The Unstructured N-terminal Region of *Arabidopsis* Group 4 Late Embryogenesis Abundant (LEA) Proteins Is Required for Folding and for Chaperone-like Activity under Water Deficit*

Cesar L. Cuevas-Velazquez\(^1\), Gloria Saab-Rincón\(^6\), José Luis Reyes\(^1\), and Alejandra A. Covarrubias\(^1,2\)

From the Departamentos de \(^4\)Biología Molecular de Plantas and \(^4\)Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, Universidad Nacional Autónoma de México, 62250 Cuernavaca, México

Late embryogenesis abundant (LEA) proteins are a conserved group of proteins widely distributed in the plant kingdom that participate in the tolerance to water deficit of different plant species. *In silico* analyses indicate that most LEA proteins are structurally disordered. The structural plasticity of these proteins opens the question of whether water deficit modulates their conformation and whether these possible changes are related to their function. In this work, we characterized the secondary structure of *Arabidopsis* group 4 LEA proteins. We found that they are disordered in aqueous solution, with high intrinsic potential to fold into α-helix. We demonstrate that complete dehydration is not required for these proteins to sample ordered structures because milder water deficit and macromolecular crowding induce high α-helix levels *in vitro*, suggesting that prevalent conditions under water deficit modulate their conformation. We also show that the N-terminal region, conserved across all group 4 LEA proteins, is necessary and sufficient for conformational transitions and that their protective function is confined to this region, suggesting that folding into α-helix is required for chaperone-like activity under water limitation. We propose that these proteins can exist as different conformers, favoring functional diversity, a moonlighting property arising from their structural dynamics.

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Low water availability caused by different environmental conditions such as drought, or low temperatures represents a vulnerable situation for many forms of life, particularly for plants. To contend with and to overcome these adverse environments, numerous complex response mechanisms have been selected in the different species of the plant kingdom. One of the most conserved responses is the accumulation of a group of proteins known as late embryogenesis abundant (LEA)\(^3\) proteins (1). LEA proteins have been found in all the orthodox dry seeds (embryos) where they have been searched (1, 2), and they also accumulate in response to water limitation in all vegetative tissues (2, 3). Most LEA proteins show high hydrophilicity, high content of small amino acids, and absence or deficit of hydrophobic residues, properties that are extended to a larger set of proteins called hydrophilins, which have been found in species from the three domains of life and that also accumulate under water deficit (2, 4). The composition of these proteins is also characteristic of a group of proteins known as intrinsically disordered proteins (IDPs) (5, 6). Consistent with the predicted structural disorder for most LEA proteins, structural analyses have confirmed this property for some of them in aqueous solution (7–13). Based on their sequence similarity, LEA proteins have been classified in seven groups or families, each one characterized by the presence of specific sequence motifs (2). In *Arabidopsis thaliana* there are 51 genes encoding LEA proteins from six of the seven families (3). Group 4 LEA (LEA4) proteins are one of the smallest families of LEA proteins in *Arabidopsis* consisting of only three members: AtLEA4-1 (At1g32560), AtLEA4-2 (At2g35300), and AtLEA4-5 (At5g06760) (3, 14). Group 4 LEA proteins are enriched in charged and small amino acid residues, whereas they lack Cys, Phe, and Trp (2, 3, 14). This group is characterized by an N-terminal region ranging from 74 to 78 amino acid residues, containing conserved amino acid sequence motifs. *In silico* analysis predicts that this particular region is able to form an amphipathic α-helix structure. The C-terminal region in this protein family is more variable in sequence and length, and it is predicted to be structurally disordered (2, 14). A phylogenetic analysis of group 4 LEA proteins revealed two subclasses in this family (subgroups 4A and 4B) (14). In *Arabidopsis*, AtLEA4-1 and AtLEA4-2 proteins belong to subgroup 4A, whereas AtLEA4-5 protein fits into subgroup 4B (14).

