A Solid-State ^{31}P NMR Study of the Interaction between Trehalose and DPPC Bilayer

Takayuki Hanamura, Naoki Asakawa, Yoshio Inoue, and Minoru Sakurai*
Department of Biomolecular Engineering, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501

(Received April 17, 1998; CL-980293)

While ^{31}P solid-state NMR experiments are being carried out for DPPC vesicles mixed with trehalose, the spectra exhibit sudden changes, implying that during dehydration the trehalose molecules bind to the head groups of lipids.

It is known that trehalose has a remarkable ability to protect biological membranes from the stress of dehydration.[1-3] Much evidence has been accumulated that trehalose interacts directly with the surface of membrane in place of bound water. For example, by addition of trehalose to dry membranes, the gel-liquid crystal transition temperature T_m is lowered below the T_m of hydrated membranes.[4-7] FT-IR studies have indicated that the PO_4 asymmetric stretching vibration greatly shifts to a lower wavenumber relative to that of hydrated membranes.[4,5,8] However, there is no report informing us when trehalose binds to a membrane surface in a drying process. To capture such an accidental phenomenon, it is necessary to continue monitoring the behavior of bound water molecules and/or the head groups of lipids during dehydration. In usual cross polarization/magic angle spinning (CP/MAS) NMR experiments, a high power alternating magnetic field is applied to a sample to average out the strong dipolar couplings between observed spins and abundant ones. The field continues heating water molecules contained in the sample, leading to evaporation of them. Thus, the NMR measurement allows us to monitor the drying process of samples. In this study, during CP/MAS experiments two kinds of NMR parameters are monitored for the ^{31}P nuclei of the lipid head groups in trehalose-containing and -lacking DPPC vesicles. And we successfully observe a phenomenon indicating the sudden binding of trehalose to the membrane surface.

A JEOL GSX270 spectrometer (109.25 MHz for ^{31}P) was used to perform CP/MAS NMR measurements, where the spin-locking of ^{31}P magnetization follows the mismatched cross polarization from ^{1}H to ^{31}P. The magic-angle sample spinning was set at about 3 kHz. To monitor the mobility of ^{31}P nuclei, we measured the spin-lattice relaxation time in the rotating frame ($T_1\rho$) and the chemical shift anisotropy (CSA). Time dependent NMR spectra were obtained with varying spin-lock time (τ). It took 50 min (accumulation of 600 FID signals) to obtain each spectrum. The total elapsed time for one series of $T_1\rho$ measurement was 350 min. The time dependence of CSA was obtained from the analysis of the seven spectra.

DPPC was purchased from Sigma Chemical Co. Inc., St. Louis. Trehalose (TRE) was gifted from Hayashibara Co., Okayama. Pure DPPC vesicles or trehalose-containing vesicles were prepared in the following way. Pure DPPC or mixtures of DPPC and trehalose was dispersed in water at 60 ℃ for 4 h, and subsequently placed at 4 ℃ for 3 days. The resulting dispersion was lyophilized. For annealing of the sample, an appropriate amount of water was added, followed by equilibration for 1 h at 70 ℃. Water content of the sample was determined by use of thermogravimetry (TG).

Figures 1(a) and (b) show the time dependence of CSA and the result of the $T_1\rho$ experiment for the trehalose-containing vesicles, where the molar ratio of TRE/DPPC is 1. Before NMR measurements, water content of the sample was 13.2 wt% per DPPC. After the measurement, this sample contained 8.1 wt% of water per DPPC: namely, approximately 5.1 wt% of water was evaporated. During a series of $T_1\rho$ experiments, the apparent shape of spectrum changed. The CSA of the respective spectrum, which was calculated from the intensity of spinning sidebands,[9] drastically changed between 2nd spectrum and 3rd one from 130ppm to 190ppm (Figure 1(a)). In accordance with the result of CSA, the $T_1\rho$ relaxation exhibited bi-exponential behavior (Figure 1(b)): the relaxation times of the fast and slow components were 3.3 ms and 16.4 ms, respectively.

Figures 2(a) and (b) show the time dependence of CSA and the results of the $T_1\rho$ experiment for pure DPPC vesicles. Evaporation of water, from 10.2 to 7.9 wt% per DPPC, was also observed. CSA was only gradually increased with elapsed time. Furthermore, the $T_1\rho$ process was described with a single

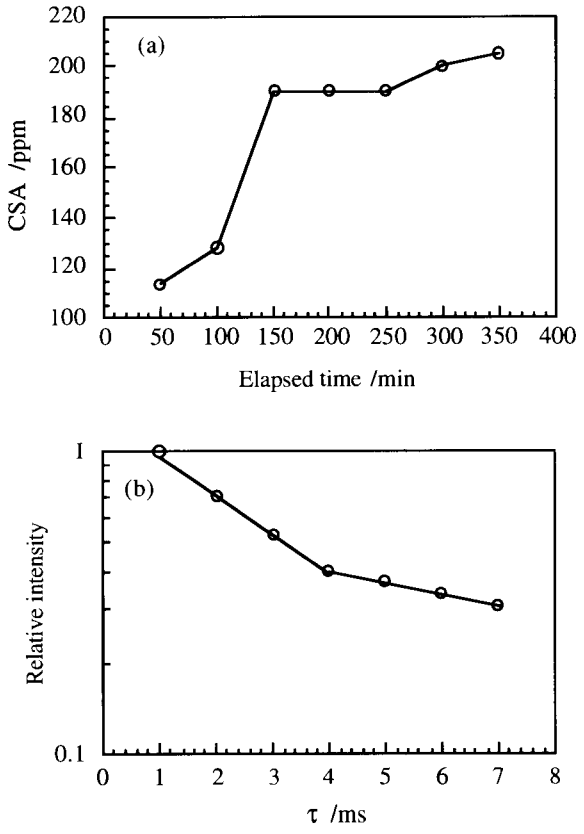

Figure 1. Time dependence of CSA and time course of signal intensity obtained by ^{31}P $T_1\rho$ measurements for trehalose-containing DPPC vesicles.

