2+}, Cu^{2+}, Fe^{3+}, SDS	dextran 2,000 kDa	28		
	II-extracellular	71	5.7	5.5	65			28		
	Fusarium sp. extracellular	69	4.6	6.5	35	Stable over pH 4.5–11.8 at T = 4°C for 24 h and T < 45°C for 10 min at pH 6.5; stable to EDTA. Partial inactivation with Ba^{2+}, Pb^{2+}	62% IM_3, D-GL, IM_2 from dextran 2,000 kDa	11	Dextrans from *L. mesenteroides* NRRL B-1416, B-1416, B-742S, B-1355s	189
	Sporotrix schencki extracellular	79	n.d.	5.0	n.d.	Stable at pH 4.5–4.75, where the half-life at T = 55°C was 18 min; stable to Co^{2+}, Mn^{2+}, Mg^{2+}, Al^{3+}, EDTA	D-GL, IM_2, IM_N from dextran 170 kDa	26	Sephadex G-50, G-200	6
Endodextranases (EC3.2.1.11) Yeasts	*Lipomyces starkeyi* ATCC 20825 extracellular					Stable at pH 2.5–6.0 at T = 25°C for 72 min; a loss of activity after 30 h of exposure at pH above 7.0; stable to EDTA, dithiothreitol, 2-mercaptoethanol	D-GL, IM_2, IM_3, IM_4 from dextran 2,000 kDa	43 for all enzymes	Dextrans from 10 to 2,000 kDa, IM_N	105, 106
	I	74	5.6	5.0	50					
	II	71	5.8	5.0	50					
	III	68	5.9	5.0	50					
	IV	65	6.1	5.0	50					
	Lipomyces starkeyi KSM 22 extracellular	100	6.0	5.0	45	Stable at pH 2.0–7.5 for 72 h at T = 22°C and at T < 50°C for 3 min at pH 5.5	D-GL, IM_2, IM_4, IM_N from dextran 2,000 kDa	33	Insoluble glucan, levan, β-fructan	95, 172
	Lipomyces starkeyi IGC 4047 extracellular	23	5.4	5.0	50	Stable at pH 3.5–7.5; after 2 h at T = 50° (pH 5.0) retained 30% activity	D-GL, IM_2, IM_3, IM_4-IM_6 from dextran 2,000 kDa	n.d.	Sephadex G-100	106, 224

Bacteria	*Bacteroides oralis* Ig4a extracellular 1	44	4.5	5.5	n.d.	Stable to Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, EDTA, iodoacetamide	D-GL, IM_2, IM_3, IM_N from dextran 2,000 kDa	n.d.	n.d.	204
	Flavobacterium sp. M-73 extracellular	114	n.d.	7.0	35	Stable at T = 4°C for 24 h over pH 6.5–12.0 and up to T = 35°C on heating for 10 min; inhibitors are Hg^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}, EDTA, Co^{2+}, 4-CMB	63% IM_3, 31% D-GL from clinical dextran	n.d.	Dextrans T-10, T-110, B-512F native dextran, highly branched dextrans IM_N	101, 202
	Pseudomonas sp. strain UQM 733					D1 is stable up to T = 51°C for 1 h at pH 5.5; D2 activity decreased at T > 43°C during 1 h at pH 5.5; D4 is stable at T < 40°C during 1 h at pH 5.5	IM_2-IM_{10} (D1); IM_4-IM_{10} (D2); D-GL, IM_N (D4); from dextran 100–200 kDa		Series of IM_N, dextran B-512 (D1, D2, D4)	29, 30, 164, 165, 166
	Extracellular D1	n.d.	n.d.	4.5–5.5	55			50		
	Extracellular D2	n.d.	n.d.	4.5–7.5	40–45			38		
	Intracellular D4	n.d.	6.9–7.3	5.5	58			183		
	Termoanaerobacter sp. strain RT 364 extracellular	140	n.d.	5.5	80	Stable during 12 h at T = 75°C and pH 5.0	n.d.	38	Starch, amylose, amylopectin	228
	Termoanaerobacter sp. AB11Ad extracellular	n.d.	n.d.	5.0–6.0	70	Half-life was 6.5 h at T = 75°C and 2 h at T = 80°C at pH 5.0 in the absence of dextran	n.d.	n.d.	n.d.	227
	Cytophaga sp. extracellular	60	4.0	5.0–6.5	50	Activity falls to 20% of its maximum value at pH 4.0 and pH 8.8 and to 0% at pH 2.5 and 11.0; falls to 35% at T = 55°C, to 4% at T = 60°C, and to 0% at T = 65°C	D-GL, IM_2 from dextran 70–2,000 (Pharmacia)	115	Sephadex G-100 G-150, G-200	83
	Streptococcus sobinus 6715					Plateau of tolerance to higher temperatures up to T = 44°C, at which point dextranase activity falls off quickly				10
	Extracellular C	175	n.d.	5.4	36		n.d.	3,190	n.d.	
	Extracellular D	160	n.d.	5.2	36			2,330		
	Streptococcus mutans K1-R extracellular	n.d.	n.d.	5.5	35	Inactivated above T = 40°C; activity loss at the rate of 4% per week when stored at T = 2°C; stable to EDTA.	IM_3 from dextran 2,000 kDa (Sigma)	1,857	Dextrans (B-512, B-1355), streptococcal glucans	162
	Bacillus circulans MT-G2 extracellular	n.d.	n.d.	6.2–6.7	35	Inhibitors: EDTA, Cu^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}	n.d.	50	Insoluble glucans, laminarin, glycogen, starch, nigeran	154
	Paenibacillus illinoisensis					Stable in solutions at T < 50°C	D-GL, IM_2, IM_N from dextran T-500	733	n.d.	88, 89
	Extracellular (I)	76	4.95	6.8 for all	50 for all					
	Extracellular (II)	89	4.2							
	Extracellular (III)	110	4.0							
Glucose-forming exodextranases										
Bacteria	*Bacteroides oralis* IG 4a					Stable to Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, EDTA, iodoacetamide (II); inhibitors $CoCl_2$, $HgCl_2$ (V)			Inactive to modified insoluble glucans (II); inactive toward glucans containing α-1,3- and β-1,4-linkages (V)	73, 204
	Extracellular II	52	6.5	6.8	n.d.		n.d.	n.d.		
	Intracellular V	105	n.d.	5.0	55		n.d.	n.d.		
	Arthrobacter globiforms I-42 extracellular	120	4.31	6.0	45	Stable at pH 5.5–7.5 and temperature lower than 55°C; inhibitors Pb^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+}, Fe^{3+}, $KMnO_4$, phenyl-α-D-glucoside	β-GL	16.5	Starch, amylopectin, maltotriose, panose, isopanose	152, 153, 180
	Pseudomonas sp. strain UQM733					Stable at T < 40°C at pH 5.5 for 1 h (G1); stable at T < 50°C at pH 5.5 for 1 h (G3)	D-GL, IM_2, IM_5, IM_6, IM_N (G1, G2, G3) from B-512 native dextran		IM_N, dextran B-512 (G1–G3)	29, 30
	Intracellular G1	n.d.	7.5	6.0–6.5	37			8		
	Intracellular G2	n.d.	7.7	6.0	33			4		
	Intracellular G3	n.d.	4.7	7.0	50			2.5		
	Streptococcus mitis ATCC 903 intracellular	54	4.45	7.2	37–40	Retain 100% of original activity after storage at T = 5–7°C for 6 mo; inhibitors are Ni^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, EDTA, SDS, sodiumfluoride	α-GL	925	Dextran 9,400 Da, isomaltose, pNPG	115
Yeasts	*Lipomyces lipofer* IGC 4042 extracellular	29	7.0	4.5–5.0	45	Stable at pH 4.5–6.0; after 2 h at T = 50°C maintained >60% of its original activity	β-GL	n.d.	Dextrans, IM_N	163
Glucan 1,6-α-isomaltosidase (EC3.2.1.94)	*Arthrobacter globiformis* T6 extracellular	69	5.2	5.3	65	Stable at pH 3.0–8.0 at T = 4°C for 24 h; retains 90% of its original activity after heating at T = 60°C for 10 min; inhibitors Ag^{2+}, Hg^{2+}, Fe^{3+}, $KMnO_4$	IM_2 from dextran 2,000 kDa	207	Panose, pullulan, dextrans T-40, T-2000, B-512, B-1397, isomalto-oligosaccharides, 13 native NRRL dextrans	150, 151, 178, 211

Continued on following page

TABLE 1—*Continued*

Enzyme family and source(s)	Enzyme source and localization	Mass (kDa)	pI	pH optimum	Temp optimum (°C)	pH stability; thermal stability (°C), chemical effectors	Enzymatic digestion products	Purity (fold)	Substrate specificity	Reference(s)
Dextran 1,6-α-isomaltotriosidase (EC3.2.1.95)	*Brevibacterium fuscum* var. *dextranolyticum* extracellular	70	4.17	7.0–7.5	n.d.	Enzyme retains maximal activity at pH 6.0–9.0; at pH 5.0, 10.0, and 11.0, T = 37°C for 12 h, activity is lost less than 10%; up to T = 50°C activity loss less than 1%; retains 70% of activity at T = 60°C; stable to Mg^{2+}, Ca^{2+}, Fe^{3+}, iodoacetic acid	IM_3 from dextran 53 kDa and reduced isomaltodextrins	7.6	Sephadex G-100, G-200	199, 200
Dextran endo-1,2-α-glucosidase (EC3.2.1.115)	*Flavobacterium* sp. strain M-73 extracellular	125	n.d.	5.6–6.0	45	Stable up to T = 40°C during 10 min and over a pH range of 6.5–9.0 on incubation at T = 4°C for 24 h inhibited by Al^{3+}, Fe^{3+}, Hg^{2+}, Cu^{2+}	D-GL, limit dextrins from B-1299 dextran	n.d.	Dextrans B-1298, B-1299, B-1397, B-512, mutan	100, 131, 132
Cycloisomalto-oligosaccharidglucanotransferase (EC2.4.1)	*Bacillus* sp. T-3040 extracellular	98	n.d.	5.5	n.d.	Stable over the range of pH 4.5–8.5 and stable at T < 40°C for 15 min at pH 5.5 activity almost completely inhibited by Cu^{2+} and Hg^{2+}	CI-7, CI-8, CI-9 from dextran 40 kDa; IM_9 from CI-8 and glucose; linear IM_N from IM_5	73	n.d.	144