From the 10 distinctive motifs found in this protein group, the high conservation of motif 2 at the N-terminal region constitutes a signature for this family. The same study also showed that both subgroups emerged from a very early duplication before branching of monocots and dicots, suggesting that this separation gave rise to a subfunctionalization of these subgroups (14). Group 4 LEA proteins and transcripts have been found in dry seeds but also in response to water deficit in vegetative and reproductive tissues (14, 15). Moreover, *Arabidopsis* mutants deficient in group 4 LEA proteins are sensitive to
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water deficit, indicating that these proteins participate in the tolerance to this stress condition (14).

Many different functions have been proposed for group 4 LEA proteins such as membrane protectors, sugar or metal binding, radical scavengers, and protein dehydro- and cryo- protectors (16–20). *A. thaliana* AtLEA4-5 protein was shown to prevent inactivation and conformational changes of reporter enzymes such as lactate dehydrogenase (LDH) and malate dehydrogenase after partial dehydration and freeze/thaw cycles from 1:1 molar ratios, indicating that group 4 LEA proteins have a chaperone-like function to protect other proteins from the effects of water deficit (19, 20).

Studies on animal and bacterial IDPs have shown that these proteins can gain structural order upon binding to a specific partner, interaction that leads to IDP folding (21–25); however, in some other examples, the function of globular chaperones is linked to an order to disorder structural transitions in response to environmental cues such as those imposed by changes in pH or redox state (26, 27). Even though it has been shown that severe dehydration can promote folding of some LEA proteins or redox state (26, 27). Even though it has been shown that severe dehydration can promote folding of some LEA proteins and other hydrophilins) and whether this structural changes could be related to their function are still open questions.

In this work, we demonstrate that even though *Arabidopsis* members of subgroups 4A (AtLEA4-2) and 4B (AtLEA4-5) LEA proteins are structurally disordered in solution, low osmotic potentials and macromolecular crowding can induce significant levels of α-helix, particularly in the conserved AtLEA4-5 N-terminal region, whereas the C-terminal region displays high structural disorder. We also show that the AtLEA4-5 N-terminal region is necessary and sufficient for the protective effect of this protein on reporter enzyme activities after freeze-thaw cycles and partial dehydration at low molar ratios. Our data support the hypothesis that cellular environment modulates the structural organization of disordered proteins and that these structural changes are related to their functions.

**Experimental Procedures**

*In Silico Analyses*—AtLEA4 proteins were aligned using T-Coffee multiple sequence alignment. Secondary structure prediction was determined using AGADIR helical content predictor (32). Intrinsically disordered tendency was predicted using DISpro (33), PONDRT (34), and DISOPRED3 (35).

