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Research article

A predicted N-terminal helical domain of a Group 1 LEA protein is required for protection of enzyme activity from drying

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Abstract

Late embryogenesis abundant (LEA) proteins have been repeatedly implicated in the acquisition of desiccation tolerance in angiosperm seed embryos. However, the mechanism(s) by which protection occurs is not well understood. While the Group 1 LEA proteins are predicted to be largely unordered in solution, there is strong evidence that upon drying these proteins undergo a structural transition that leads to an increase in α-helical content. Several studies also suggest there is a direct interaction between Group 1 LEA proteins and other molecules in the cytoplasm that may be critical for the establishment of desiccation tolerance during embryo maturation. We have produced a recombinant Group 1 LEA protein and show that it is capable of protecting the enzyme lactate dehydrogenase from the deleterious effects of drying. We have also evaluated the ability of various altered recombinant Group 1 LEA proteins to protect in the same assay. Our results suggest that the highly conserved 20 amino acid Group 1 LEA signature motif is not required for protection in our *in vitro* assay. However, introduction of two juxtaposed proline residues into an N-terminal helical domain predicted to exist in the hydrated structure significantly compromises the ability of the recombinant protein to provide protection from drying. These results suggest that the N-terminal domain of Group 1 LEA proteins may be important for proper folding during dehydration.

Keywords: Desiccation tolerance; Angiosperm seed embryo; Late embryogenesis abundant proteins; Intrinsically unordered proteins; Glycine-rich proteins; Water replacement hypothesis

1. Introduction

Most angiosperm seeds undergo dehydration to varying degrees at the end of normal development resulting in the production of an anhydrobiotic state. The accumulation of several different molecular species has been suggested to correlate with the acquisition of embryonic desiccation tolerance including proteins [1–3] and sugars [2,4,5]. Despite the widespread occurrence of this phenomenon in angiosperm seeds, its importance to seed longevity, as well as its potential exploitation for

Abbreviations: 2D, two-dimensional; BCA, bicinchoninic acid; BSA, bovine serum albumin; CD, circular dichroism; ddH_2O, distilled, deionized H_2O; ESI-MS, electrospray ionization mass spectrometry; GPMAW, general protein mass analysis of windows; GST, glutathione-*S*-transferase; IEF, isoelectric focusing; IPTG, isopropyl-β-D-1-thiogalactopyranoside; LDH, lactate dehydrogenase; LEA, late embryogenesis abundant; mol%, mole percent; MS, mass spectroscopy; nm, nanometer; PBS, phosphate buffered saline; PII, poly (Pro)II; rEm, recombinant Em; RH, relative humidity; SDS-PAG, sodium dodecyl sulfate polyacrylamide gel.

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biotechnological applications, the mechanism(s) that lead to desiccation tolerance are still poorly understood.

The proteins most frequently implicated in desiccation tolerance are those termed late embryogenesis abundant (LEA). This diverse, hydrophilic class of proteins was initially separated into at least five groups based on primary amino acid/mRNA sequence and expression pattern [6,7]. The different groups have in turn been suggested to contribute in various ways to protection from dehydration during embryo maturation. These include the Group 1 proteins that may be involved in binding or replacement of water, the proteins of Groups 3 and 5 that are proposed to function in ion sequestration, and the Group 2 and Group 4 proteins that may contribute to maintenance of protein and membrane structure, respectively [6].

Recent bioinformatic analyses based on low complexity sequence similarities have also been used to classify these proteins [8,9]. The newer classifications indicate that the previous groupings may be separable into sub-groupings (superfamilies) and also suggest potential relatedness between previously distinct groups. These alternative groupings may be useful as function is studied in more detail. However, the earlier classifications appear rather robust, at least for the three main groups. This is particularly true for the Group 1 LEA proteins.