[a] CMB, chloromercuribenzoate; pNPG, *p*-nitrophenyl-α-D-glucopyranoside; PCMB, *p*-chloromercuribenzoate; GL, glucose; IM_2, isomaltose; IM_3, isomaltotriose; IM_4, isomaltotetraose; IM_N, series isomalto-oligosaccharides; CI, cycloisomaltooligosaccharide (cyclodextran); n.d., no data. NRRL, Northern Regional Research Center (U.S. Department of Agriculture, Peoria, Ill.). Dextran IAM (from *Leuconostoc mesenteroides* strain IAM) (Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan) is the dextran containing 66% α-1,6-, 19% α-1,4-, and 15% α-1,3-glucosidic linkages.

http://afmb.cnrs-mrs.fr/CAZY/). The Carbohydrate Active Enzymes (CAZy) database describes the families of structurally related catalytic and carbohydrate-binding modules (functional domains) of enzymes that degrade, modify, or create glycosidic bonds. An analogous classification system for dextran-hydrolyzing enzymes with dextranases divided into four families has also been presented (5), but it is not presented in this review. In contrast to the IUB-MB system, the CAZy database was designed to integrate both structural and mechanical features of these enzymes; enzymes with different substrate specificities can be placed in the same family, and enzymes that hydrolyze the same substrate are sometimes placed in different families (16, 64).

According to sequence similarities, dextran-glucosidases (EC3.2.1.70) have been included in glycosylhydrolase families 13 and 15 (http://afmb.cnrs-mrs.fr/~pedro/CAZY/ghf.html). Isomaltodextranase (EC3.2.1.94) and isomaltotriosidase (EC3.2.1.95) have different structures and have been included in glycosylhydrolase families 27 and 49, respectively. Endodextranases are found in glycosylhydrolase families 49 and 66, with no sequence similarities between the two families. Also, *Bacillus circulans* CITase possessing a high homology with endodextranases is classified in family glycosylhydrolase 66.

SOURCES, MAIN PROPERTIES, INDUCTION, AND MECHANISM OF ACTION

The enzymes capable of hydrolyzing dextrans are found in various microbial groups (Table 1), in animal and human tissues (51, 161, 170), and in coleoptiles of the genus *Avena* (65, 66). Dextranase activity is also demonstrated in soil samples (40). In general, dextran-hydrolyzing enzymes have high substrate specificity. The physicochemical properties of a number of purified dextranases are presented in Table 1.

Endodextranases (EC3.2.1.11) from Fungi

Mold dextranase, 1,6-α-D-glucan 6-glucanohydrolase (EC3.2.1.11), is an enzyme, which catalyzes endohydrolysis of α-(1,6)-D-glycoside linkages in random sites of dextran. Isomaltose, isomaltotriose, and a small amount of D-glucose, together with traces of higher oligomers, are the main reaction products. However, a variation in the reaction products and substrate specificities of dextranases from different sources are evident. For example, endodextranase from a *Penicillium* sp. degrades cyclodextrans to isomaltose and glucose (143). All mold dextranases (EC3.2.1.11) can degrade the cross-linked dextran Sephadex (Table 1).

Molds are the commonest source for the extracellular endodextranases (EC3.2.1.11) and exhibit a higher enzyme activity than dextranases from bacteria and yeasts. There is only one report on intracellular mold dextranase found in *Penicillium lilacinum* NRRL 896 and *Penicillium funiculosum* NRRL 1132 (72). The hydrolysis products of *Penicillium notatum* dextranase are isomaltose and isomaltotriose with a small amount of glucose, as in most fungal dextranases. Reducing sugars are released from dextran much faster and in larger amounts by random attack of endodextranases compared to terminal end-group attack of exoenzymes (225). Thus, a 67 to 74% conversion of dextran to sugar syrup is attained by *P. notatum* dextranase.

The dextranases of two *Penicillium* species (*Penicillium lilacinum* NRRL-896 and *Penicillium funiculosum* NRRL-1132) produce, in addition to oligosaccharides that contain one glucose unit joined by an α-1,4 linkage to a glucose unit of a homolog to isomaltose, small proportions of D-glucose, isomaltose, and isomaltotriose from α-1,4-branched dextran of *L. mesenteroides* NRRL-1415 (1, 2, 191).

Mold dextranases can also hydrolyze oligosugars. D-Glucose is released from isomaltotriose and from higher homologs up to isomaltoheptaose by *Penicillium lilacinum* NRRL-896 dextranase. The hydrolysis occurs at the first linkage from the reducing end. The enzyme also catalyzes an extremely slow, concentration-dependent degradation of isomaltose. This may occur via condensation to isomaltotetraose followed by hydrolysis of the first linkages to give D-glucose and isomaltotriose (218). A similar phenomenon has been found with dextranases from *Aspergillus carneus* and *Penicillium luteum* (68) (Table 1).

The mode of action of *Aspergillus carneus* has been investigated with a series of isomaltodextrins and their derivatives as the substrates. The enzyme readily hydrolyzes these substrates, and the reaction products are similar to those of fungal dextranases. In these studies, the degree of dextran T2000 hydrolysis by *A. carneus* dextranase was about 40% (213). This value is lower than that of *P. luteum* (55%) when the reducing sugars liberated are calculated as glucose (Table 1). This difference was considered to be due to the difference in their hydrolytic abilities toward isomaltotriose, which was readily split by the latter enzyme but very slowly by the former. In contrast, *L. mesenteroides* IAM 1046 dextran, containing 66% α-1,6-, 19% α-1,4-, and 15% α-1,3-glucosidic linkages, was hydrolyzed slowly and to a lesser extent by both enzymes. The amino acid compositions of these two enzymes are closely similar (47, 67, 68, 213).

A strain of *Penicillium aculeatum* produces large quantities of dextranase in its culture broth. The crude enzyme was highly stable (117) (Table 1). About 90% of the substrate dextran was converted to isomaltose in a 4-h period at 40°C. No D-glucose was observed, and thus the results differed from those obtained with the *Penicillium luteum* and *Penicillium funiculosum* enzymes (117). *Chaetomium gracile* produced endodextranases, while the maximal dextran hydrolysis to glucose was 55% (60).

Typically, dextranase synthesis by several fungi is induced in the presence of dextran (47, 48, 49, 59, 117). However, with *Sporothrix schenckii* a tenfold increase in the dextranase production was achieved without cell mass increase when soluble bacterial dextrans were substituted with glucose as the substrate (6). When *Penicillium minioluteum* HI-4 was grown on minimal medium supplemented with different carbon sources, dextran but not starch, glucose, glycerol, lactose, or sorbitol induced high dextranase expression. Quantitation of mRNA indicated that dextran affected dextranase expression at the transcriptional level. When fungi were cultivated in the presence of both dextran and glucose or glycerol, dextranase expression was repressed at the transcriptional level. In the 5′ noncoding region of the *dexA* gene there are several sequences similar to those involved in binding of CreA, important in the D-glucose-mediated carbon catabolite repression of several genes (39). The putatively conserved nature of this regulatory mechanism in fungi suggests that *dexA* may be under the control of a CreA homolog.

The ability of yeasts to synthesize dextranases has mainly been observed in the genus *Lipomyces* (Table 1). The characteristics of the *Lipomyces starkeyi* (ATCC 20825) dextranase (EC3.2.1.11) regarding the effects of pH and temperature on activity and stability are very similar to those of the *Chaetomium* and *Penicillium* enzymes (NRRL 1768; Table 1). The purified enzyme shows the same K_m values as reported for the IGC 4047 dextranase but is not regulated through product inhibition, in contrast to the latter enzyme (105, 106) (Table 1). The *Lipomyces starkeyi* dextranase is a glycoprotein containing 8% sugar. The *Penicillium funiculosum* (34) and *Chaetomium* (60) dextranases are also glycoproteins. The specificity of *Lipomyces starkeyi* dextranase is similar to that of fungal dextranases, the final hydrolysis products being isomalto-oligosaccharides from glucose to isomaltotetraose (105).

In recent studies, a novel glucanohydrolase from mutant *Lipomyces starkeyi* strain KSM 22 has been shown to possess either dextranolytic or amylolytic activity depending on reaction conditions (207) (Table 1). Competition studies with different amounts of dextran and starch as substrates showed consistency with the hypothesis that hydrolysis of dextran and starch occurs at two independent active sites (95, 114, 172).

Endodextranases from Bacteria

Endodextranases can be obtained from several bacterial genera, including *Pseudomonas*, *Brevibacterium*, *Streptococcus*, *Bacteroides*, and *Bacillus* (Table 1). Bacterial endodextranases, like fungal endodextranases, show distinct patterns of action for each specific microorganism. An enzyme extract from the cellulose-degrading bacterium *Cellvibrio fulva* hydrolyzes dextran mainly into comparatively large fragments. Apparently no D-glucose or disaccharides from the ends of the molecule are formed (80). For example, the extracellular endodextranase of anaerobic *Lactobacillus bifidus* produces a mixture of oligosaccharides but no glucose or isomaltose when incubated with essentially unbranched dextran of *Streptomyces bovis* and branched *Leuconostoc* dextran (7, 8).

