Effect of Magnesium on Chlorophyll-Protein Complexes

By

EVA-MARI ARO and NIINA VALANNE

Department of Botany, Institute of Biology, University of Turku, SF-20500 Turku 50, Finland

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Abstract

The effect of Mg^{2+} during the isolation of chlorophyll-protein complexes was studied in two moss species (*Pleurozium schreberi* and *Ceratodon purpureus*) and New Zealand spinach (*Tetragonia expansa*). When 2 m*M* $MgCl_2$ was included in all the extraction and separation phases, the proportions of chlorophyll-protein complex I were very small in all plants studied. The withdrawal of Mg^{2+} considerably increased the proportions of CP I. The most pronounced increase in the chlorophyll present as CP I was found when Mg^{2+} was withdrawn from the gel, and this also increased the mobility of the CP II complex and free pigment zone. Exclusion of Mg^{2+} from the running buffer had very little effect. Although Mg^{2+} had little effect on the relative amount of chlorophyll in CP II, the withdrawal of Mg^{2+} from all the extraction and separation phases caused formation of polymers of CP II. In the mosses, the formation of polymers of CP II seemed to be more obvious in the species with large grana. Absence of Mg^{2+} from all the extraction and separation phases sometimes also produced a polymer of CP I.

Introduction

Magnesium is known to increase the fluorescence of photosystem II (Murata 1969, Homann 1969, Gross and Hess 1973, Davis *et al.* 1976) and regulate the energy distribution between photosystem II and I (Butler and Kitajima 1975). The addition of Mg^{2+} leads to a decrease of energy spillover from photosystem II to photosystem I, thus decreasing the energy received by system I (Murata 1969, Bennoun 1974), and increasing that received by system II. Mg^{2+} ions are thought to cause interaction between photosystem II and the light-harvesting pigment-protein complex (Arntzen and Ditto 1976).

There is evidence that the effects of divalent cations are due to changes in protein structure (Jennings and Forti 1974, Davis and Gross 1975), although the results of studies on cation effects in CP II-deficient mutants are contradictory (Vernotte *et al.* 1976, Boardman and Thorne 1976).

We have studied the effect of Mg^{2+} ions on the distribution of chlorophyll-protein complexes in polyacrylamide gels. Our observation that the withdrawal of Mg^{2+} causes a conspicuous increase in the amount of chlorophyll-protein I is probably connected with the effects of Mg^{2+} on the energy distribution between the two photosystems.

Abbreviations: Chl, chlorophyll; CP I, chlorophyll-protein complex I; CP II, chlorophyll-protein complex II; CP I*, CP II*, and CP II**, polymers of chlorophyll-protein complexes; CP II′, dissociation product of CP II; PS I, photosystem I; PS II, photosystem II; SDS, sodium dodecyl sulfate.

Materials and Methods

Tufts of the mosses (*Ceratodon purpureus* [Hedw.] Brid. and *Pleurozium schreberi* [Brid.] Mitt.) were collected from natural habitats and used immediately (if not otherwise mentioned) for the isolation of chlorophyll-protein complexes. New Zealand spinach (*Tetragonia expansa* Murr.) was cultured in a greenhouse under long day conditions at a light intensity of 5000 lux.

For isolation of chlorophyll-protein complexes, the upper parts of moss shoots or the leaves of spinach were ground in a glass homogenizer in an ice bath for 5 min. The isolation of chloroplast lamellae was performed by the method used by Thornber and Highkin (1974). The membranes were solubilized for 5 min in a glass homogenizer in an ice bath with a sufficient volume of 0.5% sodium dodecyl sulfate–50 m*M* Tris, pH 8.0, to yield a 15 : 1 ratio of SDS/Chl (w/w).

The effect of Mg^{2+} was studied by adding 2 m*M* $MgCl_2$ to the SDS extract, the gel and the running buffer, or to one or two of them in different combinations.

