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Increasing Packing Density of Hydrated Dipalmitoylphosphatidylcholine Unilamellar Vesicles Induced by Trehalose

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On the basis of the observation of $^{31}P\{^{1}H\}$ nuclear Overhauser enhancement, trehalose is shown to increase the packing density of the polar head groups of phospholipid unilamellar vesicles. This finding is not consistent with previous results obtained for Langmuir-type membranes or multilamellar vesicles. The present results explain the anti-fusogenic ability of trehalose.

Anhydrobiotic organisms survive years under severe dry conditions. These organisms contain large quantities of trehalose (TRE), which likely plays a key role in maintaining the functional and structural integrities of cellular membranes during dehydration and rehydration.[1] In particular, during the dehydration process TRE must act as an anti-fusogenic reagent, which prevents inter-cellular fusion.[2] However, its molecular mechanism remains unclear at present, although some model studies have dealt with this problem. A recent X-Ray study has indicated that the addition of TRE causes an increase in the interbilayer spacing of hydrated multilamellar vesicles (MLV) of dipalmitoylphosphatidylcholine (DPPC), but exerts no notable effects on the lipid chain packing.[3] The latter fact seems to show no explicit interactions between TRE and the bilayer surface. On the other hand, Langmuir-type monolayers of phospholipids are appreciably expanded when TRE is added to the aqueous subphase, indicating the occurrence of direct interactions between TRE and the head groups, such as hydrogen bonding.[4] Hydrated MLV may not be suitable for studying the effect of sugar on membrane structures, because added sugar molecules are excluded from the interbilayer space as a result of osmotic dehydration.[2] Similarly, it is doubtful whether the results for monolayers are directly applicable to the cases of biomembranes or not. A closer analogue of a biomembrane is a unilamellar vesicle (ULV). Here, on the basis of the results from measurements of $^{31}P\{^{1}H\}$ nuclear Overhauser effect, TRE is shown to interact with hydrated ULV of DPPC in a different manner

from the cases of MLV or monolayers. Additionally, a good correlation is found between the NOE results and the rates of vesicular fusion.

L-,α-DPPC and α-, α-TRE were purchased from Sigma Chem. Co., St. Louis. Glycerol (from Nakarai Chemical Co., Tokyo; abbreviated as GLY) was also used as a solute for comparison with the case of TRE. All the reagents were used without further purification; nominal purities were 99%. DPPC (0.1 mmol) was dissolved in 10 cm^3 of chloroform, and the solvent removed by evaporation. The resulting lipid film was further dried under vacuum for 12 hours and subsequently dispersed at 50 ^{o}C (above the gel to liquid-crystalline transition temperature, 41 ^{o}C) by hand shaking in 10 cm^3 of water which contains TRE (or glycerol), TES buffer (pH=7), D_2O for NMR lock, and 0.1 mmol EDTA. This lipid dispersion was sonicated for 40 minutes with a probe type sonicator (Tomy Seiko Co. Ltd., Tokyo) with a titanium tip, and subsequently centrifuged at 100000 g for 1 hour to remove traces of titanium tip and larger particles of the lipid.[5)]

^{31}P-NMR spectra of all samples were recorded on a JEOL GSX-270 FT NMR spectrometer operating at 109.25 MHz and 50 ^{o}C. T_1 measurements were made using 180^{o}-τ-90^{o} pulse sequence with proton decoupling. The viscosities of the samples were measured using a Cannon Fenske viscometer.[6)] ^{31}P nuclear Overhauser effect enhancements (NOEE) were determined by comparison of proton decoupled with coupled spectra.[7)] The peak intensities were evaluated by cutting and weighing copies of the spectra. The vesicular fusion was followed by observing the turbidities of the samples, measured as their absorbances at 400 nm[8)] with a Shimazu UV-2100 spectrometer.

