Relationship between Hydrophobic Index of Saccharide and Gel–Liquid Crystal Transition Temperature of the L-α-Dipalmitoyl Phosphatidylcholine (DPPC)/Saccharide/Water System

Hiromasa Nagase,*,[a] Haruhisa Ueda,[a] and Masayuki Nakagaki[b]

Department of Physical Chemistry, Hoshi University,[a] 2–4–41 Ebara, Shinagawa-ku, Tokyo 142–8501, Japan and Tokyo Institute of Colloid Science,[b] 502 Higashi-Nakano 4–4–3, Nakano-ku, Tokyo 164–0003, Japan. Received November 2, 1998; accepted January 25, 1999

An examination of the relationship between the hydrophobic index of saccharide and gel–liquid crystal transition temperatures in L-α-dipalmitoyl phosphatidylcholine (DPPC)/saccharide systems at a water content of 10 wt% and 70 wt% was performed using differential scanning calorimetry. Saccharide at 10 wt% water was found to interact with phospholipids hydrophobically *via* the hydrophobicity-rich side of the pyranose ring. Molar ratios (pyranose ring/DPPC) were determined as 2 for the DPPC/rhamnose system with 8.2 wt% water and for the DPPC/trehalose system at 15.8 wt%. Pyranose would thus appear to form a 2 : 1 complex with lipid that faces the hydrophobicity-rich side of the pyranose ring. Saccharide with 70 wt% water interacted with the phospholipid hydrophilically through hydration of the saccharide.

Key words   L-α-dipalmitoyl phosphatidylcholine; saccharide; hydrophobic index

Certain mono- and disaccharides are capable of stabilizing biological membranes[1] and liposomes[2—4] during freeze-drying. Mono- and disaccharides have also been shown to differ in their ability to stabilize membranes, monosaccharides being less effective than disaccharides.[2,3] Such differences may possibly be related to the glass transition temperature of the saccharide. Koster *et al*. found that for lipids to be stable, the saccharide must be in a glassy state when the former is a liquid crystal.[5] Glassy state saccharide effectively retains trapped materials in dry liposomes and leakage of such materials is enhanced above this temperature.[6] A saccharide with a high glass transition temperature is not always capable of stabilizing a membrane.[7] A glassy saccharide is necessary, though not sufficient in itself, to stabilize dry liposomes during freeze-drying.[7] Crowe *et al*. maintain that a glassy saccharide and direct saccharide interaction with lipid are both required for importing stability to a membrane.[7] This interaction results from hydrogen bonding between the phosphate group of the lipid and the hydroxyl groups of the saccharide.[7—9] Thus, in a dry state, saccharide interacts directly with lipid.

The mechanism for saccharide stabilization of hydrated lipid is preferential exclusion of the saccharide or preferential hydration at the bilayer surface, meaning the saccharide is excluded from the L-α-dipalmitoyl phosphatidylcholine (DPPC) bilayer surface.[10] This mechanism is considered applicable to the stabilization of protein by saccharide and other solutes.[11]

In the DPPC/saccharide/water system, the effect of saccharide on the gel–liquid crystal transition temperature (Tc) of DPPC only appeared at less than 20% water, and at more than 20% water the effect of the sugar almost disappeared.[12] In the DPPC/disaccharide/water system with a low water content (less than 5 wt%), lateral DPPC packing expanded and the Tc decreased to 24 °C.[13] In the DPPC/monosaccharide/water system under the same conditions, lateral DPPC packing showed only a slight change and Tc remained at 42 °C.[12] Using IR[7] and NMR,[14] saccharides appear to undergo hydrophilic interaction with DPPC by hydrogen bond-

ing at less than 5% water content. At 10% water content, Tcs of the DPPC/mono-, di- and trisaccharide/water system were approximately 45 °C.[12,13] Interaction of saccharides with lipids appears to occur regardless of the type of saccharide. At an intermediate water content (10—20%), the manner in which sugar interacts with lipid is not well understood.