The SDS-extracted lamellar components were resolved by SDS polyacrylamide-gel electrophoresis, using the method of Thornber and Highkin (1974). The electrophoresis was carried out at 5°C for 40 min, 9 mA/gel. Densitometer tracings were obtained with a densitometer device attached to the Perkin Elmer 402 spectrophotometer. Unstained gels were scanned at 675 nm, and the gels stained with amido

schwarz at 620 nm. The absorption spectra of the chlorophyll-containing bands were recorded with the same spectrophotometer *in situ* after stopping the densitometer at the maximal absorption of the band in the gel.

The relative proportions of the chlorophyll-protein complexes were estimated by calculating the areas under each recorder peak by a height times width at half height method.

Results

With the method used by us earlier (Alberte *et al.* 1972), we always found very low percentages of CP I (Valanne and Aro 1976) in Ceratodon purpureus. Even smaller peaks of CP I were obtained in the present study with the higher plant, Tetragonia expansa (Figure 1). The withdrawal of Mg^{2+} from the gel caused a dramatic increase in the amount of chlorophyll in CP I (from 3 to 23%). There was no change in the migration of CP I, whereas the mobility of CP II and the free pigment zone increased.

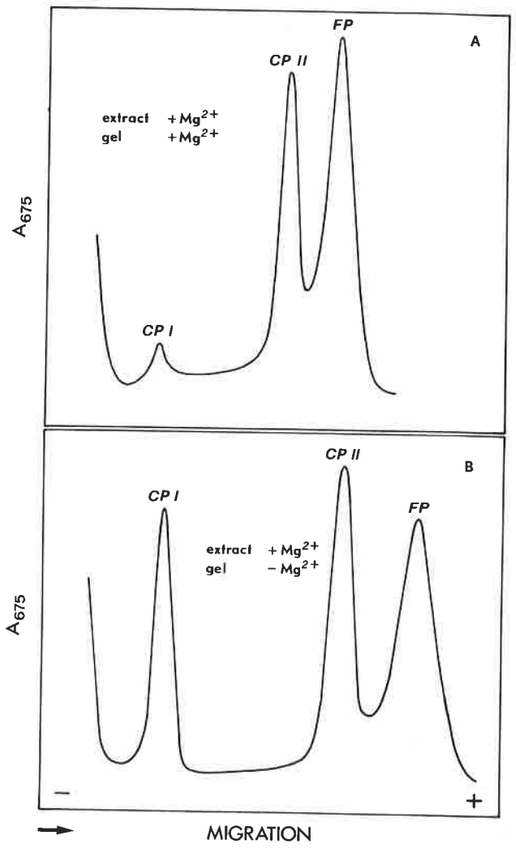

Figure 1. *Densitometer tracings of chlorophyll-protein complexes of Tetragonia expansa.* (A) 2 m*M* $MgCl_2$ in the SDS extract, the gel and the running buffer. (B) 2 m*M* $MgCl_2$ in the SDS extract and the running buffer, but no Mg^{2+} in the gel.