**Plasmid Constructions**—ORFs of AtLEA4-1, AtLEA4-2, and AtLEA4-5 genes were cloned using cDNA from RNA obtained from *Arabidopsis* dry seeds. AtLEA4-5 ORF was amplified by PCR using specific primers containing at their ends NcoI (5′-AAAACATGAGTCGATGAAGAAAC-3′) and SalI (5′-GCGGTCGACCCGTTTATCC-3′) restriction sites. This cloning strategy led to a modification in the second codon, which in the recombinant version corresponds to glutamic acid (GAG) instead of glutamine (CAG). The amplicon was cloned into pET1.2/blunt and subsequently digested with NcoI and SalI for its insertion into pTrc99A vector. To eliminate the Met33 in AtLEA4-5 ORF used in bacterial cells as an alternative translation initiation site and responsible of the production of an additional shorter AtLEA4-5 protein, directed mutagenesis of AtLEA4-5 ORF sequence was conducted using the following overlapping primers to exchange Met33 for a Leu residue: sense (5′-GGAGGAAAAAGCCGAGACTTGAA-GAC-3′) and antisense (5′-GGTTCAGCTTCTCCGGTTTTTTCCTCC-3′). The modified DNA fragment was inserted into pET1.2/blunt plasmid vector to produce pET1.2/AtLEA4-5. For protein production, AtLEA4-5 ORF was transferred to pTrc99A plasmid vector by digesting with NcoI and SalI restriction enzymes. The DNA fragments encoding AtLEA4-5 N- and C-terminal regions were obtained from pET1.2/AtLEA4-5 plasmid, using the following sense and antisense oligonucleotides: 5′-AAAAACATGGAGTCGATGAAGAAAC-3′ and antisense 5′-GCGGTCGACCCGTTTATCCGTCG-CCG-3′ and sense 5′-AAAAACATGGAGTCGATGAAGAAAC-3′ and antisense 5′-GCGGTCGACCCGTTTATCCGTCG-CCG-3′. The DNA fragments containing one tablet of cOmplete protease inhibitor mix-
tured (Roche) per 50 ml of buffer. The cells were lysed by sonication on ice, and the extract was clarified by centrifugation at 20,000 × g for 30 min at 4 °C. To obtain proteins lacking the intein tag, the clarified extract was loaded onto a chitin column following the procedure described by the manufacturer (IMPACT™-CN kit). The eluted fractions were analyzed for the presence of the recombinant proteins by SDS-PAGE. In contrast to AtLEA4-2, this analysis showed that AtLEA4-1 and AtLEA4-5, AtLEA4-5, AtLEA4-5, and AtLEA4-5, representing the two LEA4 subgroups, 4A and 4B, respectively (14). Because AtLE4–1 was mostly degraded during different expression and purification procedures, the rest of the experiments were performed only with AtLEA4-2 and AtLEA4-5, representing the two LEA4 subgroups, 4A and 4B, respectively (14).

To determine the secondary structure of AtLEA4-2 and AtLEA4-5 in solution, the purified proteins were analyzed by far UV CD. The results obtained corroborated that both proteins are mostly disordered in solution over a wide range of temperatures (Fig. 2, A and B) and pH (data not shown), with
the characteristic negative band for random coil structures around 198 nm. Both spectra showed a significant negative signal around 222 nm (typical of $\alpha$-helix), suggesting that these proteins possess residual $\alpha$-helix structure. The comparative analysis of the AtLEA4-2 CD difference spectra obtained at different temperatures ($\Delta$10 – 80 °C and $\Delta$20 – 80 °C) revealed that this protein is able to form $\alpha$-helix structures at low temperatures (10 °C), whereas at higher temperatures the protein is...
Although the proteins are able to adopt two structural conformations (Fig. 3, E and F), as was indicated by the presence of isodichroic points. These data demonstrate that AtLEA4-2 and AtLEA4-5 possess an intrinsic ability to form α-helical species, which under some conditions are in equilibrium with unfolded conformations (U4–2 ↔ F4–2, U4–5 ↔ F4–5).

AtLEA4-2 and AtLEA4-5 Fold to α-Helix in Response Low Water Availability and Macromolecular Crowding Induced In Vitro—Previous studies have shown that recombinant LEA proteins from different groups acquire secondary structure, mostly α-helix, when subjected to complete dehydration (8, 11, 13, 28–31). Given the intrinsic potential of AtLEA4-2 and AtLEA4-5 to gain α-helix conformation and because these proteins accumulate even under mild water limitation, we hypothesized that in vitro conditions limiting water availability could induce changes in their secondary structure. Addition of increasing glycerol concentrations led to a notorious progressive gain in α-helix structure in both proteins, as shown by the [θ]222 change toward positive values and a deeper minimum at [θ]198 (Fig. 3, A and B). Analysis using Dichroweb estimated a small difference in α-helix content between these two proteins: 54% α-helix for AtLEA4-2 and 46% for AtLEA4-5 at the highest glycerol concentration (80%) (Table 1). We observed the presence of isodichroic points in both cases (Fig. 3, A and B), which was also supported by their corresponding transition diagrams (Fig. 3, E and F), that together with those obtained from TFE treatments showed that AtLEA4-2 and AtLEA4-5 seem to follow the same folding pathway to α-helix under both treatments (Fig. 3, E and F).