Saccharides have hydrophobicity, as evident from the cavities in cyclodextrins, and also possess hydrophilicity. Rudolph *et al*. proposed that interactions of saccharide with phospholipid were regulated by molecular mechanics.[15] In this model, non-bonded interactions between the glucose ring and choline methyl groups occur and there is hydrogen bonding between the hydroxyl and phosphate groups. This result implies that lipid interacts with saccharide by hydrophobic and hydrophilic interactions.

Miyajima *et al*. examined and measured the hydrophobicity of saccharide based on the hydrophobic index (HI) and specific HI (SHI).[16] HIs were defined by surface-area ratios of hydrophobic and hydrophilic groups. In determining SHI, only surface area on the hydrophobicity-rich side of the pyranose ring was considered, while both sides were considered for HI.

Yano *et al*. noted a positive correlation between the HI and partition coefficients with polystyrene gel in aqueous solution.[17] Saccharide thus apparently interacts both hydrophilically and hydrophobically with membranes.

Tc of a phospholipid is related to the strength of interactions with saccharide.[18] In the present study, the relationship between HI and/or SHI of saccharides and the change in Tc of a membrane with 10% and 70% water was examined using DPPC as a model membrane.

Experimental

Sample Preparation   Mannose (Man), ribose (Rib) and sucrose (Suc) were from Wako Pure Chem. Ind., Ltd. All other saccharides (glucose (Glc), galactose (Gal), 2-deoxy-ribose (D-Rib), xylose (Xyl), arabinose (Ara), rhamnose (Rha), trehalose (Tre), maltose (Mal) and raffinose (Raf)) and DPPC were obtained from Sigma. DPPC (about 80 mg) in chloroform was dried at 20 °C by evaporation and further dried for 12 h at 60 °C under vacuum. Dried thin-film samples of DPPC were hydrated with 4 ml of water or an aqueous solution of saccharide at a molar ratio of 2.6 (mol

---

saccharide/mol DPPC). This was followed by hydration under agitation at 60 °C for 3 h. Water was removed by evaporation at 45 °C and the samples were heated to 90 °C, exposed to the air and cooled to room temperature. Samples containing monosaccharides were viscous and those from di- and trisaccharides, powdery. These samples are referred to as "powdery sample". The powdery samples were transferred to an aluminum pan for differential scanning calorimetry (DSC) and immediately sealed. Their water content was determined by the Karl Fischer method using a moisture meter (model CA-06, Mitsubishi Kasei Corp.). To obtain samples of greater water content, water was added by Micropet pipettes (Beton, Dickinson and Company) to some of the unsealed powdery samples at 24 °C. To obtain samples with less water content, the powdery samples were heated at 40, 60 or 80 °C for 30 min. After being sealed, the samples were allowed to swell for 3 h at 60 °C and water content was determined gravimetrically.

DSC Tc was determined based on the onset temperature of the DSC endothermic curve for the first scan, using a Rigaku DSC 8240D provided with a TAS 200 thermal analysis system. The heating rate was 1 °C/min from 20 °C to 70 °C for samples with a water content close to 10 wt% and 0.5 °C/min from 20 °C to 50 °C for those with close to 70 wt%. Alumina served as the reference.

Enthalpy change in the transition of saccharide from hydrate to the amorphous state was determined from the DSC curve of Tre dihydrate and Rha monohydrate and swollen samples of the DPPC/Tre/water and DPPC/Rha/water systems. These samples were cooled to 0 °C using a CryoCool CC100 II under nitrogen gas and then immediately heated from 0 °C to 110 °C at 2 °C/min. The second and third scans were conducted immediately following the first scan.

Tc of the DPPC/Saccharide System with a Water Content of 10 wt% Tc of the DPPC/saccharide/water system was constant for the most part at 20%.[12] As water content decreased to below this value, Tc of DPPC with saccharide increased and then decreased after plateauing near 10%.[12] Tc of this plateau was used to examine the relationship between Tc and HI. Water was added to a powdery sample in the DSC pan to obtain two samples containing water, one lower and above 10%, respectively. Tc for the 10% sample was obtained by linear interpolation.

Tc of the DPPC/Saccharide System with a Water Content of 70 wt% Water was added to a powdery sample in a DSC pan to obtain two samples with water content above and below 70%, as determined gravimetrically. Tc of a DPPC/water mixture has been shown to decrease linearly with increase in water content.[19] Based on this, linear interpolation was carried out to determine Tc of a system with 70% water.