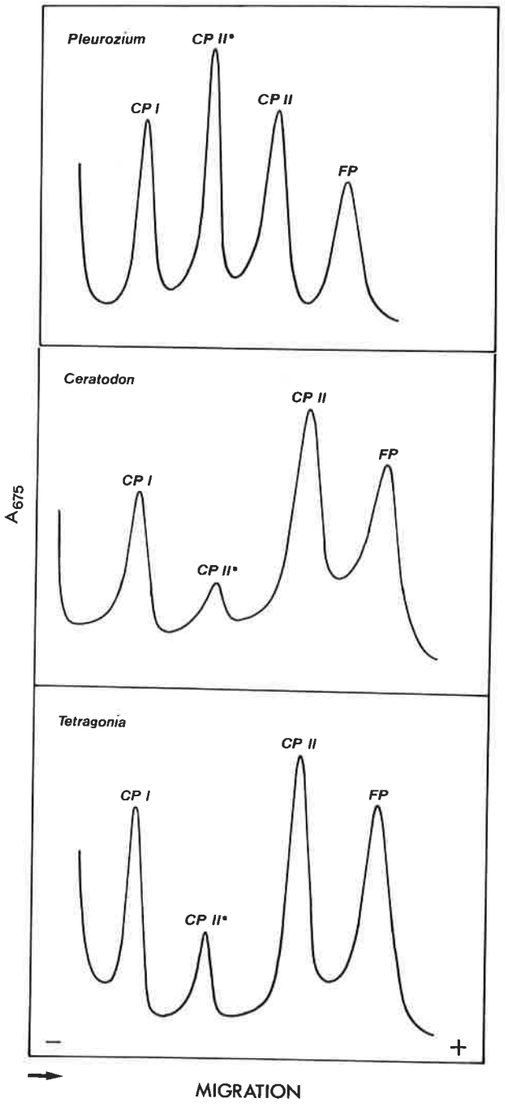

Figure 2. *Densitometer tracings of gels run with different materials.* Mg^{2+} exluded from all the separation phases.

Table 1. *The percentage of total chlorophyll in CP I, CP II* (including CP II*) *and the free pigment zone* (FP). The effects of the presence or absence of Mg^{2+} ions from different phases of the isolation of chlorophyll-protein complexes.

$MgCl_2$ in extract gel buffer			CP I		CP II		FP	
			Ceratodon	Tetragonia	Ceratodon	Tetragonia	Ceratodon	Tetragonia
+	+	+	8	3	38	42	54	55
−	+	−	11	6	37	38	52	56
+	−	−	20	22	43	33	37	44
+	+	−	9	5	45	37	46	58
−	−	−	21	21	44	41	35	38
−	+	+	8	5	35	39	58	56
+	−	+	18	23	42	34	40	43
−	−	+		23		38		39

The effects of the presence of Mg^{2+} in the SDS extract, the gel and the running buffer were studied separately using Ceratodon and spinach (Table 1). The most pronounced changes caused by the withdrawl of Mg^{2+} were the increases in the percentage of chlorophyll in CP I. The changes in the percentage of chlorophyll present as CP II were less significant, although there was some increase with Ceratodon after withdrawal of Mg^{2+} from the gel. The presence of magnesium in the running buffer did not prevent the occurrence of a large amount of chlorophyll in CP I.

In all the plants, withdrawal of magnesium from all the separation phases resulted in the appearance of a fourth chlorophyll-containing zone, between CP I and CP II (Figure 2). The spectrum of the new band (Figure 3) revealed a peak of chlorophyll *b*, and we therefore suppose it to be a polymer of CP II, CP II*. In contrast to the spectra of CP II and CP II*, the free pigment zone failed to show any peak of chlorophyll *b*. Both CP I and free pigment have a spectrum resembling that of chlorophyll *a*.

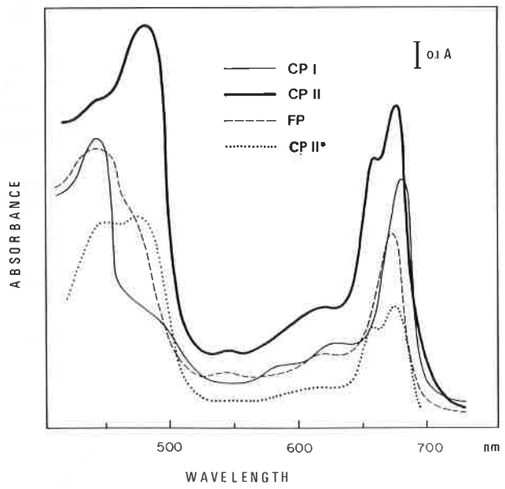

Figure 3. *Absorption spectra of chlorophyll-containing bands in a polyacrylamide gel run without* Mg^{2+}. *Pleurozium schreberi* (see above Figure 2).