Inside living cells, macromolecules are present at very high concentrations (~400 g/liter) (42–44), a condition that is typically known as macromolecular crowding (45, 46). This state is further exacerbated in cells under water deficit, reaching macromolecular concentrations up to ~900 g/liter upon severe dehydration (42). PEG was used to simulate a crowded environment in vitro. The addition of 45% PEG 5000 to AtLEA4-2 or AtLEA4-5 solutions clearly induced changes in their structural conformations (Fig. 3, C and D). Dichroweb estimations indicate 37 and 39% α-helix gains for AtLEA4-2 and AtLEA4-5, respectively (Table 1). Together, these data indicate that AtLEA4-2 and AtLEA4-5 can acquire secondary structure under low water availability or macromolecular crowding in vitro, possibly reflecting what occurs in plant cells under water deficit.

The N-terminal Region of AtLEA4 Proteins Is Necessary and Sufficient for the Conformational Changes Induced by Water Deficit—Plant group 4 LEA proteins are characterized by the presence of conserved motifs at their N-terminal region (2, 14). In silico analysis predicts that this region of 70, 74, and 77 residues in AtLEA4-1, AtLEA4-2, and AtLEA4-5, respectively, has a higher propensity to adopt α-helical conformations than the C-terminal region (Fig. 1C). To test this prediction, we performed far UV CD experiments using AtLEA4-5 truncated versions: one containing the first 77 amino acids, named AtLEA4-51–77, and a second one corresponding to the 81 amino acid C-terminal region, from residues 78 to 158 (AtLEA4-578–158) (47).

In contrast to in silico predictions, AtLEA4-51–77 behaved as a disordered protein in aqueous solution under all temper-
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FIGURE 3. Far UV CD spectra of AtLEA4-2 and AtLEA4-5 in different concentrations of glycerol or PEG. A and B, far UV CD spectra of AtLEA4-2 (A) and AtLEA4-5 (B) in glycerol/water mixtures at 0% (black), 10% (blue), 20% (brown), 30% (cyan), 40% (green), 50% (magenta), 60% (orange), 70% (purple), and 80% (red) glycerol. Difference spectra (Δ80–0% glycerol) are shown as insets in A and B. C and D, AtLEA4-2 (C) and AtLEA4-5 (D) in aqueous solution (black line) and in PEG/water mixture at 45% PEG 5000 (blue line). Difference spectra (Δ45–0% PEG) are shown as insets in C and D. E and F, transition diagrams for AtLEA4-2 (E) and AtLEA4-5 (F) were obtained using the ellipticity values at 198 and 222 nm from TFE and glycerol titrations. Dashed lines represent the linear fits of the data. These results were reproduced at least four times in autonomous experiments, using three independent purification batches of both proteins.

tures tested (Fig. 4A), with a difference spectrum profile (Δ10–80 °C) similar to that obtained for the complete AtLEA4-5 (Figs. 2B and 4A, insets). However, the addition of increasing TFE concentrations progressively induced α-helix formation in the truncated protein containing the N-terminal region (Fig. 4B). Likewise, treatments with glycerol and PEG led to the same behavior in this protein (Fig. 4C and D), reaching up to 55 and 42% α-helix at the highest glycerol and PEG concentrations, respectively (Table 2). Also complete AtLEA4-5 and AtLEA4-2, far UV CD spectra from AtLEA4-51–77 showed isodichroic points when treated with progressively increasing TFE or glycerol concentrations, indicating that this protein is in equilibrium between two states in these conditions: disordered and α-helix conformations (Fig. 4, B and C). The high linear correlation of transition diagrams confirmed this observation (data not shown).

