Acta Physiologiae Plantarum (2025) 47:53
https://doi.org/10.1007/s11738-025-03799-0

SHORT COMMUNICATION

Temperature-dependent conformational changes in *Arabidopsis* DEHYDRATION-RESPONSIVE ELEMENT BINDING PROTEIN 2A

Petri Tähtinen[1] · **Hiroaki Fujii**[2]

Received: 7 October 2024 / Revised: 13 March 2025 / Accepted: 26 March 2025

Abstract
With the recent rise in global temperatures, understanding plant heat stress responses has become an urgent challenge. The DEHYDRATION-RESPONSIVE ELEMENT BINDING PROTEIN 2A (DREB2A) is one of the key transcription factors involved in plant responses to heat stress. Previous studies show that DREB2A degrades at 23 °C, while it accumulates at 37 °C in *Arabidopsis*, leading to heat-induced gene expression. However, the direct impact of temperature on DREB2A protein itself remains insufficiently understood. This study investigates the effect of temperature on the DREB2A protein by expressing recombinant DREB2A in *Escherichia coli*. Results demonstrate that DREB2A accumulates in *E. coli* at 37 °C but not at 23 °C, a pattern also observed in *Arabidopsis*, despite the differences between these organisms. Circular dichroism (CD) spectroscopy further revealed structural alterations in DREB2A between 23 °C and 37 °C, though specific details remain unclear. Taken together, these findings suggest that temperature-induced conformational changes occur in DREB2A between 23 °C and 37 °C, which may play a role in regulating its stability. This knowledge also indicates that 37 °C-induced stability is a contributing factor to successful purification of full-length recombinant DREB2A protein.

Keywords DREB2A · Heat stress · Protein conformation · Arabidopsis

Introduction

Plants have evolved sophisticated mechanisms to cope with environmental stressors, such as heat stress. A key component of the heat stress response is the induction of specific gene expression mediated by several transcription factors (Bakery et al. 2024). Among these is DREB2A, a member of the plant-specific APETALA2/ethylene-responsive element binding factor (AP2/ERF) family. DREB2A binds to the dehydration-responsive element (DRE), thereby activating genes involved in responses to both heat and drought stress (Liu et al. 1998; Sakuma et al. 2006). Studies show that the *dreb2a* mutant exhibits hypersensitivity to heat stress, whereas transgenic *Arabidopsis* expressing a constitutively active form of DREB2A shows enhanced heat tolerance (Sakuma et al. 2006). Similarly, overexpression of DREB2A in other species, such as cowpea (*VuDREB2A*) and transgenic tobacco overexpressing finger millet DREB2A, has been shown to enhance heat stress tolerance (Kumar et al. 2022; Singh et al. 2021). Other members of the DREB2 family have also been found to confer thermotolerance in *Arabidopsis* and tobacco (Lim et al. 2007; Almoguera et al. 2009; Li et al. 2014). These findings underscore the critical role of DREB2A in heat stress responses.

The regulatory mechanisms controlling DREB2A have been studied extensively. Under non-stress conditions, full-length DREB2A is rapidly degraded via the proteasome system, whereas under heat or dehydration conditions, the protein accumulates (Liu et al. 1998; Qin et al. 2008; Morimoto et al. 2013, 2017). DREB2A contains a negative regulatory domain (NRD, amino acids 136–165; Liu et al. 1998), and deletion of this domain results in the accumulation of DREB2A under non-stress conditions, leading to a constitutively active form. Degradation under unstressed conditions is triggered by phosphorylation (Mizoi et al. 2019),

Communicated by V. P. Singh.

✉ Hiroaki Fujii
hiroaki.fujii@utu.fi

[1] Department of Chemistry, University of Turku, 20014 Turku, Finland

[2] Molecular Plant Biology Unit, Department of Life Technologies, University of Turku, 20014 Turku, Finland

Published online: 11 April 2025

while SUMOylation contributes to the stabilization of DREB2A at elevated temperatures (Wang et al. 2020). Additionally, DREB2A's transcriptional activity is influenced by proline isomerization (Theisen et al. 2024), and its interaction with DNA induces structural changes that reduce its binding affinity to Med25 (Blomberg et al. 2012). These findings highlight the importance of post-translational modifications in regulating DREB2A function.