The effect of total withdrawal of Mg^{2+} on the distribution of chlorophyll-protein complexes in polyacrylamide gels run with the three plants is compared in Figure 2. Of the two mosses, Pleurozium is a typical shade plant, with large grana (unpublished results, paper in preparation), whereas Ceratodon favours sunny habitats and has small grana (Valanne 1977). The shade moss has a far more pronounced peak of CP II* (up to 26% of total chlorophyll) than the other species (Ceratodon 6% or less, spinach 6%). There were some seasonal changes in the percentage of CP II and its polymer, but the formation of the polymer was not dependent on the percentage of CP II. For example, in autumn the combined value for CP II and CP II* in Pleurozium was around 55%, but there are isolations with Ceratodon in which 52% of the chlorophyll was present as CP II and only 1–2% as the polymer.

It seems that exclusion of magnesium increases only the chlorophyll content of CP I (Figure 4A); the staining of the gels after total withdrawal of Mg^{2+} (Figure 4B) reveals no difference in the protein content. In the case of CP II, on the other hand, the appearance of the polymer is due to a proteinaceous aggregation.

In Pleurozium material in an exceptional physiological state, withdrawal of Mg^{2+} was followed by the appearance of a second band of CP I (Figure 5). After one week's storage of Pleurozium tufts at low temperature and low light intensity, we obtained seven chlorophyll-containing bands instead of the four bands normally found in gels run totally without magnesium. Analysis of the chlorophyll spectra in these seven bands showed that all the bands around CP II had a peak of chlorophyll *b* in addition to chlorophyll *a*. When estimating the percentage distribution of chlorophyll proteins in these gels, we obtained the following values: CP I* + CP I = 25%, CP II* + CP II* + CP II + CP II′ = 55%, FP = 20%.

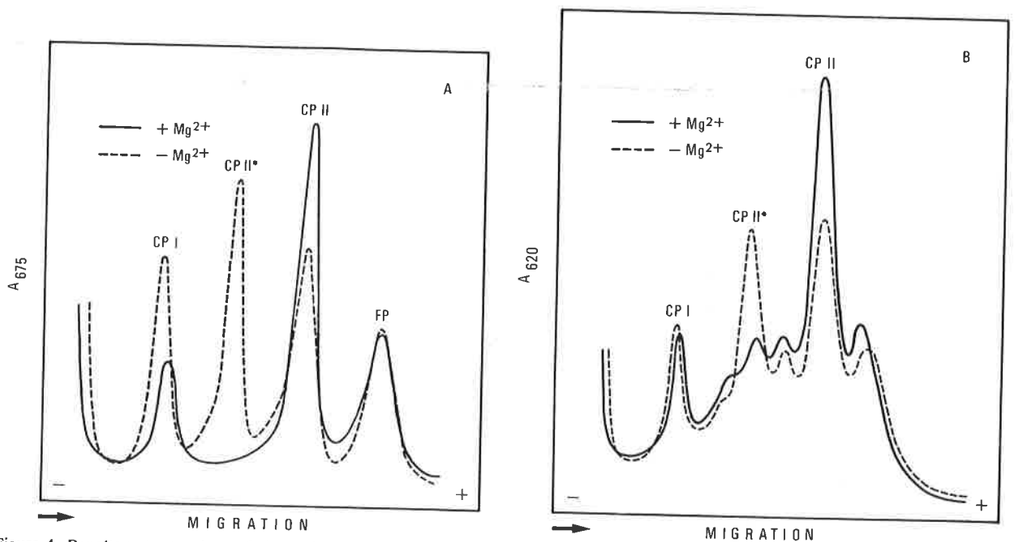

Figure 4. *Densitometer tracings of chlorophyll-protein complexes of Pleurozium.* Continuous line, Mg^{2+} in the SDS extract. Dashed line, without Mg^{2+}. In both runs, Mg^{2+} was excluded from the gel and the running buffer. (A) Unstained gels, (B) gels stained with amido schwarz.