Most of the bacterial dextranase producers have the ability to synthesize several α-glucosidases with different subcellular localizations and substrate specificities simultaneously (29, 30, 205). Two extracellular dextranases (D_1 and D_2) and one intracellular dextranase (D_4) from *Pseudomonas* sp. strain UQM 733 have been isolated. Both extra- and intracellular dextranases are induced in the presence of dextran. D_1 and D_2 differ in their physicochemical properties, which is possibly attributable to the presence of two proteins in D_2 while D_1 produces much higher yields of low-molecular-weight oligosaccharides from dextran (164). No activity of D_1 appeared with potato starch, amylopectin, amylose, glycogen, or sucrose. However, the enzyme was capable of slowly attacking the α-1,6 linkages in pullulan. Based on studies with reduced and tritiated oligosaccharides, a model for three active sites of the enzyme was postulated (165). The intracellular dextranase, D_4, was very similar to D_1 in molecular weight, pH, and temperature optima as well as mode of action (Table 1). Dextranase activity has also been detected in both intra- and extracellular fractions of *Bacteroides oralis* Ig4a obtained from human dental plaque (205) (Table 1).

Extracellular isomaltotriose-producing dextranases occur in

Streptococcus mutans K1-R and *Flavobacterium* sp. strain M-73 with a strict specificity for consecutive α-1,6-glucosidic linkages. The final yields of isomaltotriose produced from clinical dextran by the endo-action of these two enzymes were 63% for the *Flavobacterium* sp. and 99 to 100% for *S. mutans* dextranase. Dextranase-producing bacterial strains in the genus *Bacillus* have also been isolated from soil samples. One strain, determined by 16S RNA analysis as *Paenibacillus illinoisensis* exhibiting a stable dextranase activity, was characterized (89). The chromatography of products from dextran T-500 with crude enzyme suggested a random endo-type hydrolysis resulting in liberation of long-chain oligomers together with glucose and isomaltose units (Table 1).

The production and characteristics of thermostable dextranases have been reported (69, 227, 228). Among the isolates, *Thermoanaerobacter* sp. strain Rt364 produces dextranase with a high thermostability. The production of endodextranases inducible by dextran has been found in two *Arthrobacter* strains, *Arthrobacter globiformis* T-3044 (147) and *Arthrobacter* sp. strain CB-8 (155).

Glucose-Forming Exodextranases

Exodextranases, such as glucodextranase (EC3.2.1.70; glucan 1,6-α-glucosidase), catalyze stepwise hydrolysis of the reducing terminus of dextran and derived oligosaccharides to yield solely β-D-glucose; i.e., hydrolysis is accompanied by inversion at carbon-1 in such a way that new reducing ends are released only in the β-configuration. Only few bacteria and yeasts are known to produce glucodextranases. Dextran-inducible extracellular glucodextranase occurs in *Arthrobacter globiformis* strains I42 (Table 1) and T-3044 (146, 147). Although glucodextranase I42 releases glucose from dextran and isomaltose and also from starch, maltose, nigerose, and kojibiose, its activity to α-1,4-glucosidic linkages is much less than to α-1,6-glucosidic linkages (134, 153). This might indicate that glucodextranase and glucoamylase activities are due to enzymes functioning differently in different conditions (153). Besides that, glucodextranase I42 converts α- and β-D-glucosyl fluorides to β-D-glucose and hydrogen fluoride, providing additional evidence for the functional flexibility of the catalytic groups of the carbohydrases (97). Glucodextranase T-3044 exhibits properties, pH optimum, and mass similar to those of glucodextranase I42 (147).

Intracellular dextran glucosidases (EC3.2.1.) producing α-D-glucose from dextran exist in several strains of *Streptococcus mitis* (115, 216, 217). *S. mitis* ATCC 903 exoglucanase was purified 925-fold, and some properties were studied (Table 1). The enzyme was active with isomaltose and dextran but nonactive with substrates of α-1,1, α-1,2, α-1,3, and α-1,4 glucosidic linkages. Sucrose, fructose, and mannose had no effect on the activity, while 400 mM glucose almost completely inhibited the enzyme (115). The *S. mitis* 439 intracellular enzyme has an activity pattern closely similar to that of glucodextranase (EC3.2.1.70) but the glucose residues released from isomaltopentaose and dextran by the action of this enzyme are in the α-configuration, demonstrating that it is a glucosidase (216). *S. mitis* 439 dextran glucosidase acts on molecules with a glucose joined through α-1,6 bonds to either a maltosaccharide or an isomaltosaccharide and acts more readily on panose than on isomaltose. A comparable intracellular dextran glucosidase, DexB, in *S. mutans* LT11 releases free glucose from the α-1,4,6 branch points in panose (226). The growth of the strain on panose-induced medium and the rate of hydrolysis of panose were equivalent to those of isomaltotriose and higher than those of isomaltose (226).

Exoenzyme activity that releases glucose from dextran has been detected in animal tissues and in bacteria (51, 161, 170) (Table 1). An enzymatic complex capable of hydrolyzing dextrans to D-glucose as the sole or major product has been found in intestinal anaerobic bacterium of the *Bacteroides* genus. This complex evidently contains two different dextranases active at pH 5.0 to 5.5 (186). Exodextranases have also been isolated from extra- and intracellular fractions of *Bacteroides oralis* IG4 (205) (Table 1).

Inoculation of dextran-containing medium with a soil sample resulted in accumulation of several *Bacillus* species, which were isolated and characterized as *Bacillus subtilis* and *Bacillus megaterium*. The cleavage mechanism of the cell-bound exodextranase of *Bacillus* species involved endwise cleavage of D-glucose residues from the terminal groups, leaving the rest of the dextran molecule intact. Isomaltodextrins were hydrolyzed at a higher rate than dextrans of 100 kDa and 2,000 kDa under the same conditions (231).

Three intracellular glucosidases (G1, G2, and G3) from *Pseudomonas* sp. strain UQM 733 have also been described (Table 1). The action of purified G1, G2, and G3 on pure isomaltooligosaccharides shows that the glucosidases have optimal activity on isomaltotetraose and are, therefore, classified as oligoglucanases (30). Glucosidases G1 and G2 exhibit general properties different from those of glucosidase G3 (29) (Table 1).

Pig spleen acid α-D-glucosidase, possessing dextranase activity, is an exoglucanase with broad specificity (161). The enzyme of ≈106 kDa was purified over 2,000-fold. It hydrolyzed reducing α-D-glucosyl disaccharides and almost completely degraded dextrans that contain α-1,3 and α-1,6 linkages. The pH optimum of dextran glucosidase activity was pH 4.8 to 5.0. Studies with various pHs, temperatures, and inhibitors caused changes in the activity of the α-D-glucosidase against oligo- and polysaccharide substrates, suggesting that the enzyme has multiple substrate-binding sites (161).

Isomaltose-Forming Exodextranases

The soil bacterium *A. globiformis* T6 isomaltodextranase (EC3.2.1.94; 1,6-α-D-glucan isomaltohydrolase) is a novel extracellular exoenzyme capable of hydrolyzing dextran by removing successive isomaltose units from the nonreducing ends of the dextran chains (177, 179) (Table 1). The properties of the enzyme are unusual for it is able to split not only α-1,6 linkages of glucooligosaccharides but also α-1,2-, α-1,3-, and α-1,4-links to yield isomaltose (211); it can split dextran so that the α-configuration of the anomeric carbon atoms is retained in the hydrolysis products (150); it has transfer and condensation activities on isomaltose to produce isomaltotetraose in concentrated solutions (94, 178); and it can split α-1,4-glucosidic linkage of panose and α-1,6-glucosidic linkage of isomaltotriose and pullulan as well (151, 206). It was concluded that there is a single active site on the enzyme molecule for

hydrolysis of α-1,6- and α-1,4-glucosidic linkages responsible for both the isomaltodextranase and isopullulanase activity (206). This isomaltodextranase hydrolyzes 13 dextrans to various extents (11 to 64%, 13 days) at initially high but gradually decreasing rates.

Dextran B-1355 fraction S, unlike the other dextrans, has been found to be hydrolyzed initially at the lowest rate among the dextrans used, but the rate was maintained for a long period of time with little decrease in a manner that 85% of dextran was converted within 13 days (181). Extracellular isomaltodextranase (optimal pH 5.0) from the actinomycete *Actinomadura* sp. strain R10 and that from *Arthrobacter* demonstrate similar modes of action on dextran, but the enzyme is more active on the 1,6-α-D-glucopyranosidic linkages while the relative activity increases within the degree of polymerization. In contrast, the relative activity of the actinomycete enzyme is almost constant throughout the same series of substrates and much higher on 1,3-, and 1,4- linkages than the *Arthrobacter* enzyme (182).

Isomaltotriose-Forming Exodextranases

Exoisomaltotriohydrolase (EC3.2.1.95) is produced by *Brevibacterium fuscum* var. *dextranolyticum* (Table 1). The enzyme is a glycoprotein that removes isomaltotriose from the nonreducing ends of dextran and reduced isomaltodextrins (200). Isomaltotriodextranase does not hydrolyze other than α-1,6-glucosidic linkages. The purified recombinant enzyme shows the same optimum pH, lower specific activity, and a similar hydrolytic pattern to the native enzyme (133) (Table 1).

Debranching Exodextranase

Branched dextran exo-1,2-α-glucosidase (EC3.2.1.115) was found in the culture supernatant of the soil bacterium *Flavobacterium* sp. strain M-73 by Mitsuishi et al. (131). The general properties of dextran 2-glucohydrolase were examined with an electrophoretically homogeneous preparation (Table 1). The enzyme had a strict specificity for 1,2-α-D-glucosidic linkage at the branch points of dextrans (containing 12 to 34% of 1,2-α linkages) and related polysaccharides producing free D-glucose as the only reducing sugar. The enzyme did not hydrolyze disaccharides or oligosaccharides containing linear 1,2-α-glucosidic bonds (100, 131, 132, 202).