Using the chemically synthesized peptide corresponding to the AtLEA4-5C-terminal region (AtLEA4-578–158), far UV CD analysis confirmed that AtLEA4-578–158 is highly disordered in aqueous solution at different temperatures (Fig. 4E). Unlike AtLEA4-2, AtLEA4-5, or AtLEA4-51–77, the difference spectrum Δ10–80 °C of AtLEA4-578–158 indicated the possible formation of poly-L-proline-like structures (Fig. 4E, inset). In contrast to the N-terminal region of these proteins, addition of up to 60% TFE had only minor effects on the structure of AtLEA4-578–158 (Fig. 4F), inducing 4% to 19% α-helix (Table 2). It was not until a high TFE concentration (90%) was reached that AtLEA4-578–158 became helical (Fig. 4F), indicating a low
intrinsic competence to acquire ordered structures. This finding was further supported by the results obtained from the addition of glycerol or PEG to AtLEA4-578–158 solutions, which showed no effect on its structure (Fig. 4, G and H), given that the negative band at \([\theta]_{222}\) did not show any change and that only a slight increase in the \([\theta]_{198}\) signal was detected. Under the highest glycerol (80%) and PEG (45%) concentrations, AtLEA4-578–158 only showed 5% \(\alpha\)-helix formation (Table 2). Comparison

**FIGURE 4.** N-terminal conserved region of AtLEA4-5 is necessary and sufficient to induce folding to \(\alpha\)-helix under the different conditions tested. Far UV CD spectra of AtLEA4-51–77 (A–D) and AtLEA4-578–158 (E–H) under different temperatures (A and E), different TFE (B and F) and glycerol concentrations (C and G), and under 45% PEG 5,000 (D and H). Difference spectra (\(\Delta\)100 °C; \(\Delta\)90 – 0% TFE; \(\Delta\)80 – 0% glycerol; \(\Delta\)45 – 0% PEG) are shown as insets in each graph. These results were reproduced at least four times in autonomous experiments, using three independent purification batches of AtLEA4-51–77 protein and one batch for AtLEA4-578–158 polypeptide.
between the difference spectra for AtLEA4-5_{1–77} and AtLEA4-5_{78–158} in TFE, glycerol and PEG showed the magnitude of the AtLEA4-5_{1–77} folding to α-helix, compared with AtLEA4-5_{78–158} (Fig. 4, A–H, insets). In accordance, the transition diagram for glycerol titration of AtLEA4-5_{78–158} showed no linear behavior (data not shown). Together, these data demonstrate that the AtLEA4-5 N-terminal region (AtLEA4-5_{1–77}) is necessary and sufficient to drive α-helix conformations in this protein under low water availability or macromolecular crowding conditions.

The N-terminal Region of AtLEA4 Proteins Is Necessary and Sufficient to Prevent Inactivation of Lactate Dehydrogenase Caused by Freeze-Thaw Cycles and Partial Dehydration—Because the motif conservation and ability to fold into α-helical conformations of the AtLEA4-5 are preferentially located at the N-terminal region as compared with its C-terminal region, we asked about the competence of these individual segments to protect the reporter enzyme LDH from deleterious effects caused by freeze-thaw cycles and partial dehydration, as was previously shown for the complete protein (19, 20). For this purpose, LDH in the presence or absence of AtLEA4-5, AtLEA4-2, AtLEA4-5_{1–77}, or AtLEA4-5_{78–158} was subjected to in vitro freeze-thaw cycles and partial dehydration treatments. AtLEA4-2, the smallest protein of this group, mostly consisting of the N-terminal region (Fig. 1A), showed a similar protective effect on LDH activity as AtLEA4-5 after partial dehydration (Fig. 5A). Interestingly, a comparable protection was produced by AtLEA4-5_{1–77}, contrasting with the negligible protective levels showed by the AtLEA4-5 C-terminal region (AtLEA4-5_{78–158}), whose values were rather close to those shown by lysozyme, an unrelated globular protein (Fig. 5, A and B). A similar conclusion can be drawn when different molar ratios of these proteins were used in freeze-thaw in vitro assays, where it is evident that the various proteins containing the N-terminal region showed an equivalent protective trend, in opposition to the C-terminal region represented by AtLEA4-5_{78–158} or to lysozyme (Fig. 5B). This analysis revealed the highest and lowest protective efficiencies for AtLEA4-5 and AtLEA4-5_{78–158} respectively, among the different proteins tested (Fig. 5B). Because the hydrophilicity index and length are similar between the N-terminal and C-terminal regions, these data are consistent with the hypothesis that the protective activity of these proteins is rather dependent on the conserved motifs present in the N-terminal region and/or on its ability to fold into α-helical conformations.