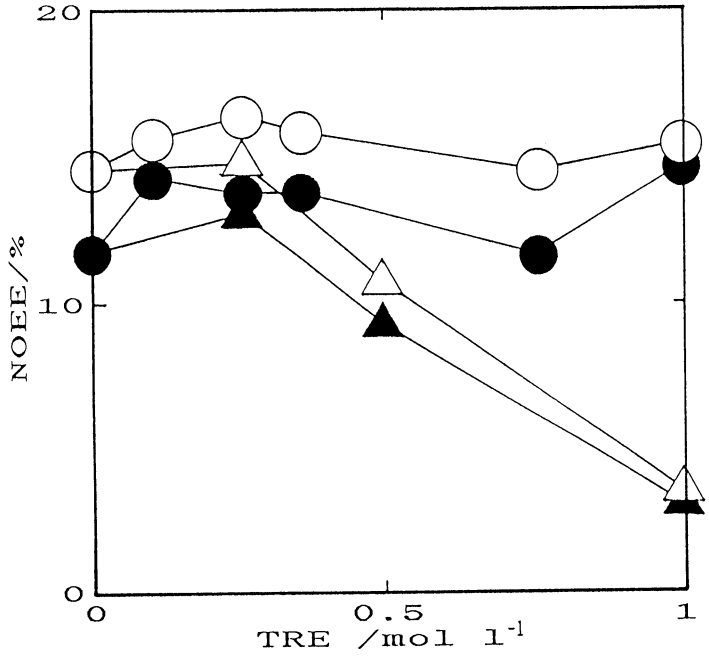

Fig. 1. The $^{31}P\{^{1}H\}$ NOEE vs. TRE concentration. The circles and the triangles represent the data for TRE- and GLY-solutions, respectively. Each triangle is plotted at the concentration of the TRE-solution which has the same viscosity as that of the given GLY-solution. The open and solid circles represent the data for completely proton decoupled and selectively choline methyl proton decoupled spectra, respectively.

The ^{31}P relaxation in a phospholipid ULV is known to be mainly governed by dipole-dipole interactions with the surrounding protons. Then, the NOEE of the ^{31}P nuclei is a function of the correlation time of the motion modulating the dipolar coupling. If the so-called extreme narrowing

conditions are satisfied, the maximum value of NOEE is calculated to be 1.24.[9] As shown in Fig. 1, the NOEE value of the pure DPPC dispersion is 0.14, indicating the considerably restricted mobility of the DPPC head groups. This is consistent with the result of a previous work by Yeagle et al.[7]

The value of NOEE exhibits no apparent variation by the addition of TRE, whereas it decreases with an increase in the concentration of GLY. The addition of these solutes caused an increase in the viscosities of the samples. In view of this, the trend observed for the GLY-containing solutions are interpreted as a result of a decrease in the molecular motion with increasing viscosity. This is supported by the fact that the T_1 value of the ^{31}P nuclei decreases with an increase in viscosity (Fig. 2). However, the results for TRE can not be simply interpreted. The NOEE values for the TRE-containing solutions are larger than those for the GLY-containing ones when both of the solutions have the same degree of viscosity.

Theoretically, $^{31}P\{^1H\}$ NOEE depends on the efficiency of cross correlation causing NOE of interest when the