Cycloisomalto-oligosaccharide Glucanotransferase

Cycloisomalto-oligosaccharide glucanotransferase (CITase) is a novel enzyme that catalyzes the conversion of dextran to cyclodextran by intramolecular transglycosylation (cyclization). CITase has been purified to homogeneity from the culture filtrate of *Bacillus circulans* T-3040 (Table 1). CITase produces three cyclic isomaltooligosaccharides (cycloisomalto-heptaose, -octaose, and -nonaose) with a total yield of about 20%, wherein cycloisomalto-octaose is the main product. Coupling, disproportionation, and hydrolytic reactions are also observed. The enzyme does not act on amylopectin and pullulan (143, 144). Immobilization of CITase and its application in the production of cycloisomalto-oligosaccharides from dextran have been studied more recently (87). Cyclodextrans almost equally inhibit both reducing sugar and dextran producing activities of the dextransucrase reaction. The inhibition is dependent on the cyclodextran concentration (104).

Since the general characteristics of CITase and cyclomaltodextrin glucanotransferase (EC2.4.1.19) resemble each other, the enzymatic mechanism of CITase can be postulated. First, the main domain (A) of cyclomaltodextrin glucanotransferase closely resembles the structure of α-amylase. Second, the starch-binding "groove" on domain A contains a similar catalytic Asp-Glu residue pair as in α-amylases. Finally, cyclomaltodextrin glucanotransferase contains the starch-anchoring domain E as well as domain B that partially protect the catalytic Asp-Glu dyad from the attack of water molecules. Whenever the average length (and concentration) of the starch is high, the hydrolytic function dominates, but when the length is decreasing the transglycosylation reaction becomes prevalent (see discussion in ref. 124). Decreasing water activity by the addition of organic solvents shifts the equilibrium towards cyclization (37, 125). It is also possible to predict that CITase and endodextranase (glycosylhydrolase family 66) have related structural relationship similar to what has been detected between amylase and cyclomaltodextrin glucanotransferase.

BIOLOGICAL FUNCTION OF DEXTRANASES

Role of Dextranases in Dextran-Producing Microorganisms

It is reasonable to believe that the biological role of dextrans, for the benefit of microbes that produce them, is not only to provide protective and adhesive effects, but also to provide sugar storage for those microbes that are capable of depolymerizing them. Interestingly, certain extracellular exodextranases have special domains for anchoring dextrans into the cell surface from where the glucose units can be economically delivered to the cell (see discussion in reference 134). The ability to maintain food storage outside the cell is especially favorable in conditions when microbes are within reach of vast amounts of oligosugars (e.g., sucrose in mouth). In such conditions dextran is apparently synthesized fast by extracellular enzymes (probably already specifically bound to dextran polymers), while the monosugars generated from the transglycosylation are consumed immediately for metabolism. From the viewpoint of a microbe or a microbial association, optimization of the dextraneous environment in respect of the synthesis of polymerization-depolymerization activities and specificities is complex.

Sucrose is the major constituent of the human diet and both water-soluble and water-insoluble glucans are synthesized from it by oral streptococci (*Streptococcus mutans*, *S. sanguis*, *S. sobrinus*, *S. cricetus*, and *S. rattus*). They are believed to be responsible for the formation of dental plaque and the induction of caries on the surface of teeth and have, therefore, been a subject of numerous studies (11, 23, 46, 55). The glucan-producing streptococci *S. sanguis*, *S. bovis*, and *S. mutans* are also the most frequent organisms associated with endocarditis in humans. The chemical and physical properties of these glucans distinguish them from each other (43, 58, 140). Both soluble and insoluble glucans are important in cell-cell and cell-surface adhesive interactions in dental plaque (25, 55, 230). On the cleaned tooth surface, *S. sanguis*, *S. mitis*, *S. oralis*,

and *S. gordonii* predominate among the first colonizing bacteria, and it is believed that these species help to establish conditions for development of the plaque biofilm (25, 108, 116, 155).

S. sanguis, which synthesizes little or no dextranase, produces not only soluble glucans but also large amounts of α-1,6-linked insoluble glucans that are subject to extensive hydrolysis by exogenous dextranase (13, 54, 220). Strains of *S. mutans* serotype d produce water-insoluble glucans that are resistant to further hydrolysis by exogenous dextranase (56, 220). It has been demonstrated that α-1,6-linked side chains allow the insoluble glucan to adhere to the surface of teeth, while the α-1,3 regions render the glucan insoluble in water and contribute to the resistance to exogenous dextranases (41).

The total amount of glucans and their structures are influenced not only by the activities of the glycosyltransferases but also by extracellular dextranases. Oral streptococci are predominant producers of the dextranases (24, 46, 55, 220). Strains of *S. mutans* constitutively produce both endo- and exodextranases (162, 171, 220), whereas *S. sobrinus* synthesizes only endodextranase (10, 222). Endodextranase activity is present in the culture filtrates, while dextran glucosidase is predominantly cell associated (220). Endodextranase may regulate glucan synthesis by altering the ratio of α-1,6 to α-1,3 linkages and modify the glucan substrate to a firmer form, hence influencing its solubility and adhesive properties (25, 46, 220). Therefore, dextranase activity influences sucrose-dependent adherence of bacterial cells.

Dextranase-deficient mutants of *S. mutans* ($DexA^-$) are more adherent to a smooth surface than the parent strain, but no difference in sucrose-dependent cell-cell aggregation has been observed (24). Endodextranase activity evidently provides primer or branch points for glucosyltransferases and thus contributes to the complexity of the glucan structure (25, 44). Possible intermediates in glucan synthesis could also be the products of exodextranase activity, which have been determined in intra- and extracellular extracts of oral strains of *S. mitis* (115, 215, 216, 217).

The current knowledge of sugar metabolism of *S. mutans* strains combined with genomic data suggest that this organism is capable of metabolizing a wider variety of carbohydrates than any other gram-positive organism, and thus, carbohydrate metabolism is the key survival strategy for *S. mutans* (4, 226). The dextranase of *S. mutans* breaks down glucans to isomaltooligosaccharides, which are then transported into the cell via the products of the multiple sugar metabolism (*msm*) operon. In the cell, the oligosaccharides are further degraded to glucose by the products of the *dexB* gene, a dextran glucosidase (24). *S. sobrinus* and *S. salivarius* do not have such a mechanism and are unable to utilize dextrans or isomaltosaccharides as the sole carbon source (44, 112).

The second role of dextran glucosidase is in facilitating the total degradation of glycogen-like intracellular polysaccharide storage (IPS) to glucose by removing the α-1,6 glucose stubs. IPS is believed to be of significance in the absence of dietary carbohydrates. The third possible function suggested for the dextran glucosidase is in the metabolism of α-limit dextran products from the degradation of extracellular starch by human salivary α-amylase or plaque-derived amylase (226).

The dextranase produced by *S. sobrinus* appears to be regulated in an entirely different way than the dextranase of *S. mutans*, exemplifying a different kind of strategy within dextran-producing microbes. A heat-stable, glucan-binding protein called Dei, which has the ability to inhibit dextranase activity with high specificity, has been detected in *S. sobrinus* but not in *S. mutans*. This inhibition causes the accumulation of water-soluble glucan, which inhibits plaque formation and adherence of the mutans group of streptococcal cells. Dei derived from *S. sobrinus* can only inhibit dextranase from *S. sobrinus* (serotypes d and g), *S. downei* (previously *S. sobrinus* serotype h), and *S. macacae* (serotype h) (201). Under conditions of carbohydrate limitation of *S sobrinus*, Dei levels are high and little active dextranase can be detected. When growth rates increase, the relative proportions and binding of dextranase and Dei alter and free dextranase becomes available (25). This finding suggests that Dei exists in some serotypes of mutans group of streptococci and participates in sucrose metabolism through its interaction with dextranase (201).

Role of Dextranases in Non-Dextran-Producing Microorganisms

The majority of the non-dextran-producing microorganisms use dextrans either as the sole or as a secondary carbon source. Typically, the dextran-degrading enzyme synthesis of several fungi and soil bacteria is induced when grown in the presence dextran (29, 30, 47, 48, 59, 117, 147, 155). As mentioned above, most of the bacterial producers are able to synthesize simultaneously a few α-glucosidases with different subcellular localizations (Table 1). A wide range of bacterial species, such as *Bacteroides* spp., *Bifidobacterium* spp., and *Fusobacterium* spp. associated with dental plaque, produce inducible dextran-hydrolyzing enzymes (26, 76, 86, 196, 205).

Three D-glucan-hydrolyzing enzymes from *Bacteroides oralis* Ig4a have been found. Extracellular endodextranase hydrolyzes polysaccharides in dental plaque to produce oligosaccharides that are small enough to enter the cells. The others, cytoplasmic exodextranase and mutanase hydrolyze the oligosaccharides to monosaccharides, thus permitting the use of dental plaque polysaccharides for microbial growth (205). Interestingly, an enzyme identical to dextranase (EC3.2.1.11) is also associated with the cell walls of growing coleoptiles of a plant, *Avena*. The enzyme plays a prominent role in the growth process, hydrolyzing certain cell wall components and providing necessary plasticity to the cell walls to extend (66).