Despite the wealth of information regarding DREB2A regulatory mechanisms, the direct effects of temperature on the DREB2A protein itself remain unclear. In this study, recombinant proteins are produced in *Escherichia coli* at different temperature and analyzed thereafter with circular dichroism (CD) spectroscopy to investigate the structural features of DREB2A at critical temperatures.

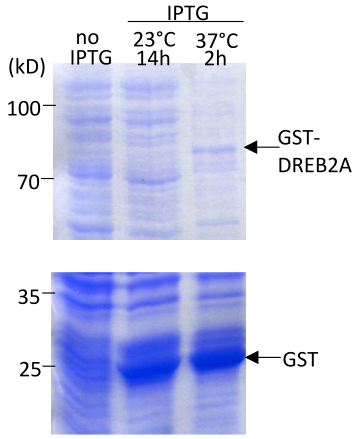

Fig. 1 Expression of recombinant DREB2A proteins in *E. coli* Coomassie staining of crude *E. coli* extracts separated by SDS-PAGE. Extracts were collected from *E. coli* expressing either GST-DREB2A (top panel) or GST (bottom panel) before and after IPTG induction at 23 °C for 14 h or 37 °C for 2 h. The 37 °C sample lane shows a strong band at the size of GST-DREB2A, whereas the 23 °C sample lane does not

Materials and methods

Plasmid constructs

The full-length *DREB2A* cDNA was amplified by PCR using the primers DREB2A-F (5′-AGAATTCATGGC AGTTTATGATCAGAGTGG- 3′) and DREB2A-R (5′-AGTCGACTTAGTTCTCCAGATCCAAGTAAC- 3′). The resulting PCR product was cloned into the pGEX-4T-1 vector (Amersham, Buckinghamshire, UK) at EcoRI and SalI sites and verified through DNA sequencing.

Purification of recombinant proteins from *E. coli*

E.coli carrying GST-DREB2A plasmids was grown overnight at 37 °C, transferred to fresh 20 × volume of Luria–Bertani media, and further cultured for 1 h. Recombinant protein expression was induced by 0.2 mM isopropyl beta-D-thiogalactopyranoside (IPTG) for 2 h at 37 °C. To estimate the induction, the culture was incubated for 2 h at 37 °C or for 14 h at 23 °C after IPTG induction, and then, *E. coli* pellets were heated in Laemmli sample buffer for 5 min at 95 °C, followed by SDS-PAGE. Recombinant GST-DREB2A proteins were purified as described by Fujii and Zhu (2009), with modifications to maintain the samples at 37 °C throughout the procedure. The proteins bound to glutathione-conjugated beads were washed six times with 1 ml of STE buffer (150 mM NaCl, 10 mM Tris–HCl pH 8.0, and 1 mM EDTA). Laemmli sample buffer was then added directly to the beads for SDS-PAGE analysis, followed by Coomassie staining. For CD spectroscopy, the bound proteins were eluted from the beads using 40 mM reduced glutathione (pH 8.0). The eluted proteins were subsequently dialyzed overnight in 10 mM Na_2HPO_4/NaH_2PO_4 buffer, pH 7.4, at 37 °C.

CD spectroscopy measurement

CD spectra were measured using a Chirascan CD spectrometer (Applied Photophysics, UK). The chamber temperature was adjusted as indicated using a Quantum Northwest CD250 rectangular single-cell holder with a Peltier element coupled to a Julabo AWC- 100 recirculating cooler controlled via a Quantum Northwest TC125 temperature control unit. The purified proteins were maintained in a heat retention box at 37 °C prior to the measurements. Proteins were measured in 10 mM Na_2HPO_4/NaH_2PO_4 buffer (pH 7.4) using a 1 mm pathlength quartz cuvette with 3 s response time, 1 nm bandwidth, and 1 nm step size. Spectra were recorded over the 190–240 nm range. To obtain pure protein spectra, the buffer spectrum was subtracted from each sample spectrum. Five separate measurements were taken at each temperature and subsequently averaged.

