Protective Effects of Model Peptides for Group-3 Late Embryogenesis Abundant Proteins on Desiccation-Induced Damage of Liposomes

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To investigate whether or not short model peptides work as substitutes for group-3 LEA (Late Embryogenesis Abundant) proteins, two peptides were tested: 1) PvLEA-22, which consists of two tandem repeats of the 11-mer motif characteristic to LEA proteins from an African sleeping chironomide, and 2) its control, i.e. the peptide with the amino acid composition identical with that of PvLEA-22, although its sequence is scrambled. Liposomes were prepared with DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine). Both of the two peptides were found to suppress the desiccation-induced aggregation of the liposome. When the liposome was dried in the presence of anyone of the two peptides, its gel-to-liquid crystalline temperature, measured by differential scanning calorimetry (DSC) and temperature-controlled Fourier transform infrared (FT-IR) spectroscopy, was lowered compared to that of the dried liposome without additives. The P=O stretching band position was found to shift from that of the pure liposome in the dried state. The peptide secondary structure was composed of several different structures as well as α-helix in the dried peptide/liposome mixture, being different from the pure dried PvLEA-22, in which α-helix was predominant. These results are suggestive of the direct interactions between the added peptide and the membrane polar headgroup. Taken together, these two peptides are able to act as a kind of molecular shielding reagent by binding to the surfaces of the dried liposomes, and thereby to avoid their desiccation-induced damage.

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INTRODUCTION

Group-3 late embryogenesis abundant proteins (G3LEA) have been widely recognized to play important roles in successful induction and maintenance of anhydrobiosis, occurring in non-plant species such as an African sleeping chironomide (*Polypedilum vanderplanki*), nematode, as well as in plant species1). 

The primary structure of G3LEA proteins consists of several times repeat of the so-called 11-mer motif, whose sequence depends on the origin of the corresponding G3LEA protein. Previously we reported that the 22-mer peptides with two tandem repeats of the 11-mer motif derived from *P. vanderplanki* and so on had structural and thermal properties in support of several putative functions of G3LEA proteins in desiccated cells, such as cytoskeleton formation, biological glass former, and ionic species sequestration2,3). Most recently, the 22-mer peptides were found to have anti-aggregation
effects on proteins under desiccation stress in concentration-dependent manners, a fact which is likely explained on the assumption that the target protein is shielded by the 22-mer peptides in the dried state.\(^0\)

The cellular membrane protection against drought stress is also one of the hypothetical roles of G3LEA proteins.\(^1\) Evidence for the interaction of several native G3LEA proteins with phospholipid model membrane has been obtained in the dried state.\(^5,7\) However, it remains unclear whether the 11-mer motif repeat region in the given G3LEA protein is directly interacting with the membrane surface or not.

As described above, the 22-mer peptides as models for native G3LEA proteins are very useful for gaining an insight into the functional mechanism of native G3LEA proteins and have potential utility as protective reagents for biological molecules against desiccation-induced aggregation. In the present study, two issues will be addressed: 1) the possibility of the model peptides to reduce the desiccation-induced damage of liposomes, and 2) effects of the model peptides on the gel-to-liquid crystalline transition of liposomes in the dried state.

**MATERIALS AND METHODS**

The following two 22-mer peptides were tested: (1) G3LEA model peptide consisting of two tandem repeats of the 11-mer consensus motif, Ala-Lys-Asp-Gly-Thr-Lys-Glu-Lys-Ala-Gly-Glu, which is characteristic of G3LEA proteins from *P. vanderplank* \(^2\) [Hereafter denoted as PvLEA-22], and (2) control, a peptide with the amino acid composition identical with that of PvLEA-22, although its sequence was randomized. For comparison, trehalose was also tested as one of the well-known membrane protectants.\(^8\) DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) was used as phospholipid to form liposomes.

Liposomes were prepared by extruding aqueous suspensions of DPPC at 20 mg/mL through a polycarbonate filter having a pore size of 100 nm in the diameter, with the aid of a commercially available device, Mini-extruder (Avanti Co.). To the resulting liposome solution, the selected additive in a desired amount was added, and then the solution was sufficiently stirred. Its droplet of 10-20 \(\mu\)L was dried at 55 °C and subsequently placed in a desiccator with silica gel under reduced pressures at ambient temperatures until use.

When liposome damage occurs during dehydration, such as aggregation and/or concomitant collapse, the corresponding solution after rehydration has higher turbidity than before drying. The assay was performed by measuring apparent absorbance due to light scattering at 400 nm (denoted as \(A_{400}\)), according to ref. 9.

DSC measurements were carried out using a Q-100 instrument (TA instruments, New Castle, DE). 1 mg or less amount of dried sample was sealed in an aluminum DSC pan. An empty pan was used as a reference. Heating rate was 5 °C min\(^{-1}\).