STRUCTURE-FUNCTION ANALYSIS OF DEXTRANASES

At present, there are 13 annotated sequences of endodextranases from species of *Streptococcus*, *Arthrobacter*, *Paenibacillus*, and *Penicillium* in the databanks (EMBL/GenBank and SWISS-PROT). Only one crystal structure of an endodextranase from *Penicillium minioluteum* (Dex49A) and one from glucodextranase (exodextranase; iGDase) from *Arthrobacter globiformis* have been published. There have been some attempts to solve three-dimensional structures by computer modeling using sequence data and three-dimensional structures of homologous proteins. Compared to the knowledge of structural relationships and mechanisms of action of enzymes involved in synthesis and degradation of starch, cellulose, and

TABLE 2. Dextranase genes and their products

Microbial species	Size of gene (bp) and enzyme (kDa)	Reference(s)
Streptococcus sobrinus	3,999 bp/80–130 kDa (*E. coli* expressed); 160–260 kDa (native)	9, 222
Streptococcus mutans Ingbritt (endodextranase)	1,610 bp/70, 105, 120 kDa (native)	171
Streptococcus salivarius PC-1	−/70, 90, 190 kDa (*E. coli* expressed); 110 kDa (native)	112
Arthrobacter sp. strain CB-8	1,920 bp/62 kDa (native)	155
Arthrobacter globiformis T6	1,926 bp/66 kDa (native)	82
Streptococcus mutans Ingbritt (exodextranase)	2,550 bp/88; 104, 118, 133 kDa (*E. coli* expressed); 96, 108, 167 kDa (native)	24, 74
Streptococcus salivarius M-33	2,469 bp/86 kDa (native)	149
Penicillium minioluteum HI-4	2,109 bp/67 kDa (*E. coli* expressed)	48
Streptococcus suis	1,629 bp/62 kDa (native)	185
Bacillus circulans T-3040	3,000 bp/110 kDa (*S. gordonii* expressed)	145, 190
Arthrobacter globiformis T-3044	3,153 bp/120 kDa (*A. globiformis* and *E. coli* expressed)	147
Brevibacterium fuscum var. *dextranolyticum* strain 0407	1,923 bp/68 kDa (*E. coli* expressed)	133
Penicillium minioluteum HI-4	1,859 bp/—	50
Streptococcus downei	3,891 bp/220 kDa (native); 210 kDa (*E. coli* expressed) + multiple smaller forms	77
Streptococcus rattus	2,760 bp/100 kDa	79

chitin, the data on dextran metabolism are still elusive. However, it seems plausible that the basic understanding of the structures and mechanisms of other carbohydrate enzymes may be applicable to the structure-function analysis of dextranases.

Sequence Comparison Studies

Microbial genes encoding proteins associated with sugar metabolism are clustered, which indicates that their expression is coordinated. The sequences of some of the genes encoding endo- and exo-type dextran-hydrolyzing enzymes have been determined and analyzed to deduce the primary structure of dextranase enzymes and to produce them in heterologous systems (Table 2.). Endodextranases are found in two glycosylhydrolase families, 49 and 66 (CAZy web server http://afmb.cnrs-mrs.fr/CAZY). Family 49 comprises bacterial dextranases from *Arthrobacter* species (CB-8 and T-3044) and fungal dextranases from *Penicillium* and dextran 1,6-α-isomaltotriosidase from *Brevibacterium fuscum* var. *dextranolyticum*.

The sizes of dextranase genes and their protein products are highly divergent (Table 2). This is exemplified by aligning the 13 dextranase protein sequences that are currently available in the protein data banks. Multiple sequence alignment for proteins was created using ClustalW (version 1.82) (http://www.ebi.ac.uk/clustalw/) using default values. The phylogram (Fig. 1) indicates sequences that are related, but due to the great difference in sequence length, the alignment as such is not well presented. This indicates that dextranases form a group of proteins that possess similar enzyme activities even though they have highly different primary protein structure.

Nucleotide sequence analysis of the gene for *Arthrobacter* sp. strain CB-8 dextranase and analysis of the N-terminal amino acid sequence of the dextranase protein reveals that the enzyme is initially synthesized as a polypeptide precursor consisting of 640 amino acid residues, including a 49-residue-long, alanine-rich N-terminal sequence that is cleaved during secretion. In *Escherichia coli*, the activity is mostly detected in the periplasmic space. The active dextranase expressed in *Streptococcus sanguis* is not secreted from cells (155). The enzyme shows 93% and 65% sequence identity with the deduced endodextranase 1 and endodextranase 2 amino acid sequences of *Arthrobacter globiformis* T-3044, respectively. The N terminus of the purified endodextranase from *A. globiformis* T-3044 is similar to the deduced amino acid sequence of the *dex1* gene. Endodextranase can therefore be translated from mRNA as a secretory precursor with a 32-amino-acid-long signal peptide (147).

cDNA clones expressed in dextran-induced cultures of *Penicillium minioluteum* HI-4 have been identified by differential hybridization (48). Analysis of selected clones revealed nonhomologous cDNAs corresponding to four different genes, *dexA*, *dexB*, *dexC*, and *dexD*. One of these cDNAs (*dexA*) encodes endodextranase (EC3.2.1.11). According to the structure prediction of DexA, the enzyme has a mechanism of hydrolysis with net inversion of anomeric configuration and it is, therefore, included in glycosylhydrolase family 49 (63, 159, 160). The enzyme shows 29% and 33% sequence identity with *Arthrobacter* sp. strain CB-8 dextranase and *Brevibacterium fus-*

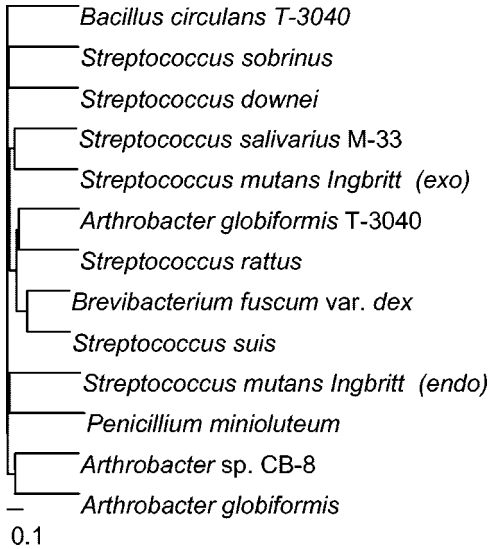

FIG. 1. Neighbor-joining tree showing phylogenetic clustering of representative collection of divergent group of dextranases shown in Table 2.

cum var. *dextranlyticum* strain 0407 isomaltotriodextranase, respectively (48, 133).

The *dexA* cDNA sequence comprises 2,109 bp plus a poly(A) tail, coding for a protein of 608 amino acids, including 20 N-terminal amino acid residues that might correspond to a signal peptide. The predicted molecular mass is 64 kDa, which is somewhat smaller than that estimated for the native enzyme (67 kDa). The *dexB* (1.6 kb) gene shows sequence homology to sugar transporter proteins, whereas the *dexC* (1.8 kb) gene belongs to glycosylhydrolase family 13, showing high sequence identity (51%) to oligo-1,6-glucosidase of *Bacillus cereus*. The *dexD* (1 kb) product is homologous to α-amylase proteins according to structural comparisons. Taking into account that the *dex* genes (with the exception of *dexD*) are grouped in the same 9-kb BamHI fragment, it is tentative to speculate that the genes necessary for dextran assimilation and hydrolysis are clustered in the genome of *P. minioluteum* (50, 111).

The gene encoding extracellular isomaltotriodextranase, an exo-type enzyme (EC3.2.1.95), designated DexT, has been cloned from the chromosomal DNA of *Brevibacterium fuscum* var. *dextranolyticum* strain 0407 and expressed in *Escherichia coli*. A single open reading frame (ORF) consisting of 1,923 base pairs that encoded a polypeptide composed of a signal peptide of 37 amino acids and mature protein of 604 amino acids (M_r 68,300) was found. The primary structure of DexT had significant similarity (78.5% and 63.0%) with two other reported endo-type dextranases isolated from two *Arthrobacter* strains, CB-8 and T-3040, respectively (82, 133).

Isomaltodextranase (EC3.2.1.94), other than DexT, from *A. globiformis* T6 is an exo-type enzyme (Table 1). However, it possesses an entirely different structure, and isomaltodextranase is, therefore, classified in glycosylhydrolase family 27. It contains enzymes having a mechanism of hydrolysis with an overall retention of anomeric configuration. The *imd* gene is 1,689 bp in length and encodes a protein with a molecular mass of 66 kDa. Isomaltodextranase has weak homology with the C terminus of α-galactosidase from *Cyamopsis tetragonoloba* and practically no homology with dextranases from *Arthrobacter* sp. strain CB-8 (155) and *S. mutans* (171), which implies that the isomaltodextranases have different specificities (82).

Glucose-producing exo-type dextranases have been included in glycosylhydrolase families 13 and 15. Glycosylhydrolase family 13 comprises enzymes responsible for the hydrolysis of α-1,2, α-1,3, α-1,4, and α-1,6 glucosidic linkages. Few sequences of dextran glucosidase genes from *Streptococcus* and *Arthrobacter* species are available. Isomaltodextranase from *Arthrobacter globiformis* T6 belongs to glycosidehydrolase family 27, but this isomaltodextranase has poor sequence homology to this family. The enzymes cleave the glycosidic bond of the substrate by either retaining or inverting the anomeric configuration. It is believed that a retaining enzyme is involved in a two-step, double-displacement mechanism utilizing active-site carboxylic acids as the nucleophile and general acid/base catalysts in the hydrolytic reaction. The critical amino acid residues at the isomaltodextranase active site that catalyze the hydrolysis reaction of dextran have been identified, and the roles of nine amino acid residues in this isomaltodextranase were studied by site-directed mutagenesis. Out of 15 enzymes that were mutagenized, eight had reduced dextran-hydrolyzing activities. Aspartic acid-227 and Asp-342, which are part of the apparent catalytic site, are essential for hydrolase activity toward dextran (210).

The gene encoding dextran glucosidase from *Arthrobacter globiformis* T-3044 has been cloned and expressed in *Escherichia coli* (147). The enzyme gene consists of a unique ORF of 3,153 bp. The comparison of the DNA sequence data with the N-terminal and six internal amino acid sequences of the purified enzyme secreted from *A. globiformis* T-3044 suggests that the enzyme is translated from mRNA as a secretory precursor with a signal peptide of 28 amino acid residues, whereas there was no evidence for a signal peptide in *S. mutans* Ingbritt dextran glucosidase (*dexB*) (147, 171). The deduced amino acid sequence of the mature glucodextranase enzyme contained 1,023 residues, resulting in a polypeptide with a molecular mass of 107,475 Da that showed about 38% sequence identity to that of glucoamylase from *Clostridium* sp. strain G0005. Although the glucodextranase that was produced from the transformant was shorter than the authentic enzyme by two amino acid residues at the N terminus, its enzymatic properties were practically the same as those of the authentic enzyme.

