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Structural transitions in the intrinsically disordered plant dehydration stress protein LEA7 upon drying are modulated by the presence of membranes

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ABSTRACT

Dehydration stress-related late embryogenesis abundant (LEA) proteins have been found in plants, invertebrates and bacteria. Most LEA proteins are unstructured in solution, but some fold into amphipathic α-helices during drying. The Pfam LEA_4 (Group 3) protein LEA7 from the higher plant *Arabidopsis thaliana* was predicted to be 87% α-helical, while CD spectroscopy showed it to be largely unstructured in solution and only 35% α-helical in the dry state. However, the dry protein contained 15% β-sheets. FTIR spectroscopy revealed the β-sheets to be largely due to aggregation. β-Sheet content was reduced and α-helix content increased when LEA7 was dried in the presence of liposomes with secondary structure apparently influenced by lipid composition. Secondary structure was also affected by the presence of membranes in the fully hydrated state. A temperature-induced increase in the flexibility of the dry protein was also only observed in the presence of membranes. Functional interactions of LEA7 with membranes in the dry state were indicated by its influence on the thermotropic phase transitions of the lipids and interactions with the lipid headgroup phosphates.

1. Introduction

LEA proteins comprise a large and heterogeneous group of proteins that were first found to accumulate in plant seeds during maturation [1]. Their accumulation often coincides with the onset of desiccation tolerance in seeds [2,3]. In addition to their presence in plant seeds, LEA proteins are accumulated in response to dehydration stress in vegetative plant organs (e.g. [4]) and in some species of bacteria [5], nematodes [6], rotifers [7], insects [8] and cyanobacteria [9]. Recently, systematic *in silico* investigations [4,10] identified 51 LEA protein encoding genes in the genome of the model plant *Arabidopsis thaliana* that were divided into nine phylogenetically unrelated groups according to amino acid sequence similarity. Most of these proteins were predicted to be intrinsically disordered (IDPs) [4], i.e. proteins without a stable secondary structure in solution [11]. Experimental evidence for this lack of stable secondary structure has been obtained for several LEA proteins from diverse biological sources (see [12] for a recent review).

The expression of many LEA proteins has been linked to the acquisition of desiccation tolerance in seeds, pollen and anhydrobiotic organisms (reviewed in [13]), but the functional role of LEA proteins in cellular stress protection is still largely unresolved. Based on in-vitro studies, a number of functions have been proposed, such as water binding, ion sequesteration and stabilization of DNA, RNA, proteins and membranes ([12,13] and references therein). Some LEA proteins are able to prevent the inactivation of enzymes during freezing or drying by preventing protein aggregation [14–17]. A LEA protein from the desiccation tolerant nematode *Aphelenchus avenae* showed anti-aggregation activity even under fully hydrated conditions when the protein was introduced into desiccation sensitive mammalian cells [16] and two plant LEA proteins showed chaperone function towards sensitive enzymes during heat treatment [18].

Some LEA proteins acquire secondary, mainly α-helical, structure during drying [7,19–24]. Structural modeling and FTIR spectroscopy indicated that four different LEA proteins interact with membranes in the dry state by folding into amphipathic α-helices and these interactions lead to the stabilization of membranes in the dry state [7,22,24,25].

However, a shortcoming of these previous studies is that protein structural determinations were performed on isolated proteins without taking a possible influence of the interaction with membranes on protein structure into account. Here we present a detailed FTIR spectroscopy investigation of dehydration-induced structural

Abbreviation: CD, circular dichroism; EPE, egg phosphatidylethanolamine; EPG, egg phosphatidylglycerol; FTIR, Fourier-transform infrared; GRAVY, grand average of hydropathy; IDP, intrinsically disordered protein; LEA, late embryogenesis abundant; LG, β-lactoglobulin; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine; T_m, gel to liquid-crystalline phase transition temperature

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alterations of the LEA7 protein of the higher plant *Arabidopsis thaliana* in the presence and absence of lipid membranes of varying composition. From the same spectra we also determined some structural characteristics of the involved lipids. LEA7 is a member of the Pfam LEA_4 (PF02987) family of LEA proteins, previously described as group 3 [26]. It is a highly hydrophilic (GRAVY = −1.317), neutral protein (pI = 6.96) with a molecular mass of 18 kDa and is therefore rather typical of LEA Group_4 proteins that have been found in plants, invertebrates and bacteria [12]. The encoding gene is highly expressed in seeds and strongly induced in leaves by drought, salt stress and by the phytohormone abscisic acid [4].

2. Materials and methods

2.1. Materials

POPC was obtained from Avanti Polar Lipids (Alabaster, AL), EPE from Lipid Products (Redhill, Surrey, UK) and EPG from Sigma. D_2O (99.98%) was purchased from Deutero GmbH (Kastellaun, Germany).

2.2. Expression and purification of recombinant LEA7

A full length cDNA clone for the *Arabidopsis thaliana* gene *LEA7* (At1g52690; clone RAFL 05-04-I14) was obtained from the RIKEN (Tokyo, Japan) RAFL collection [27,28]. The cDNA sequence was amplified by PCR and inserted into the Gateway pENTR.SD.D-TOPO vector (Invitrogen, Karlsruhe, Germany). The identity of the insert was checked by sequencing. The gene was transferred into the expression vector pDEST17 (Invitrogen) to express the protein with an N-terminal 6xHis-tag under the control of the T7 expression system.