Results and discussion

The production of recombinant full-length DREB2A has been studied previously (Schramm et al. 2008; Blomberg et al. 2012), though it has sometimes been challenging (Mizoi et al. 2019). To investigate whether temperature affects the production of DREB2A, *E. coli* harboring a

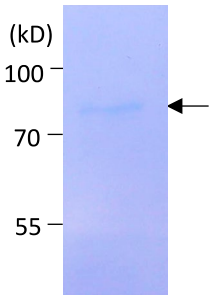

Fig. 2 Purified recombinant GST-DREB2A. Coomassie-stained gel of purified GST-DREB2A protein. The arrow indicates a band with the expected molecular weight of GST-DREB2A

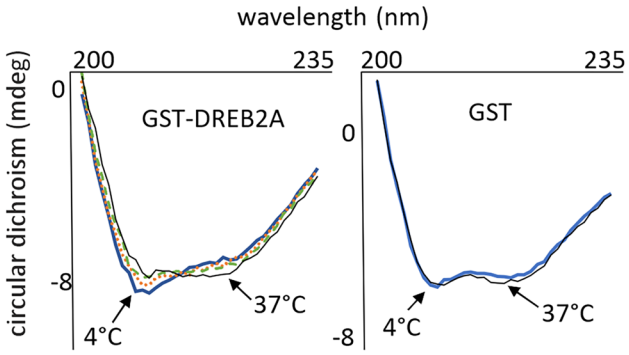

Fig. 3 Structural analysis of recombinant DREB2A. CD spectra of GST-DREB2A (left panel) and GST (right panel) recorded at 37 °C (thin solid line), 23 °C (dashed line), 14 °C (dotted line), and 4 °C (bold solid line). Signals have U-shape at all temperatures. Signals of GST-DREB2A at 4 °C have a negative peak at around 209 nm, while signals of GST-DREB2A at 37 °C have a wide peak between 209 and 221 nm. Signals of GST-DREB2A at 14 °C and 23 °C appear between those at 4 °C and 37 °C. Signals of GST at 4 °C nearly overlap to signals of GST at 37 °C. Data represent a typical experiment, with similar results obtained from three independent purifications of protein

plasmid containing GST-DREB2A cDNA was incubated in the presence of IPTG at either 37 °C for 2 h or 23 °C for 14 h. Coomassie-stained SDS-PAGE of crude extracts revealed an induced protein band of the expected size for GST-DREB2A in the 37 °C sample but not in the 23 °C sample (Fig. 1 and Supplemental Figure). As a control, *E. coli* carrying the same backbone plasmid with GST cDNA successfully produced GST under both temperature conditions, indicating that the transcription system and GST stability were not responsible for the reduced expression at 23 °C (Fig. 1). Furthermore, GST-DREB2A was successfully purified using glutathione-conjugated beads at 37 °C (Fig. 2 and Supplemental Figure), indicating that the induced band was GST-DREB2A. These findings suggest that GST-DREB2A accumulates at 37 °C but not at 23 °C, which is consistent with the previous reports (Mizoi et al. 2019), where production of GST-DREB2A was unsuccessful at lower temperatures (15 °C).

Although protein accumulation is often dependent on the host expression system and may not always carry physiological significance, this temperature-dependent instability may be of interest, especially given that a similar temperature-sensitive behavior of DREB2A has been observed in vivo in *Arabidopsis*. Although the protein degradation systems in *E. coli* differ entirely from the plant proteasome system (Dong et al. 2021), and 37 °C is optimal for *E. coli* but unfavorable for *Arabidopsis*, the critical temperature range for DREB2A degradation in both systems lies between 23 °C and 37 °C. One possible explanation, though speculative, is that the DREB2A protein adopts distinct conformations at 23 °C and 37 °C, which may influence its stability in both systems. To explore this, CD spectroscopy experiments (Greenfield 2006) were performed. At 37 °C, GST-DREB2A exhibited a negative peak around 221 nm (Fig. 3). As the temperature was reduced to 23 °C, 14 °C, and 4 °C, the signal at 209 nm decreased further to a more negative value, while the signal at 221 nm increased toward a more positive value (Fig. 3). These changes were not observed with GST alone (Fig. 3). The results suggest that GST-DREB2A undergoes a gradual conformational shift between 4 °C and 37 °C, though specific details remain unclear.