A comparison of the deduced amino acid sequence of *Streptococcus mutans* Ingbritt dextran glucosidase encoded by *dexB* with the sequences of other proteins revealed regions of local similarity that correspond to highly conserved regions of α-amylases (171). These regions are also found in enzymes that attack the α-1,6 interchain linkages in starch and pullulan: pullulanase, isoamylase, and neopullulanase. It is believed that these regions are involved in substrate binding and catalysis of these carbohydrases (82). In addition, DexB and isoamylase have a common region in their C-terminal ends. The highest degree of homology was found between DexB and dextranase (DexS) from *Streptococcus suis* with 34% identity and 74% similarity, as well as a significant amount of overall sequence similarity between DexB and cyclodextrin glucanotransferase of *Klebsiella pneumoniae* (171, 185). An analysis of the *dexB* ORF revealed that it codes for a 536-amino-acid protein with a predominantly hydrophilic character and a predicted mass of 62,000 Da. There was no evidence for the existence of a signal peptide. Furthermore, the size of DexB detected by SDS-PAGE in recombinant *E. coli* was 62 kDa, close to that predicted from the sequence data (62,103 Da) (171). DexB is intracellular and does not undergo any posttranslational modification (171).

The primary amino acid sequence of the *dexB* gene product of *S. mutans* strain LT11 showed significant similarity to *Bacillus* oligo-1,6-glucosidase. *dexB* is a a member of the multiple sugar metabolism (*msm*) operon, which contains genes for both transport and subsequent breakdown of products from hydrolysis of extracellular polysaccharides (226). Structure prediction and hydrophobic cluster analysis have also shown that *S. mutants* LT11 dextran glucosidase and *Bacillus* sp. oligo-1,6-glucosidase have similar domain structures, with a catalytic $(\beta/\alpha)_8$-barrel and a smaller C-terminal domain (226).

A glucodextranase (iGDase; EC3.2.1.70) from *Arthrobacter globiformis* I42 was classified in glycosidehydrolase family 15. The iGDase gene was cloned and the primary structure was deduced. A homology search with other proteins revealed that dextran glucosidase from *A. globiformis* T-3044 was the most homologous protein (80% similarity) over the primary structures. Apart from this homology, the N- and C-terminal parts

of iGDase are individually homologous with different kinds of proteins. The N-terminal region showed high similarity to bacterial glucoamylases (52% similarity), whereas the C-terminal region showed 29% identity to the S-layer homology domain of pullulanase (134).

The bacterial endodextranases from the *Steptococcus* species and cycloisomalto-oligosaccharide glucanotransferase from a *Bacillus* sp. showed significant similarity and were classified in glycosylhydrolase family 66. Genes encoding extracellular dextranases were cloned from *S. mutans*, *S sobrinus*, *S. salivarius*, *S. suis*, *S. downei*, and *S. rattus*. A single copy of the dextranase (*dex*) gene was detected in *S. mutans* (24), *S. sobrinus* (10), and *S. salivarius* (112).

The dextranase gene (*dexA*) from *S. mutans* Ingbritt (serotype c) encoded a dextranase (Dex) protein consisting of 850 amino acids with a molecular mass of 94.5 kDa. Thus, it was smaller than the SDS-PAGE-estimated masses of the native dextranase (120 kDa) produced by *S. mutans* Ingbritt and the recombinant DexA (133 kDa) produced by *E. coli* cells (74, 75). The same phenomenon was observed in *S. sobrinus* UAB66 (serotype g) dextranase, where the deduced molecular mass of Dex from the nucleotide sequence (143 kDa) was smaller than the molecular mass of native Dex (175 kDa). The molecular mass of purified enzyme from clone pYA902 was estimated to be 130 kDa. Since this size is exactly half of that obtained by gel filtration (260 kDa), native Dex might exist in a dimeric form (171, 222).

Recombinant S. *sobrinus* dextranase (Dex) is mainly transported into the periplasmic space of *E. coli* cells, whereas *S. mutans* recombinant dextranase is located in the cytoplasm. The deduced N-terminal amino acid sequences of extracellular dextranases of *S. mutans* Ingbritt (*dexA*) and *S. sobrinus* UAB66 (*dex*) showed 57.8% homology. No cross-reactivity exists between the dextranase of *S. sobrinus* UAB66 and surface protein antigen A (SpaA), which appears to be one of the proteins necessary for sucrose-independent adherence (222). The ORF of a dextranase gene from *S. rattus* is 2,760 bp and it encodes a protein consisting of 920 amino acids with a mass of 100,163 Da and pI of 4.67. The physicochemical properties of *S. rattus* dextranase purified from recombinant *E. coli* cells are similar to those of *S. mutans* and *S. sobrinus* dextranases (79).

The dextranase-encoding gene from *S. salivarius* strain M-33 has been cloned and sequenced. One of the clones is a 4.3-kb KpnI fragment containing the gene coding for an 826-amino-acid polypeptide with a molecular mass of 87.9 kDa, which corresponds to that of native Dex (86 kDa) from the *S. salivarius* M-33 culture supernatant (149). A comparison of the *S. salivarius* M-33 Dex sequence with that of *S. mutans* Ingbritt dextran glucosidase (DexB) (171) and *Arthrobacter* sp. CB-8 Dex (155) reveals no homology between these proteins (149). Another gene encoding extracellular endodextranase was cloned from *S. salivarius* strain PC1, and its native product was recovered from culture media as a single 110-kDa polypeptide whereas the recombinant strain produced a 190-kDa protein and two lower-molecular-mass polypeptides (90 and 70 kDa) (112).

DNA fragments encoding the *S. downei* dextranase were amplified by PCR and inverse PCR based on comparisons between the dextranase gene sequences from *S. sobrinus*, *S. mutans*, and *S. salivarius*, and the complete nucleotide sequence of the *S. downei dex* gene was determined. A 3,891-bp ORF encodes dextranase protein consisting of 1,297 amino acids with a molecular mass of 139,743 Da and isoelectric point of 4.49. The deduced amino acid sequence of *S. downei* Dex has homology to those of *S. sobrinus*, *S. mutans*, and *S. salivarius* Dex in the conserved region. A DNA hybridization analysis showed that a *dex* DNA probe of *S. downei* hybridized to the chromosomal DNA of *S. sobrinus* but not to the other species of *S. mutans*. The recombinant plasmid, which harbored the *dex* ORF of *S. downei*, produced a recombinant Dex enzyme in *E. coli* cells. An SDS-PAGE analysis of the recombinant enzyme indicated that there are multiple active dextranase forms (77). Comparison of the amino acid sequences of the Dex proteins and glucosyltransferases indicated that the amino acid sequences of the Dex enzymes produced by *S. mutans*, *S. sobrinus*, *S. salivarius*, and *S. downei* were similar to those of the catalytic sites of glucosyltransferases of mutans streptococci (78, 137).

Dextranase Isoforms

Multiple forms of dextranases have been commonly reported in the literature (Tables 1 and 2). The gram-negative oral bacteria *Prevotella oralis*, *Prevotella melaninogenica* (76), and *Thermoanaerobacter* sp. strain RT 364 (228) excrete multiple dextranase forms. Two active fractions have been obtained from culture broth of *Prevotella funiculosum* as well as from that of *A. carneus* (Table 1). Purified dextranase from *Paenibacillus illinoisensis* showed three isoforms, the molecular mass of which in denaturing conditions differed by only 15 to 20 kDa. At least five N-terminal amino acids in them appeared to be identical. Thus, if the isoforms were a result of proteolysis, it must have taken place at the C termini without any significant loss of activity. Protease inhibitors added to the cultivation medium did not have any effect on the content of the isoforms (89) (Table 1).

Observations suggest that the higher-molecular-mass forms of dextranase gradually lose activity during storage, while the activities of the smaller forms remain unaffected (171). This loss of activity can be prevented by protease inhibitors. The very large variability of molecular masses of dextranases from different microbial sources and the common existence of catalytically active isoforms suggest exceptional, not yet identified structural/functional features that deserve further attention from the scientific community.