Results

DPPC/Saccharide System with a Water Content of 10 wt% Figure 1 shows DSC thermograms for the DPPC/monosaccharide and DPPC systems with a water content of about 10%. In the DPPC/Glc and Man systems, two endothermic peaks were observed. The higher transition temperature (larger ΔH) was found to be that for a gel–liquid crystal transition. In the DPPC/Ara system, the lower transition temperature showing larger ΔH was that of a gel–liquid crystal transition. These results were confirmed by powder X-ray diffraction. The gel phase displayed a relatively sharp peak at 21° (2θ) and the liquid crystal phase a broad peak at 19°.

Figure 2 shows phase diagrams of the DPPC/monosaccharide/water and DPPC/water systems. The change in Tc of the DPPC/water system was in agreement with that reported previously.[20,21] Above 27% water content, Tc of the DPPC/water system was constant. Below 27%, Tc gradually increased and at 20%, the increase was quite remarkable. In the DPPC/monosaccharide/water system, Tc gradually increased and remained essentially constant with decrease in water content. The phase diagram of DPPC with monosaccharide showed a plateau region between 8 and 15% water content, as reported.[12] Tc values of the samples with saccharide were lower compared to those without saccharide, indicating that ΔTc (Tc without saccharide−Tc with saccharide) is posi-
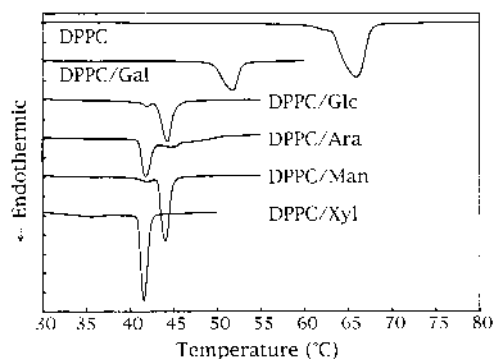

Fig. 1. DSC Endothermic Curves of the DPPC/Water and DPPC/Monosaccharide Systems with about 10 wt% Water
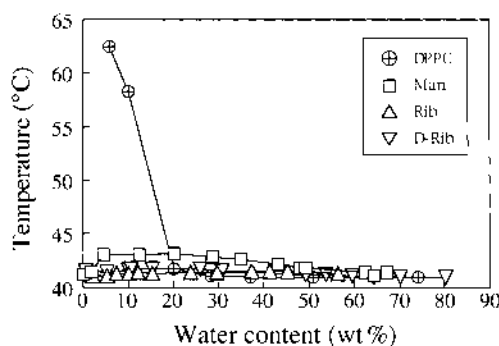

Fig. 2. Phase Diagram of the DPPC/Monosaccharide/Water System
⊕: DPPC/water system. □: DPPC/Man/water system. △: DPPC/Rib/water system. ▽: DPPC/D-Rib/water system.
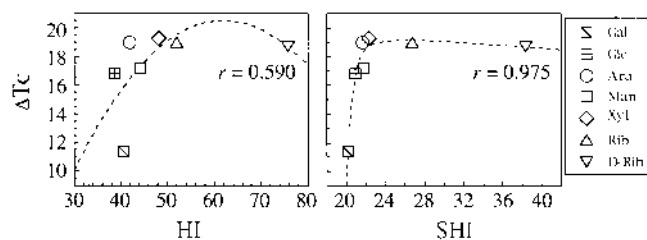

Fig. 3. Relationship between ΔTc and HI or SHI in the DPPC/Monosaccharide System with 10 wt% Water
ΔTc=Tc (without saccharide)−Tc (with saccharide). A hyperbolic function was used for curve fitting of the data points. The correlation coefficient, r, is indicated.

tive.