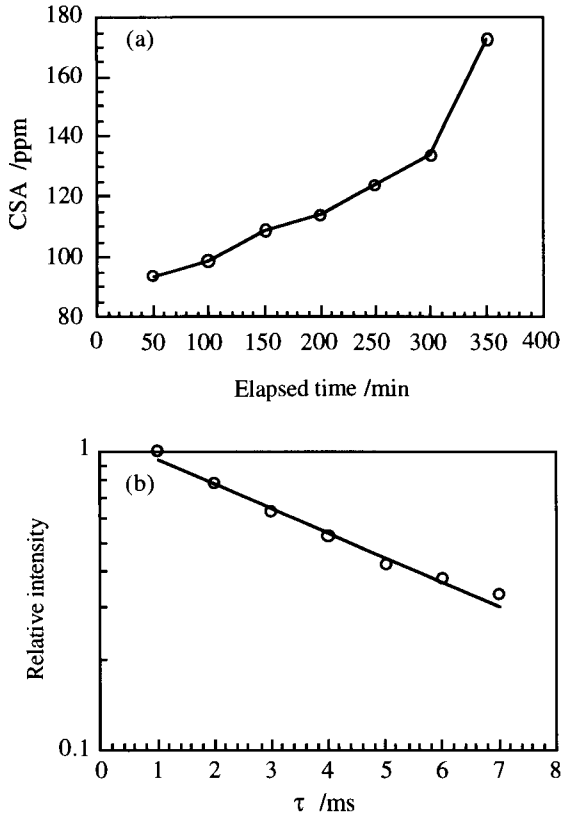

Figure 2. Time dependence of CSA and time course of signal intensity obtained by ^{31}P $T_1\rho$ measurements for trehalose-lacking DPPC vesicles.

relaxation time of 5.4 ms.

In the case of ^{31}P nuclei, the $T_1\rho$ relaxation time is given as a function of CSA and the dipolar coupling between ^{31}P and ^{1}H nuclei.[10] To interpret the results of Figures 1 and 2, it is necessary to clarify which factor is dominant in the present cases. According to the NMR relaxation theory,[10] the relaxation time is inversely proportional to the value of CSA. However, as can be seen from Figures 1 (a) and (b), the observed $T_1\rho$ value increases in response to the sudden increase of the CSA values at 150 min. This indicates that the effect of CSA on the $T_1\rho$ relaxation is less important. It is thus reasonable to interpret that the $T_1\rho$ relaxation is dominated by the dipolar coupling term. Probably, the sudden increase in the $T_1\rho$ value after the time of 150 min is caused by a lowering of the mobility of the head groups. This interpretation also explains the behavior of CSA: namely, the increase in CSA does not indicate an actual change of the intrinsic CSA, but is explained as an apparent event caused by the lowering of mobility. The results for the pure DPPC sample indicates that the mobility of the head group is only gradually lowered with an evaporation of water in the sample. This is quite naturally understood considering that water acts as a plasticizer.

By contrast, in the TRE/DPPC sample, the mobility of the head group was suddenly lowered, a phenomenon that is due to not only water evaporation but also some effect of trehalose on the head groups of DPPC. The fast $T_1\rho$ component for the TRE/DPPC sample was close to the value of $T_1\rho$ for the pure DPPC sample, which implies that in the initial state residual water molecules might be interacting directly with the DPPC head groups. If this is true, the sudden increase of the $T_1\rho$ and CSA values, found for the TRE/DPPC sample, could be understood only by the occurrence of a direct perturbation from trehalose. One possible interpretation is that trehalose binds directly to the head groups of lipids in the place of the bound water molecules. This is consistent with the so-called water replacement hypothesis[3] as mentioned above.

References

1 J. H. Crowe, L. M. Crowe, J. F. Carpenter, A. S. Rudolph, C. A. Wistrom, B. J. Spargo, and T. J. Anchordoguy, *Biochim. Biophys. Acta*, **947**, 367 (1988).
2 J. H. Crowe, J. F. Carpenter, L. M. Crowe, and T. J. Anchordoguy, Cryobiology, **27**, 219 (1990).
3 M. Sakurai and Y. Inoue, *Hyomen (Surface)*, **34**, 213 (1996).
4 J. H. Crowe and L. H. Crowe, *Science*, **223**, 701 (1984).
5 L. M. Crowe and J. H. Crowe, *Biochim. Biophys. Acta* **946**, 193 (1988).
6 M. Nakagaki, H. Nagase, and H. Ueda, *J. Membrane Science*, **73**, 173 (1992).
7 K. Tanaka, T. Takeda, K. Fujii, and K. Miyajima, *Chem. Pharm.Bull.*, **40**, 1 (1992).
8 J. H. Crowe, S. B. Leslie, and L. M. Crowe, *Cryobiology*, **31**, 355 (1994).
9 J. Herzfeld and A. E. Berger, *J. Chem. Phys*, **73**, 6021 (1980).
10 A. Abragam, "The Principles of Nuclear Magnetism", Oxford, London (1961), Chap. 8.