Secondary and Tertiary Structures of Dextranases

The secondary structure of a dextranase produced by *Penicillium minioluteum* was determined by database comparisons and circular dichroism measurements and found to be compatible with galactose oxidase, methanol dehydrogenase, and sialidase folds (159). The enzyme contains 15% of N-linked oligosaccharide chains at positions Asn 39, 571, and 574 (48). Enzyme activity and fluorescence studies indicate that the recombinant dextranase from *P. minioluteum* HI-4 loses its biological activity at neutral pH without total disruption of its conformation. The enzyme preserves its conformation even at 60°C but is then thermally denatured with aggregation at temperatures above 75°C. Two disulfide bridges (Cys^{9}-Cys^{14} and

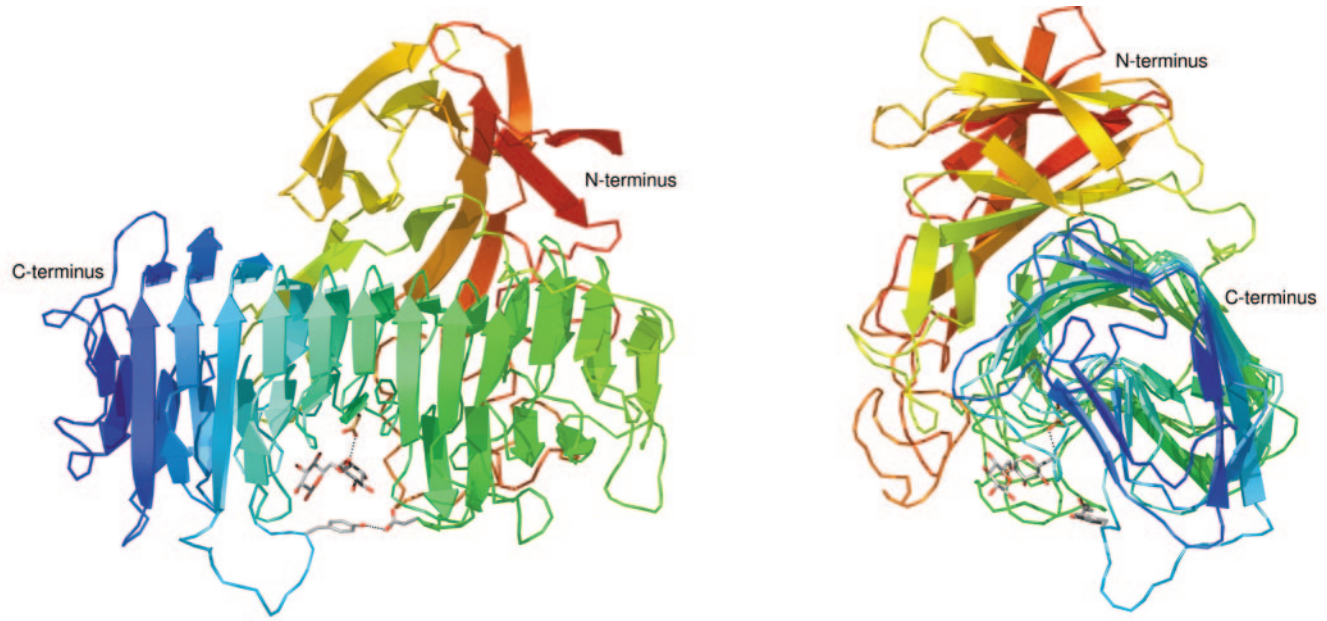

FIG. 2. Crystal structure of endodextranase Dex49A from *Penicillium minioluteum* (111). The right-side structure is turned 90° counterclockwise around the vertical axis. Reprinted from reference 111 with permission from Elsevier.

Cys^{484}-Cys^{488}) and two free Cys residues (Cys^{336} and Cys^{415}) are not conserved between bacterial and fungal dextranases in the glycosylhydrolase 49 family. It was concluded that Cys residues are not essential for maintaining enzyme conformation (12).

Based on common sequence patterns in the structural core region between DexC (glycosylhydrolase family 13) of *P. minioluteum* and *Bacillus cereus* oligo-1,6-glucosidase, a three-dimensional structure was predicted. Even though *dexA* and *dexC* have no significant sequence similarity and the predicted three-dimensional structures are different, their products catalyze chemically equivalent reactions (50).

Computer modeling studies indicated that *S. rattus* dextranase has two variable regions, an N-terminal signal peptide and a C-terminal cell wall-sorting signal. The main molecule contains two functional domains, a catalytic domain (12 amino acids) and a substrate-binding domain (120 amino acids residues) at the C-terminal side. This structural organization is quite similar to the dextranases from *S. mutans*, *S. sobrinus*, and *S. downei* (79). Asp385 of the Dex of *S. mutans* Ingbritt is essential for enzyme activity, and the catalytic and substrate-binding sites are located at different sites within the Dex molecule (78, 137). Replacement of Asp385 of DexA from *S. mutans* Ingbritt results in complete disappearance of enzymatic activity, while the enzyme retains its ability to bind dextran (78). Deletion of the N terminus abolishes enzyme activity but does not affect dextran-binding ability, while deletion of the C-terminal 120 amino acids fully abolished the ability to bind dextran (137).

The dextranases from *S. mutans*, *S. sobrinus*, and *S. downei* have a putative cell wall-anchoring region at the C terminus (75, 77, 222). However, the cell wall-bound form of dextranase has not been detected, and the enzyme has always been reported as extracellular. The presence of a cell wall-anchoring region in Dex may suggest that it is temporarily cell wall associated and then released extracellularly by an unknown modification(s) (77).

The crystal structure of a glycosylation-free mutant form of endodextranase from *P. minioluteum* (termed Dex49A) has been solved in unliganded (at 1.8 Å resolution) and product-bound (at 1.65 Å resolution) forms (111). The enzyme forms 10 right-handed parallel β-helix domains that are connected to an N-terminal β-sandwich domain (Fig. 2). In the structure of the product-bound form, isomaltose was found to bind in a crevice on the surface of the enzyme. The nonreducing end sugar forms a hydrogen bond to the Asp395 carboxylate, which is the plausible catalytic acid for the 1,6-glycosidic bond. Asp395 is conserved within glycosylhydrolase family 49, as are Asp376 and Asp396. The latter two aspartyl residues are hydrogen bonded to a water molecule, which is suitably positioned for nucleophilic attack. The structures most similar to DexA are the galacturonases found in glycosylhydrolase family 28, which is suggestive of a new glycosylhydrolase clan for glycosylhydrolase families 28 and 49.

The crystal structures of *A. globiformis* I42 glucodextranase (iGDase; EC3.2.1.70) in the unliganded state and in complex with acarbose at 2.42 Å resolution have also been solved (134). The structure of iGDase is composed of four domains, N, A, B, and C. Two, one, and three calcium ion binding sites are located at domains N, A, and C, respectively. Domain A forms an $(\alpha/\alpha)_6$-barrel structure, and domain N consists of 17 antiparallel β-strands, and both domains are conserved in bacterial glucoamylases. These domains appear to be mainly involved in catalytic activity. The structure of iGDase complexed with acarbose reveals that the positions and orientations of the residues at subsites −1 and +1 are nearly identical between iGDase and glucoamylase. However, the residues corresponding to subsite +3 that form the entrance of the substrate binding pocket and the position of the open space and constriction of iGDase are different from those of glucoamylases.

Glu430 and Glu628 are considered the catalytic residues. Domains B and C appear to be relatively independent from N and A, whereas domains B and C are not found in the bacterial glucoamylases. The primary structure of domain C is homologous with a surface layer homology domain of pullulanases, and the three-dimensional structure of domain C resembles the carbohydrate-binding domain of some glycohydrolases. It was suggested that domains B and C serve as cell wall anchors (134).

METHODS FOR MEASURING DEXTRAN-HYDROLYZING ACTIVITY

To measure the enzyme activity of a dextran-hydrolyzing enzyme can sometimes be difficult because of the large variability of available substrates and because the reaction product is an undefined mixture of sugar polymers. This also makes it laborious to assess the validity of the reaction conditions. Since the selection of the assay method is a compromise between factors such as convenience, speed, and accuracy, we give here an overview of the spectrum of methods used. Because there are no commonly accepted methods in the field, it is hard to compare enzyme activities between different investigations. Hence it would be desirable to put more effort into developing standardized and kinetically valid methods with generally acceptable formulations of the enzyme units. In particular, a correlation of a method of choice against high-pressure liquid chromatography data when analyzing one or more of the end products would be informative (119).

The first methods to be used for measuring dextranase activities were based on viscosimetric analysis (59, 71, 80). One unit of viscosity-reducing activity was defined as the amount of enzyme which reduced the specific viscosity of the mixture by half in 10 min. The nephelometric method defines dextranase activity as a dextran solution's loss of opalescence (7). These methods are apparently suitable when dextranase cleaves the dextran molecule at random to produce long oligosaccharides. Solutions of high-molecular-weight dextrans show a strong apparent UV absorption at 220 nm (102), which allows direct determination of the amount of high-molecular-weight dextrans produced differently by endo- and exodextranases (103).

In saccharogenic methods, one unit of enzyme has been defined as the amount of enzyme producing 1.0 μmol of glucose (35), 1.0 μmol of isomaltose (158), 1.0 mg of isomaltose monohydrate (20), or 1 mg of isomaltotriose (7, 8) per unit time under the assay conditions. The liberated reducing sugars in a reaction mixture are frequently analyzed with the Somogyi assay (44, 195) with 3,5-dinitrosalicylic acid reagent (49, 129), thiourea borax-modified O-toluidine color reagent (35), or alkaline potassium ferricyanide solution (225). In principle, the measurement of total reducing sugars is universal and allows comparisons between different methods. Unfortunately, however, there is no common substrate to assay the activity. Dextran T2000 (47, 68, 76), T-260 (3), T110 (158), and T-40 (196) have been widely applied. In addition, the incubation time has varied from 10 min (47, 67) to 2 h (20, 225). The hydrolytic activity of exo- and endodextranases has also been monitored with *p*-nitrophenyl-α-D-glucopyranosides as the substrates (115, 121). The method is simple but needs strict verification for the absence of other interfering enzymes.

Endodextranase activity can be measured spectrophotometrically by using chromogenic substrates with negligible interference from endogenous glucose or isomaltose (107, 120, 123). The assay is fast, sensitive, quantitative, and especially suitable for enzymes releasing relatively long dextran oligomers. The more sensitive fluorometric assay using aminodextran-70 coupled with the fluorescent dye BODIPY (4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid, succinimidyl ester) has been described (232). Other assay procedures include the use of chemically modified insoluble substrates and measure of the release of soluble chromo- or fluoro-coupled fragments with sizes different from that of the substrate (70, 88, 174). Soluble and insoluble substrates are easy to separate, but the conditions of the mass transfer from solid to liquid phase must be kept constant.