Although the physiological implications of the observed conformational change remain unclear, it is possible that *E. coli* recognizes the lower temperature conformation and targets it for degradation; however, further validation is needed. Additionally, in *Arabidopsis*, post-translational modification changes the stability of DREB2A. Phosphorylation of DREB2A in the NRD is essential for its proteasomal degradation under normal conditions (Mizoi et al. 2019), whereas SUMOylation of K163, adjacent to the NRD, suppresses its interaction with a ubiquitin ligase component, consequently increasing DREB2A protein stability under high temperatures (Wang et al. 2020).The observed conformational differences in this study may modulate post-translational modifications, leading to the stabilization of DREB2A at 37 °C, although direct experimental evidence in planta is currently unavailable. These observations may provide valuable insights for future studies. The development of a reliable protocol to produce full-length DREB2A is essential for further research.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11738-025-03799-0.

Acknowledgements The authors gratefully acknowledge financial support from Turun Yliopistosäätiö and the Jane and Aatos Erkko Foundation (2020–2024).

Author contributions PT conducted the CD spectroscopy measurements together with HF, who performed all other work.

Funding Open Access funding provided by University of Turku (including Turku University Central Hospital). Turun Yliopistosäätiö, Jane ja Aatos Erkon Säätiö, (2020–2024).

Data availability The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest Nothing to declare except financial support from Turun Yliopistosäätiö and the Jane and Aatos Erkko Foundation.

References

Almoguera C, Prieto-Dapena P, Díaz-Martín J, Espinosa JM, Carranco R, Jordano J (2009) The HaDREB2 transcription factor enhances basal thermotolerance and longevity of seeds through functional interaction with HaHSFA9. J BMC Plant Biol 9:75. https://doi.org/10.1186/1471-2229-9-75

Bakery A, Vraggalas S, Shalha B, Chauhan H, Benhamed M, Fragkostefanakis S (2024) Heat stress transcription factors as the central molecular rheostat to optimize plant survival and recovery from heat stress. New Phytol 244:51–64. https://doi.org/10.1111/nph.20017

Blomberg J, Aguilar X, Brännström K, Rautio L, Olofsson A, Wittung-Stafshede P, Björklund S (2012) Interactions between DNA, transcriptional regulator Dreb2a and the Med25 mediator subunit from Arabidopsis thaliana involve conformational changes. Nucleic Acids Res 40:5938–5950. https://doi.org/10.1093/nar/gks265

Dong S, Chen H, Zhou Q, Liao N (2021) Protein degradation control and regulation of bacterial survival and pathogenicity: the role of protein degradation systems in bacteria. Mol Biol Rep 48:7575–7585. https://doi.org/10.1007/s11033-021-06744-9

Fujii H, Zhu JK (2009) An autophosphorylation site of the protein kinase SOS2 is important for salt tolerance in Arabidopsis. Mol Plant 2:183–190. https://doi.org/10.1093/mp/ssn087

Greenfield NJ (2006) Using circular dichroism spectra to estimate protein secondary structure. Nat Protoc 1:2876–2890. https://doi.org/10.1038/nprot.2006.202

Kumar S, Muthuvel J, Sadhukhan A, Kobayashi Y, Koyama H, Sahoo L (2022) Enhanced osmotic adjustment, antioxidant defense, and photosynthesis efficiency under drought and heat stress of transgenic cowpea overexpressing an engineered DREB transcription factor. Plant Physiol Biochem 193:1–13. https://doi.org/10.1016/j.plaphy.2022.09.028

Li X, Zhang D, Li H, Wang Y, Zhang Y, Wood AJ (2014) EsDREB2B, a novel truncated DREB2-type transcription factor in the desert legume Eremosparton songoricum, enhances tolerance to multiple abiotic stresses in yeast and transgenic tobacco. BMC Plant Biol 14:44. https://doi.org/10.1186/1471-2229-14-44