**Discussion**

During the last nearly 20 years, research on the so-called IDPs has challenged the classical structure-function paradigm (48). IDPs that either completely lack a well defined three-dimensional structure or contain short regions of disorder, known as intrinsically disordered regions (IDRs) within folded
domains are highly abundant in eukaryotic proteomes and perform important functions (6). In plants, IDPs participate in developmental control, light perception, transcriptional regulation, and response to abiotic stress (12). LEA proteins are the plant protein family with the highest number of proteins either predicted or characterized as IDPs (12). There is experimental evidence showing that LEA proteins from different groups acquire α-helix after complete drying (8, 11, 13, 28–31). These findings suggest that these proteins form α-helix in the dry seed, but little is known about LEA proteins structural behavior under less severe conditions, such as those present in vegetative tissues under drought. Mouillon et al. (49) showed that three Arabidopsis group 2 LEA proteins (Cor47, Lti29, and Lti30) are IDPs in solution and that they remain disordered when subjected to low water potential or macromolecular crowding simulated in vitro with glycerol and PEG, respectively. Even though Cor47 showed a slight folding into α-helix under high concentrations of glycerol and PEG, the authors propose that dehydrins have evolved to stay disordered under cellular conditions and that disorder might be required to properly fulfill their function (49). A similar effect was also observed experimentally for α-casein, MAP2c, and p21Cip1, three different animal IDPs that show no conformational change when subjected to macromolecular crowding, supporting the idea that the physiological state of these IDPs is also disordered (50).

Even though, most LEA proteins can be considered as IDPs, the analysis of their amino acid sequences indicates variety in their potential to attain different levels of secondary structure (2). Because LEA proteins accumulate not only under severe dehydration, such as that occurring in the dry seed, but also in different tissues under a wide range of water limitation, we investigated the possibility that these proteins could adopt secondary structures under conditions prevailing upon water loss, not necessarily as extreme as those occurring in dry seeds. Group 4 LEA proteins in Arabidopsis represented a suitable set of proteins for this purpose, given that there are two protein subtypes (subgroups 4A and 4B): a long variant conformed by two distinctive N-terminal and C-terminal regions (AtLEA4-5, representative of subgroup 4B) and a short one with only the conserved N-terminal region (AtLEA4-2, representative of subgroup 4A) (14). Furthermore, in silico analysis showed that in contrast to the C-terminal region, the N-terminal region sequence has the potential to fold into α-helix conformations offering an appropriate system to experimentally compare their structural properties under different environments. In this work, we demonstrate not only that the N-terminal regions of AtLEA4-2 and AtLEA4-5 have an intrinsic capacity to fold into α-helix as shown by the far UV CD spectra in the presence of TFE but also that they gain significant helicity in low water potential and macromolecular crowded solutions. Interestingly, the AtLEA4-5 C-terminal region, which we show is disordered in aqueous solution as predicted, remains disordered under all conditions tested. These results indicate the presence of two functional domains in this LEA protein group. The N-terminal region can become ordered under conditions of water restriction, short of absolute dryness, whereas the persistent disorder of the C-terminal region could indicate a requirement to expose some amino acid residues for a more effective or additional function. Such possibility could be related to the abundance of His residues in this region, which seems to be involved in their binding to metal ions (18), suggesting multifunctionality in some LEA protein families.