Discussion

It is difficult to say whether the effects of magnesium ions observed here in polyacrylamide gels have anything to do with the cation control and divalent cation binding observed in chloroplast lamellae *in vitro* (*e.g.*, Gross and Prasher 1974). However, cation binding has also been found with purified preparations of CP II (Davis and Gross 1975), and our results after total withdrawal of Mg^{2+} show that the CP II is in an unstabilized condition without magnesium. The appearance of two or more bands with similar spectra may correspond to observations (Arntzen and Ditto 1976) that

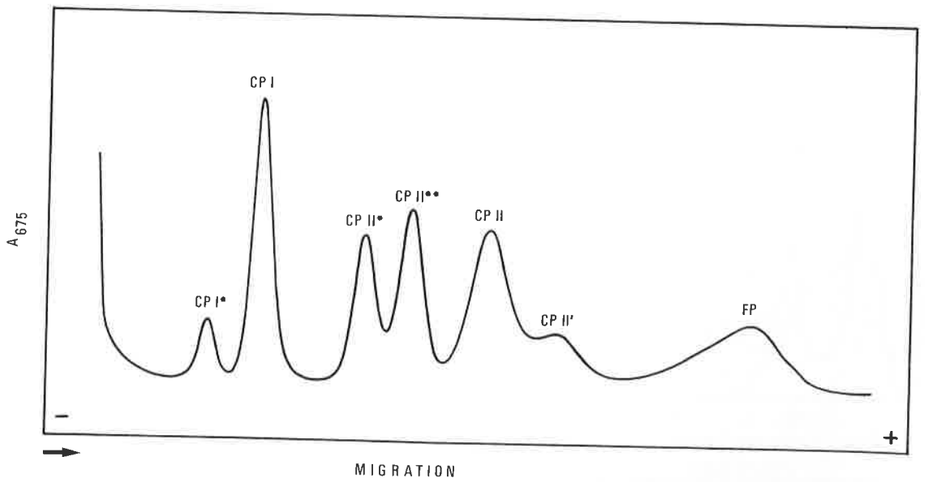

Figure 5. *Densitometer tracing of a gel with total withdrawal of Mg^{2+}. Pleurozium schreberi*; the material had been stored in the cold for 5 days.

the heavy membrane fraction is dissociated into two similar components in the absence of Mg^{2+}. In any case, the occurrence of chlorophyll *b* in the spectra of the chlorophyll-containing bands around the CP II proper indicates that this cannot be a question of the separation of the reaction center complex and the light-harvesting complex of PS II.

Earlier studies have revealed additional bands due to a low SDS/Chl ratio (Herrmann and Meister 1972, Hayden and Hopkins 1976). We have also observed this effect, but avoided it by using a higher SDS/Chl ratio. However, the dimer of Hayden and Hopkins (1976) has some similarities to our CP II* polymer. Recently the same investigators (Hayden and Hopkins 1977) observed a new band very near the CP II proper. If their complex IV has only the spectrum of chlorophyll *a*, it is not comparable with our bands. Our fastest band, CP II′, had the same location as the extraordinary band observed by Genge *et al.* (1974).

The large number of chlorophyll-containing bands observed here with stored material of Pleurozium was observed by Herrmann and Meister (1972) in connection with a low SDS/Chl ratio. The spectra of their bands are also very similar to those observed by us after withdrawal of magnesium. These bands are interesting because they occur in the same regions of the gel where we always find weak zones of protein after staining the gels with amido schwarz. It may be too early to discuss the significance of all these bands and whether they have anything to do with the suggested third chlorophyll protein (see discussion of Thornber 1978).

In contrast to CP II, CP I seems to be more stable without Mg^{2+}. Our percentages of chlorophyll associated with CP I are rather high in comparison with the more common 6–13% (Alberte *et al.* 1976), although the range lies between 4 and 30% (Brown *et al.* 1975). On the other hand, scanning of the Chl-protein complexes at 675 mm favours CP I. We have found it valuable to use a method which yields a large amount of CP I when studying the effect of environmental factors on chlorophyll proteins in different plant materials.

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