The relation between the HIs of monosaccharides and the change in ΔTc is shown in Fig. 3. Below a HI of 44 and a SHI of 22, ΔTc increased along with HI and SHI. After passing through a maximum, ΔTc decreased slightly above these HI and SHI values. Each instance of a decrease in ΔTc was fitted to the hyperbolic function, ΔTc$=aX-b-c\times$sqrt$[(X-d)^2+e]$, where a, b, c, d, and e are parameters and X, HI or SHI. This function is an experimental equation and provided by far the best fit, compared to any other conventional functions such as a parabola, exponent or logarithm. The correlation coefficients for HI and SHI were 0.590 and 0.975, respectively. The curve for SHI showed a much better fit than that for HI. Saccharide may thus possibly interact hydrobically with the DPPC bilayer *via* the hydrophobicity-rich side of the pyranose ring, with a consequent decrease in Tc.

DPPC/Saccharide System with a Water Content of

70 wt%　Figure 4 shows DSC thermograms of the DPPC and DPPC/saccharide systems with a water content near 70%. Tc was about 42 °C. At 70% water content, Tc of the system with saccharide exceeded that without saccharide, thus indicating ΔTc to be negative. Figure 5 (ΔTc vs. HI and SHI) shows that an increase in hydrophobicity lessens the effect of saccharide on DPPC. Changes in Tc were fitted to a hyperbolic function and the correlation coefficients for HI and SHI were 0.893 and 0.879, respectively.

DPPC/Tre/Water and DPPC/Rha/Water Systems　Figure 6 shows DSC thermograms of the DPPC/Tre system with 15.8 wt% water, and the DPPC/Rha system with 8.2 wt% water. The top thermograms in Figs. 6A and 6B show that the endothermic transitions of Tre dihydrate and Rha monohydrate to an amorphous state occur at 96.3 °C and 91.6 °C, respectively. The highest transition temperatures in the first scan of the DPPC/Tre/water and DPPC/Rha/water systems were 96.1 °C and 93.0 °C, respectively. These endothermic peaks could not be seen in the second scan, which is consistent with the DPPC/Tre/water system prepared by swelling by adding water after heating in a vacuum.[22] The highest temperature transition of DPPC/Rha/water or DPPC/Tre/water systems may thus be concluded to be that of hydrate to anhydrate. Transitional enthalpy change, ΔH(s) of the transition from hydrate to anhydrate was determined from the peak area of pure Rha monohydrate and Tre dihydrate. The amount of saccharide which formed a hydrate with the addition of water to the DSC pan was determined by ΔH(s) of the peak in the first scan of the DPPC/saccharide/water system. The amount of saccharide in the sample remaining after this formation was determined based on total amount of saccharide in the samples. Assuming that saccharide which does not form hydrate interacts with DPPC, the molar ratios (Rha/DPPC, Tre/DPPC) of saccharide to DPPC were found to be 1.96 and 1.09, respectively. That is, the number of pyranose rings for each DPPC molecule was determined to be 2 for either the DPPC/Rha system with a water content of 8.2% or DPPC/Tre system with a water content of 15.8%.

Discussion

Miyajima *et al.* showed that HIs correlated closely with hydrophobic interactions of monosaccharides and cholates in aqueous solutions.[16] HI were defined by the surface-area ratio of the hydrophobic and hydrophilic groups. In calculating SHIs, only the surface area on the hydrophobicity-rich side of the pyranose ring was considered, while both sides were considered for HI.

At 10% water content, ΔTc of the DPPC/monosaccharide/water system (see Fig. 3) shows that for HI below 44 and SHI below 22, monosaccharide with a small HI interacts more weakly with DPPC than monosaccharides with large HIs. This is because the stronger the interaction between saccharide and lipid, the lower is the Tc for DPPC, or the larger the ΔTc. Thus, interaction of monosaccharide with DPPC strongly depends on the hydrophobicity of the former. Based on molecular mechanics of the phospholipid/saccharide system, Rudolph *et al.* were able to demonstrate non-bonded hy-
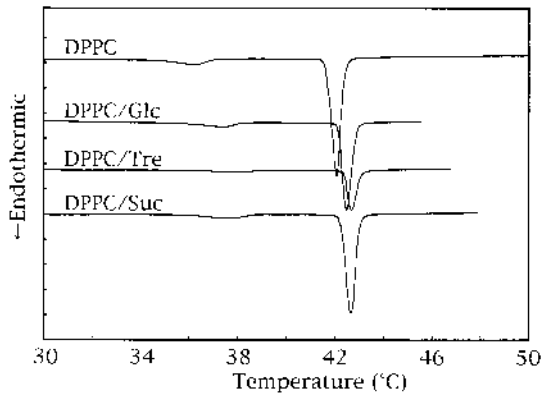