All LEA proteins studied to date from groups 1, 2, 3, 4, and 6 show structural disorder in aqueous solution, but most of them also have an intrinsic potential to acquire helical conformations in the presence of TFE (12). These results suggest that there are conditions where LEA proteins exhibit such structural transformation. For most of these LEA proteins, folding has been detected after extreme dehydration (8, 11, 13, 28–31), but extreme conditions may not be necessary in all cases to induce structural transformation. We show in this work that group 4 LEA proteins reach up to 54 and 39% α-helix under less severe environments regarding water availability and macromolecular crowding, respectively. The levels of α-helix formation for group 4 LEA proteins are significantly higher than those estimated for LEA proteins from other groups under similar treatments (12, 39), indicating that the ability to fold is not necessarily the same for different IDPs or intrinsically disordered regions. This is also supported by the wide range of α-helix formation observed among different LEA proteins upon comparable treatments (9, 12, 39, 49, 51, 52). Other proteins seem to be unable to gain ordered conformations such as Rab18, a group 2 LEA protein (9). This information indicates diversity in their structural plasticity, action mechanisms, and/or in their function.

The results from the protection activity assays demonstrate that the protective role of LEA 4 proteins on LDH under low water availability and/or molecular crowding is confined to the conserved N-terminal region of this protein family. There is no apparent participation of the C-terminal region, which completely lacks this safeguard function. These observations imply that the ability of a LEA4 N-terminal domain to gain helicity is related to this chaperone-like activity, particularly under conditions prevailing in water-deficit environments. Based on in vitro evidence, it has been proposed that one mechanism for this activity involves protein–protein interactions (20, 53–55). A comprehensive view brings into consideration the existence of different LEA4 conformers in equilibrium (partially folded or unfolded) under crowded or water-deficit environments, which supports the existence of preformed secondary structural elements, denominated as prestructured motifs that could be implicated in the recognition of different and specific binding partners (56). Our findings strongly suggest that water deficit leads to the stabilization of particular conformations in group 4 LEA proteins that may allow the exposure of different motifs necessary for the binding of their target molecules, hence supporting the idea that LEA proteins of this group function as a structural ensemble, whose dynamism can be modulated by environmental conditions (57–60). This proposed mode of action also exhibits possible binding promiscuity, in consonance with their role as chaperone-like molecules needed during water scarcity. At this point, we cannot discount the possibility that different conformers in these proteins could favor different functions such as protection of proteins and/or membranes, as well as metal binding, a moonlighting property arising from their structural dynamics.
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In conclusion, the findings reported here indicate that the in vitro chaperone-like function of the intrinsically disordered group 4 LEA proteins is closely associated to their ability to adopt ordered structural conformations under prevailing conditions in water-deficit environments. The high correlation found between accumulation under water deficit and intrinsic structural disorder, common features in typical LEA proteins and other hydrophilins (4), suggests a functional advantage for this attribute throughout evolution, not only to maintain structural plasticity that could avoid undesirable consequences under stressful environments, such as dramatic structural modifications leading to a functional breakdown, but also to gain functional and mechanistic diversity given their conformational freedom.

Author Contributions—C. L. C.-V. and A. A. C. designed the experimental strategy. C. L. C.-V. conducted cloning, protein expression, and protein purification. C. L. C.-V. and G. S.-R. performed circular dichroism spectroscopy experiments. C. L. C.-V. and J. L. R. carried out in vitro protection assays. A. A. C. supervised research. C. L. C.-V., G. S.-R., and A. A. C. analyzed data. C. L. C.-V. and A. A. C. wrote the article, which was read and approved by all authors.

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