Lim CJ, Hwang JE, Chen H, Hong JK, Yang KA, Choi MS, Lee KO, Chung WS, Lee SY, Lim CO (2007) Over-expression of the Arabidopsis DRE/CRT-binding transcription factor DREB2C enhances thermotolerance. Biochem Biophys Res Commun 362:431–436. https://doi.org/10.1016/j.bbrc.2007.08.007

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell 10:1391–1406. https://doi.org/10.1105/tpc.10.8.1391

Mizoi J, Kanazawa N, Kidokoro S, Takahashi F, Qin F, Morimoto K, Shinozaki K, Yamaguchi-Shinozaki K (2019) Heat-induced inhibition of phosphorylation of the stress-protective transcription factor DREB2A promotes thermotolerance of *Arabidopsis thaliana*. J Biol Chem 294:902–917. https://doi.org/10.1074/jbc.ra118.002662

Morimoto K, Mizoi J, Qin F, Kim JS, Sato H, Osakabe Y, Shinozaki K, Yamaguchi-Shinozaki K (2013) Stabilization of Arabidopsis DREB2A is required but not sufficient for the induction of target genes under conditions of stress. PLoS ONE 8:e80457. https://doi.org/10.1371/journal.pone.0080457

Morimoto K, Ohama N, Kidokoro S, Mizoi J, Takahashi F, Todaka D, Mogami J, Sato H, Qin F, Kim JS, Fukao Y, Fujiwara M, Shinozaki K, Yamaguchi-Shinozaki K (2017) BPM-CUL3 E3 ligase modulates thermotolerance by facilitating negative regulatory domain-mediated degradation of DREB2A in *Arabidopsis*. Proc Natl Acad Sci USA 114:E8528–E8536. https://doi.org/10.1073/pnas.1704189114

Qin F, Sakuma Y, Tran LS, Maruyama K, Kidokoro S, Fujita Y, Fujita M, Umezawa T, Sawano Y, Miyazono K, Tanokura M, Shinozaki K, Yamaguchi-Shinozaki K (2008) Arabidopsis DREB2A-interacting proteins function as RING E3 ligases and negatively regulate plant drought stress-responsive gene expression. Plant Cell 20:1693–1707. https://doi.org/10.1105/tpc.107.057380

Sakuma Y, Maruyama K, Qin F, Osakabe Y, Shinozaki K, Yamaguchi-Shinozaki K (2006) Dual function of an Arabidopsis transcription factor DREB2A in water-stress-responsive and heat-stress-responsive gene expression. Proc Natl Acad Sci USA 103:18822–18827. https://doi.org/10.1073/pnas.0605639103

Schramm F, Larkindale J, Kiehlmann E, Ganguli A, Englich G, Vierling E, von Koskull-Döring P (2008) A cascade of transcription factor DREB2A and heat stress transcription factor HsfA3 regulates the heat stress response of Arabidopsis. Plant J 53:264–274. https://doi.org/10.1111/j.1365-313x.2007.03334.x

Singh S, Chopperla R, Shingote P, Chhapekar SS, Deshmukh R, Khan S, Padaria JC, Sharma TR, Solanke AU (2021) Overexpression of EcDREB2A transcription factor from finger millet in tobacco enhances tolerance to heat stress through ROS scavenging. J Biotechnol 336:10–24. https://doi.org/10.1016/j.jbiotec.2021.06.013

Theisen FF, Prestel A, Elkjær S, Leurs YHA, Morffy N, Strader LC, O'Shea C, Teilum K, Kragelund BB, Skriver K (2024) Molecular switching in transcription through splicing and proline isomerization regulates stress responses in plants. Nat Commun 15:592. https://doi.org/10.1038/s41467-024-44859-2

Wang F, Liu Y, Shi Y, Han D, Wu Y, Ye W, Yang H, Li G, Cui F, Wan S, Lai J, Yang C (2020) SUMOylation stabilizes the transcription factor DREB2A to improve plant thermotolerance. Plant Physiol 183:41–50. https://doi.org/10.1104/pp.20.00080