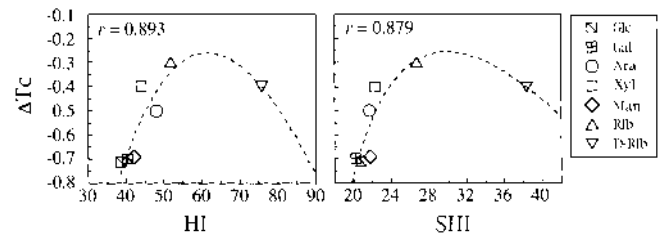Fig. 4.　DSC Endothermic Curves of the DPPC/Water and DPPC/Saccharide/Water Systems with about 70 wt% Water

Fig. 5.　Relationship between ΔTc and HI or SHI in the DPPC/Monosaccharide System with 70 wt% Water

ΔTc=Tc(without saccharide)−Tc(with saccharide). A hyperbolic function was used for curve fitting. The correlation coefficient, *r*, is indicated.
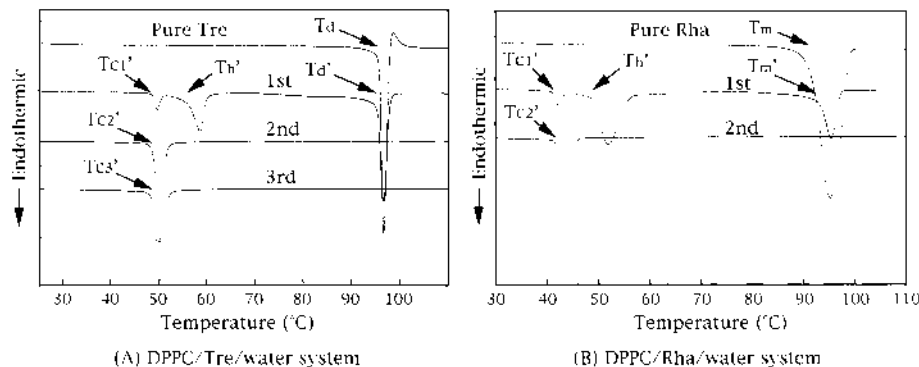

(A) DPPC/Tre/water system

(B) DPPC/Rha/water system

Fig. 6.　DSC Thermograms of (A) the DPPC/Tre System with 15.8 wt% Water and (B) the DPPC/Rha System with 8.2 wt% Water

Td and Tm are the temperatures for transition of hydrate to anhydrate of Tre dihydrate and Rha monohydrate, respectively, and Td' and Tm' are for transition of hydrate to anhydrate of Tre dihydrate and Rha monohydrate in the DPPC/Tre/water and DPPC/Rha/water systems, respectively. Tc*n*' is Tc of the DPPC/saccharide/water system in the *n*-th DSC scan. Th' is the higher transition temperature of the DPPC/saccharide/water system at the first DSC scan.
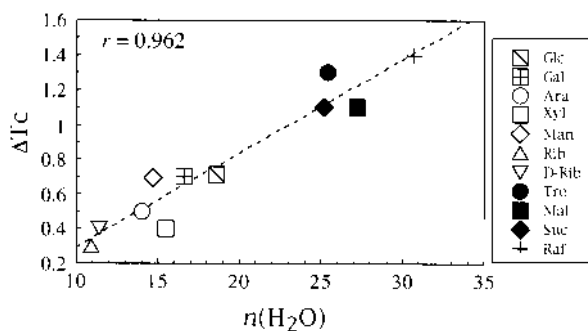
Fig. 7. Relationship between ΔTc of the DPPC/Saccharide System with 70 wt% Water and Hydration Number, n(H$_2$O)

ΔTc=Tc (without saccharide)−Tc (with ssaccharide). A straight line was used for the curve fitting. The correlation coefficient, r, is indicated.

drophobic interaction between a pyranose ring and choline methyl group.[15] This is consistent with the present observation that saccharide interacts hydrophobically with phospholipid. The closer correlation of ΔTc with SHI than with HI, as shown in Fig. 3, indicates monosaccharides possibly interact hydrophobically with the DPPC bilayer *via* the hydrophobicity-rich side of the pyranose ring.