Solid-phase (plate) assays using agar gels may be useful for screening dextranase activities. In short, a suspension of Sephadex in a buffer is supplemented with agar, sterilized, and poured onto petri dishes. Small wells are punched and filled with the test solutions, followed by incubation. The extent of clearance around the hole due to the opalescence of Sephadex provides an estimate of dextranase activity (6, 54, 173). A convenient assay for identification of chromatographic fractions of dextranase can also be based on agar slabs containing dextran. Treatment of the slabs with ethanol precipitates dextran and the hydrolysis zones become visible (113). Remarkably, if polyacrylamide is doped with blue dextran, dextranase activity can be detected on SDS-PAGE gels after renaturation of the enzyme activity. Enzyme activities are seen as clear zones on a dark blue background (9). This method is especially useful for detection of dextranase isoenzymes (89).

APPLICATIONS OF DEXTRANASES

The dextrans themselves are polydisperse and as such mostly not suitable for technological applications. However, enzymatically processed fractionated dextrans possess a significant commercial interest in cosmetics, drug formulations, and vaccines, as cryoprotectants, and as stabilizers in the food industry. Selected dextran fractions in combination with polyethylene glycol solutions form a two-phase system. In addition to using dextranases for processing dextrans, the enzymes themselves are increasingly important in the food, dental, and detergent industries (see below). Finally, dextran-hydrolyzing enzymes are important for elucidating the fine structure of dextran and certain other polysaccharides (1,2, 15, 28).

Clinical Applications of Dextran and Dextranases

Initial interest in dextranases was raised in regard to their possible application in commercial production of clinical dextran, i.e., a sterile solution of dextran of a specific molecular weight to be used to restore blood volume in patients suffering shock as a result of blood loss (80, 90, 113, 127). Relatively low-molecular-weight clinical dextrans have previously been produced from dextran by controlled acid hydrolysis followed by organic solvent fractionation. However, the yields are low (10 to 12%) due to losses during hydrolysis and fractionation. The enzymatic method seemed to have potential for replacing the acid hydrolysis for clinical dextran production and such

processes were patented in the 1950s. The enzymatic method needs less energy and simpler equipment and results in a more uniform product with a 25% to 52% yield (21, 141, 142).

The highest yield of clinical dextran, 94% of total dextran produced, has been obtained in mixed-culture fermentation of *Lipomyces mesenteroides* and a constitutive dextranase mutant of *Lipomyces starkeyi* in the presence of sucrose. A simple industrial fermentation was developed to produce controlled-size dextrans with a small polydispersity index (36, 90, 93). Dextrans of molecular weights between 900 and 1,800 were considered less likely to cause anaphylactic reactions than the higher-molecular-weight dextrans (214). Due to the extremely strict regulatory demands of the intravenously administered clinical dextrans and their stagnant market, significant technological progress has not yet managed to overcome the traditional chemical processes.

The advantages of processed dextrans for biomedical applications are the biocompatibility, slow biodegradability, and feasibility of incorporation of molecules into the matrices formed by dextrans (99, 127, 193). Dextran hydrogels and their chemical modifications have been evaluated as carriers for controlled release of drugs to targeted organs by slow dextranase hydrolysis. Remarkably, biodegradable dextran hydrogels containing polyethylene glycol have exhibited regulated insulin release (138). A substantial number of pharmacokinetic studies on dextran conjugates with therapeutic and imaging agents have been carried out in animals (31, 42, 127, 138, 139).

Dextranase can be used as universal targeting method for therapeutic agents (57). In the case of cancer, for example, a bispecific antibody has been created against cancer antigen and dextranase. The bispecific antibody is first injected into the blood circulation, where it binds to cancer cells. Dextranase is then injected and subsequently captured by the antibody-bound cancer cells. Finally, a cytotoxic therapeutic agent conjugated to dextran is injected into the bloodstream, and the conjugate is cleaved by the action of dextranase to release the cytotoxic drug selectively into the cancer cells (57).

In endocarditis, an exopolysaccharide product from viridans streptococci (glycocalyx, composed predominantly of dextran) has been associated with a delayed antimicrobial efficacy in cardiac vegetations. Enzymatic digestion of the glycocalyx by dextranase has been shown to enhance the antibiotic activity of penicillin and temafloxacin (33, 128).

Dextrans also contribute to human health since they are resistant to mammalian digestive enzymes in the small intestine but are readily fermented in the large intestine, particularly by probiotic bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium*. Prebiotic oligosaccharides, including isomalto-oligosaccharides, are believed to promote the growth and proliferation of these microbes most efficiently. Immobilized dextransucrase with soluble dextranase has been used for synthesis of prebiotic oligosaccharides (109).

Applications of Dextranases in Treatment of Dental Plaque

Dental plaque, the bacterial film adhering to tooth surfaces, is composed of closely packed bacteria and noncellular material. Roughly 20% of the dry weight of dental plaque is water-insoluble glucans (121). Degradation and removal of these glucans have been suggested to prevent oral diseases such as dental caries. Dextranase can inhibit the synthesis of insoluble glucans (121, 183, 196, 215) as well as the adherence of streptococci (183, 184). Simultaneous use of several enzymes, such as dextranase and mutanase, could be advantageous (140, 219). A novel glucanhydrolase, DXAMase from *Lipomyces starkeyi*, appears to be effective in reducing synthesis of insoluble glucans, inhibiting sucrose-dependent adhesion to glass, and removing bacterial films previously formed in the presence of sucrose. These in vitro properties of DXMase are considered propitious for dental plaque agent (172).

For the treatment of dental plaque, various compositions that comprise enzymes hydrolyzing or inhibiting glucans have been proposed (95, 96, 188, 212). Another approach to the control of dental caries would be genetic engineering of oral commensal organisms to antagonize the cariogenicity of *S. mutans* strains. The genes encoding dextranase and mutanase have been cloned and expressed in oral streptococci (110, 122). The transformant *S. gordonii* has been found to repress the firm adherence of water-insoluble glucan in a cocultivation experiment with cariogenic bacteria in the presence of sucrose (110). However, it has not yet been demonstrated that such a strategy is effective in vivo. A novel transformant technique, resident plasmid integration for cloning of foreign DNA in oral streptococci, has been used to clone the gene coding for cycloisomalto-oligosaccharide glucanotransferase (CITase) that produces cycloisomalto-oligosaccharide, a potent inhibitor of oral streptococcal glucosyltransferases. CITase has been isolated from the *Bacillus circulans* T-3040 chromosome (145) and transferred into *S. gordonii*, and the gene product was secreted into the culture medium at low levels (190).

Use of Dextranases in the Sugar Industry

One of the major industrial applications of dextranases is the reduction of sliming in sugar production processes. The growth of *Leuconostoc* and *Lactobacillus* spp. is the most important factor in contributing to the postharvest deterioration of cane sugar and frost-damaged beet sugar (18, 113, 207). Problems caused by dextran in raw sugar include sucrose loss, increased viscosity of process syrups, and poor recovery of sucrose due to inhibition of crystallization. Dextranases are used in various analytical methods for measuring glucan content in sugar juices and in raw sugar (18, 19, 167, 194, 197). Cane dextran isolated from deteriorated cane juices and raw sugars possesses an average molecular mass of 5,000 kDa and are polydisperse by nature. Dextrans isolated from various sugar cane products possess a very similar structure, 95% α-1,6 linkages and 5% branching, probably through α-1,3 bonds (18).

The majority of the methods used to remove dextran from sugar solutions rely on its enzymatic hydrolysis. Dextranases reduce the molecular mass and therefore the viscosity of juices (22, 32, 81, 118, 233). Trademarks like Novo dextranase of *P. lilacinum* (Denmark) and dextranase Hutten DL-2 of *Chaetomium gracile* (Japan) have been successfully used to treat dextran-contaminated sugar process streams (169, 233). Even at relatively low levels of dextran in raw juice (i.e., 75 mg/liter) the filtration rate is markedly dropped and, consequently, the slicing capacity is decreased by 50%. A dosage of 10 ppm dextranase NOVO 50 L enzymes to the extraction is sufficient to restore slicing to 90% of the nominal capacity (17).

By analogy with glucoamylase in the context of cyclodextrin production, glucodextranase can be used for the isolation of cyclodextrans (cycloisomalto-oligosaccharides) from the conversion mixture of dextran. Glucodextranase hydrolyzes only dextran and linear isomaltosaccharides but not cycloisomaltooligosaccharides, concomitantly decreasing the viscosity of the solution. Commercially available glucodextranase is, however, expensive because it is isolated from *A. globiformis* and its separation from the endodextranase produced by the same bacterial species is tedious. Therefore, efforts to produce recombinant glucodextranase are under way (147).

CONCLUSIONS AND FUTURE DIRECTIONS

Sugar polymers are of enormous diversity and widely distributed in different organisms and cell types. Sugar polymers have multiple roles in pathogen detection, immunity, and cell-cell interactions. Dextran belongs to a group of sugar polymers that evolved mainly to benefit a narrow range of microbial species. However, dextran is not unique among these species, and most organisms can hydrolyze it to a certain extent. Although the structures of dextrans and, e.g., starch differ considerably, the enzymes hydrolyzing them have many similarities in mechanism of action and structure. It may, therefore, be necessary to investigate the specific characteristics of a larger number of enzyme groups before common evolutionary linkages can be formulated. Only a few structures of dextranases have been solved, and there is practically no information of dextranase isoforms that may possess unique structure-function characteristics.

The few dextranases detected in higher organisms are expected to have novel functions. Purified and specific enzymes can be used in basic research for the analysis of more complicated structures of carbohydrates as well as in biotechnological applications to produce and modify glycosylated residues in receptor proteins. The number of applications of dextran and dextran enzymes is expected to increase in the near future. For example, the sugar industry requires more effective thermostable dextranases for various processes. Processed dextrans have prebiotic properties and advantageous effects on the texture and consistency of foodstuffs, features that the food industry has not yet exploited. Although dextranases have been studied most profoundly in the context of dental disease, breakthrough technologies still wait to be found. The most interesting dextran-related applications may be to create hydrolysis of microbial dextran capsules to make microbes more prone to antibiotics, and these deserve further studies.

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