FT-IR spectra were obtained using an FT-IR spectrometer (JASCO FT/IR 6100 and IMV-4000, Tokyo, Japan). The dried sample of interest was pressed between two KBr plates. All the spectra were measured with a spectral resolution of 2 cm\(^{-1}\) and 128 scans. The temperature of the sample was controlled with an LK-600 (Linkam Scientific Instruments, Surry, UK), mounted on the stage of the above spectrometer.

**RESULTS AND DISCUSSION**

For the liposome solution without addition of any peptides, turbidity recorded as \(A_{400}\) increased after drying (Fig. 1), suggesting that the desiccation stress induced aggregation and/or concomitant collapse of liposomes. Interestingly, when the peptide, PvLEA-22, was added at molar ratios of 0.25 or higher, such a turbidity increase...
was hardly observed. Similar result was also obtained for the control peptide. These results demonstrate that both of the two peptides have substantial protective activity for the dried liposomes. When trehalose was used as an additive, its amount with higher molar ratios relative to DPPC (>1) was required to suppress the increase of turbidity after drying.

The gel-to-liquid crystalline phase transition is an important observable to provide an insight into the interactions between the membrane and the additive of interest. As shown in Fig.2, the presence of the additive caused the significant shifts of the endothermic peaks to the lower temperature side. The dried binary mixture of DPPC liposome with the control peptide exhibited the DSC curves similar to the case of PvLEA-22 given in Fig. 2.

The gel-to-liquid crystalline transition is known to reflect the conformational changes in the hydrocarbon chains of phospholipids. In the liquid crystalline phase, the phospholipids are loosely packed and their hydrocarbon chains are in gauche conformation, whereas in the gel phase the phospholipid packing becomes dense and tight, with the hydrocarbon chains being stiff in trans-zigzag. The peak positions for the symmetric CH\text{2} stretching, hereafter denoted as vCH\text{2}, in FT-IR spectra are ca. 2854 cm\textsuperscript{-1} for gauche and ca.

Fig. 1. Turbidity of aqueous solutions with different molar ratios of DPPC to the peptide, PvLEA-22.

Fig. 2. Representative DSC thermograms of dried liposomes. Each number in a parenthesis indicates the molar ratio relative to DPPC.

Fig. 3 Temperature-dependent FT-IR spectra (vCH\text{2}) for dried liposomes in the presence of PvLEA-22 with the molar ratio of 0.1 relative to DPPC. Baseline was corrected.

Fig. 4 Temperature dependence of vCH\text{2} for dried liposomes. Numbers inside the figure indicate the molar ratios of PvLEA-22 relative to DPPC.

The onset and end temperatures for the vCH\text{2} versus temperature curves do not agree with those of the corresponding DSC curves. This is likely due to the difference in the physical phenomena that could be observed by these two measurements: FT-IR spectroscopy detects the microscopic changes in the molecular structure of a given
sample, while DSC observes the absorption and release of the thermal energy of a sample, corresponding to a macroscopic property of the sample.

The absorption band around 1210 \textasciitilde 1280 cm\(^{-1}\) in IR spectra of phosphate compounds arises from the asymmetric stretching mode of the P=O atomic group. The corresponding peak position, \(v\) [P=O], for the dried liposome in the presence of trehalose or PvLEA-22 was located at a lower wavenumber by ca. 10 cm\(^{-1}\) relative to that for the dried pure liposome (Data not shown). Such \(v\) [P=O] shift for dried DPPC liposome mixed with trehalose was consistent with a previous study\(^8\), which indicated that the hydrogen bonds were formed between trehalose and the polar headgroup of phospholipids. Therefore, the trehalose or PvLEA-22-induced shifts of the gel-to-liquid crystalline transition temperature would be due to the direct interaction between the added protective compound and liposome in the dried state.

The amide I spectrum for dried PvLEA-22 alone had a predominant component at 1653 cm\(^{-1}\), indicating the occurrence of an \(\alpha\)-helix rich structure\(^8\). However, for the dried PvLEA-22/liposome mixture, the amide I band included several spectral components, such as \(\alpha\)-helix (ca. 1658 cm\(^{-1}\)), \(\beta\)-sheet (ca. 1622 cm\(^{-1}\), 1637 cm\(^{-1}\)), and \(\beta\)-turn (ca. 1673 cm\(^{-1}\)). Such a mixed population of different secondary structure in the dried peptide seems to be caused by the direct interaction between the peptide and liposome in the dried state.

The FT-IR experiments for the control peptide/liposome mixture gave the spectral features similar to those for the PvLEA-22/liposome mixture mentioned above. This is consistent with the results of turbidity measurements.

CONCLUSION

In the dried state, anyone of the model peptides studied here binds directly to the membrane surface, and then works as a physical barrier between dehydrated liposomes. Namely, the model peptides are capable of acting as a molecular shielding reagent in the dried state, and thereby protecting liposomes against desiccation-induced damage such as aggregation.

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