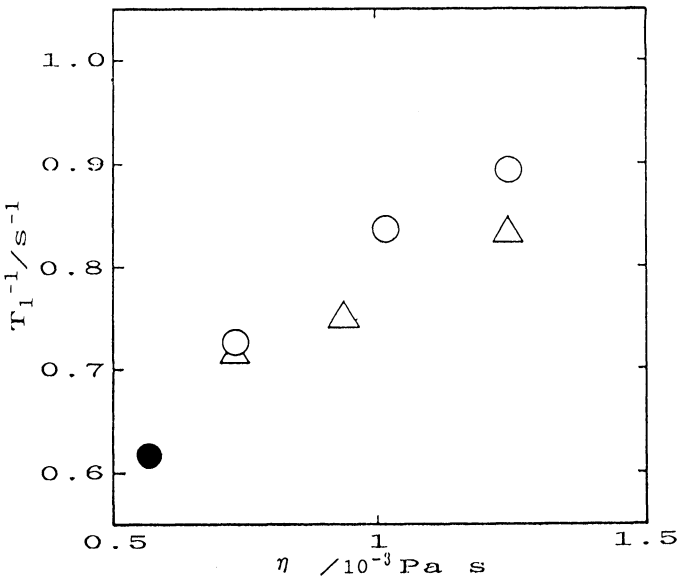

Fig. 2. The reciprocal of relaxation time ($1/T_1$) vs. viscosity of the sample (η). The open circles and triangles represent the data for TRE- and GLY-solutions, respectively. The solid circle represents the result for a control.

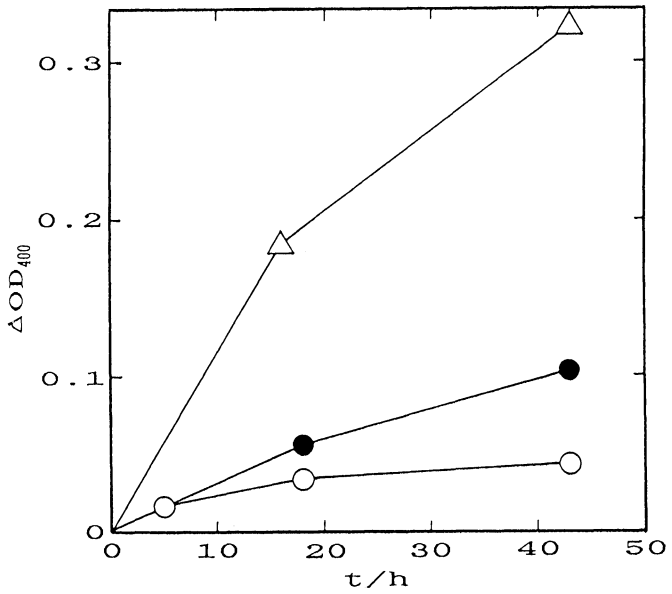

Fig. 3. The change in absorbance (at 400 nm) caused by vesicle fusion vs. time after sample preparation. The sample was stored at room temperature (23 °C). The open triangles represent the data for the sample prepared with 15 wt% GLY. The solid and open circles represent the data for the samples prepared with 0.25 M and 0.75 M TRE, respectively.

T_1 value is given. And this efficiency is inversely proportional to the sixth power of the distance between the given ^{31}P and the surrounding protons. In order to identify the protons contributing to NOEE, selective proton decoupling was carried out. The results indicated that the choline methyl group predominantly contributes to the enhancement of peak intensity of the ^{31}P nucleus (Fig. 1), being consistent with the previous report.[7] Additionally, no appreciable contribution was observed from the sugar protons. It has been revealed that the choline methyl group intermoleclarly interacts with the phosphate group of the neighboring phospholipid in a ULV.[7] On the basis of these results, it is reasonable to conclude that TRE causes a decrease in the inter-head group distance between neighboring phospholipids, resulting in enhanced efficiency of the cross correlation.

From the present data alone, it is not clear whether TRE directly interacts with the phosphate groups or not. Water-structure making reagents like sugars may perturb a hydrated layer surrounding a phospholipid vesicle, indirectly causing structural changes of the bilayer surface.[10] At any rate, the decrease in the inter-head group distance may result in an increase in the lipid packing density on the bilayer surface. Thus, the addition of TRE may affect the stability of ULV, and thereby contribute to protection of vesicular fusion. In fact, this is strongly supported by Fig. 3 showing that the rate of fusion is lowered by TRE much more than by GLY.

The measurement of $^{31}P\{^1H\}$ NOE provides valuable information for a better understanding of the anti-fusogenic effect of TRE. It should be noted that the present results for ULV are not consistent with those for MLV and monolayer systems. We are now investigating the origin of these discrepancies.

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