Group 1 LEA proteins display overrepresentation of the amino acid glycine (~18 mol%), and are rich in ionizable and hydroxylated amino acids. In addition, these polypeptides contain a signature 20 amino acid motif near the middle of the protein that can be present one to four times in tandem [10]. The biased primary amino acid sequence suggests that these proteins exist largely as random-coil structure in an aqueous environment like the cytoplasm. This prediction is supported by several structural analyses using circular dichroism (CD) that estimate the contribution of random-coil configuration to overall Group 1 LEA structure to be between 70 and 82.5% [11,12]. These same studies estimate α-helix and β-sheet content of 8–13% and 5–17%, respectively.

These predictions, however, do not take into account other possible structural elements. Analysis of the CD spectra of a recombinant Group 1 LEA protein at multiple temperatures revealed the presence of an isodichroic point indicating a transition between two structural states [12]. The difference spectrum provided evidence for the presence of left-handed, extended helical or poly(Pro) II-like (PII) structure in the recombinant Group 1 LEA protein and little, if any, α-helix. As PII-like structures have been repeatedly implicated in protein–protein and protein–peptide interactions [13–17], the presence of this structural element in Group 1 LEA proteins may be important in the amelioration of dehydration-related stresses.

We have expressed a canonical Group 1 LEA protein, the Em protein from wheat, in *E. coli* as a glutathione-*S*-transferase (GST) fusion and purified it to homogeneity. Consistent with earlier studies, our characterization of the recombinant Em protein (rEm) indicates a largely random-coil configuration. We further show that this protein is capable of protecting an enzyme, lactate dehydrogenase (LDH), from inactivation as a result of drying. Analysis of additional rEm proteins in which various predicted secondary structural elements are altered or deleted has allowed us to identify at least one region that is important for this protective effect.

2. Materials and methods

2.1. Plasmid constructions

E. coli strain DH5αMCR [18] was used for all molecular cloning procedures. All DNA manipulations were done using standard procedures [19]. Primers used for various steps in plasmid construction are listed in Table 1. Plasmid pGEX-6P-2 was digested with *Xho*I, treated with the Klenow fragment of *E. coli* DNA polymerase I to render the ends blunt and religated to remove the unique *Xho*I site. This plasmid, pGEX-6P-2-No*Xho*I, served as the base vector for construction of all expression plasmids. The coding region of the wheat Group 1 LEA protein Em was removed from plasmid pBM313 [20] as an *Nco*I/*Kpn*I fragment and treated with T4 DNA polymerase to render the ends blunt. The resulting fragment was ligated into pGEX-6P-2-No*Xho*I that had been digested with *Bam*HI followed by treatment with the Klenow fragment of DNA polymerase I to produce blunt ends. Plasmids containing the insert in the proper orientation (pBM440) result in an N-terminal in-frame fusion of GST to the *Em* coding region (GST-Em). This construct, as well as all those described below, were initially identified by restriction enzyme digestion and verified by sequence analysis.

Several altered Em protein coding regions were constructed for expression in pGEX-6P-2-No*Xho*I. Specifically, these are deletion of the 20 amino acid Group 1 LEA signature motif (Δ20 rEm), deletion of the region between the two predicted helical domains in the N-terminal half of the protein (ΔLinker rEm), and introduction of two juxtaposed proline residues into the predicted helical domain closest to the N-terminal end (Proximal-PP rEm).

2.1.1. Δ20 rEm

The 20 amino acid signature motif was removed from the coding region by ligation of an amino terminal-encoding fragment to a carboxy terminal-coding fragment. To isolate the amino terminal fragment, a 263 bp region was PCR amplified from pBM313 using primers T7 and 258615. This fragment

Table 1
Oligonucleotide primers

Primer no.	Primer sequence (5′ to 3′)
T7	TAATACGACTCACTATAGGG
258615	GCGCTGCAGCGGCTGC
258616	GGGGGGCTGAGCACCAACG
223357	GTTGCATACACCACACACGC
406671	CTGAACTACATTATTCGGTACGTAGAC
793650	CCTCCCGGGCCTTGC
793651	CCTCCCGGGCCTTGCGTGGCGGCTGCG
793652	AAGGCCGGGAGGGC
793653	AAGGCCCGGGAGGGCGAGGGCGGCAAGTCCCTCGAGGCGC
793654	GCGCCTCGAGGGACTTGCCGCCCTCGCCCTCCCGGGCCTT