The pDEST17.LEA7 construct was transformed into the *Escherichia coli* strain BL21 Star (Invitrogen). Bacterial cell lysates containing the recombinant protein were incubated in a boiling water bath for 10 min as a first purification step. LEA7, like several other LEA proteins, was stable upon boiling and remained in solution. Precipitated proteins were removed by centrifugation at 4000*g* for 15 min at 4 °C. The supernatant was filtered through a 0.2 μm filter and applied to a 1 ml HisTrap HP column (GE Healthcare) equilibrated with 20 mM sodium phosphate, 0.5 M NaCl, 20 mM imidazole (pH 7.4) with a flow rate of 1 ml/min. The column was washed with increasing concentrations of imidazole and LEA7 was eluted with 250 mM imidazole. Wash and elution fractions were analyzed by SDS-PAGE and the fractions containing LEA7 were pooled and dialyzed against ddH_2O in QuixSep Micro Dialyzer capsules (Roth, Karlsruhe, Germany) with Spectra/Por dialysis membranes (3500 molecular weight cut-off). After dialysis, the protein was >95% pure as estimated from SDS-PAGE and Coomassie blue staining. Purified protein was lyophilized and stored at −20 °C.

2.3. CD spectroscopy

CD spectra were obtained with a Jasco-715 spectropolarimeter (Jasco Instruments), as described in detail recently [24]. The mean-residue circular dichroism was calculated as:

$$\Delta\varepsilon_{MR} = \frac{\Delta A \cdot \mathrm{mMW}}{d \cdot c}$$

$\Delta\varepsilon_{MR}$ = mean-residue circular dichroism (M^{-1} cm^{-1}), ΔA = circular dichroism, mMW = mean molecular mass of an amino acid in the investigated protein (g mol^{-1}), d = pathlength (cm), c = concentration (g l^{-1}).

Spectra were analyzed with the CDPro software [29] using three different algorithms: CONTINLL, CDSSTR and SELCON3. Sets of reference spectra containing denatured proteins were chosen for the analysis. Since the results were similar in each case for the three parallel samples and all algorithms, averages are shown.

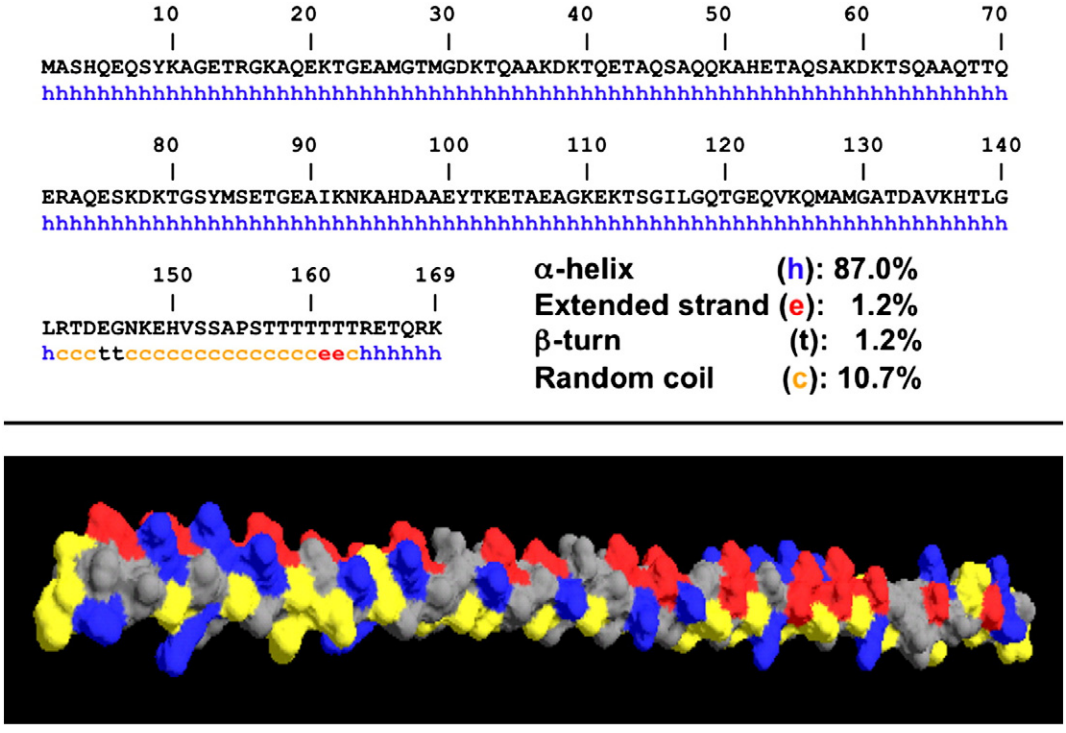

Fig. 1. Secondary structure prediction for LEA7. Secondary structure components of the full-length LEA7 protein were predicted with the SOPMA tool (upper part of the figure) and were visualized using the Swiss PDB viewer (lower part of the figure). Physico-chemical properties of the amino acids are color coded in the Swiss PDB viewer for negatively charged amino acids (red; D/E), positively charged amino acids (blue; R/H/K), apolar amino acids (yellow; A/I/L/M/V/Y) and others (grey; S/T/N/Q/G/P). LEA7 contains no F/W (apolar) and C (others).