The polar group of DPPC consists of hydrophilic constituents, such as CO$_2$, PO$_4^-$ and N$^+$ groups and hydrophobic CH, CH$_2$ and CH$_3$ groups, but the N$^+$ atom is not able to function as a polar group due to being surrounded by three methyl groups. The hydrophobic groups of DPPC interact with saccharide hydrophobically as well as hydrophilically. Maximum ΔTc at 44 (for HI) or 22 (for SHI) suggests interaction with the hydrophilic group of DPPC is apparently dependent on the proportion of hydrophilic to hydrophobic constituents in DPPC.

In the DPPC/Tre/water system, saccharide forms a complex with lipid and excess saccharide forms a hydrate by the addition of water.[12,22] In this study, the ratio of mol pyranose ring/mol lipid was determined as 2, which is consistent with that of the DPPC/Tre/water system previously reported.[22] The surface area of the pyranose ring has been shown to be 20 Å^2 (based on the crystal structure of β-D-glucose),[23] and that of DPPC, 40.6 Å^2 in the gel phase and 47.0 Å^2 in the liquid crystal phase.[24] The surface area of two pyranose rings, 40 Å^2, is somewhat less than that of DPPC in gel and liquid crystal phases. Thus, at 10% water content, saccharide interacts hydrophobically with the DPPC bilayer *via* the hydrophobicity-rich side of the pyranose ring at a molar ratio of 2 (two pyranose rings/one lipid).

As evident from Fig. 5, ΔTc of DPPC with monosaccharide decreased with a decrease in HI and SHI below 52 for HI and 27 for SHI, indicating that saccharide has a hydrophilic rather than hydrophobic effect on lipid at a water content of 70%. The correlation coefficient of HI was slightly larger than that of SHI, indicating that saccharide has an effect on lipid *via* either side of the pyranose ring. Saccharides should thus exert effects on phospholipids through the hydrated forms of the saccharide. Such interaction has actually been observed in the hydrated DPPC/Mal/water system.[19]

The hydration of saccharide can be described in terms of the dynamic hydration number of the saccharide, determined by NMR and denoted by n(H$_2$O).[25] n(H$_2$O) showed a good linear relationship with the mean number of equatorial hy-

droxyl groups.[26] The relation between n(H$_2$O) and ΔTc for a sample with a water content of 70% is shown in Fig. 7. Changes in ΔTc were fitted to a straight line and the correlation coefficient was 0.961. ΔTc increased linearly with the hydration number of the saccharide. The good correlation between ΔTc and hydration number of the saccharide supports the notion that the effect of saccharide is due to hydration. Hydrophobic interactions between saccharides and lipids in the DPPC/saccharide system with a water content of 10% could not be detected in the DPPC/saccharide system with 70% water.

Conclusions

At low water content, saccharides interact directly and hydrophobically with phospholipids *via* the hydrophobicity-rich side of the pyranose ring. The experimental value of the molar ratio (pyranose ring/DPPC) for the complex of saccharide with DPPC was determined to be 2. At high water content, no hydrophobic, but rather hydrophilic interactions occur *via* water bound to the saccharide molecules.

References
 1) Crowe J. H., Crowe L. M., Jackson S. A., *Arch. Biochem. Biophys.*, **220**, 477—484 (1983).
 2) Crowe L. M., Womersley C., Crowe J. H., Reid D., Appel L., Rudolph A., *Biochim. Biophys. Acta*, **861**, 131—140 (1986).
 3) Tanaka K., Takeda T., Fujii K., Miyajima K., *Chem. Pharm. Bull.*, **40**, 1—5 (1992).
 4) Strass G., Schurtenberger P., Hauser H., *Biochim. Biophys. Acta*, **858**, 169—180 (1986).
 5) Koster K. L., Webb M. S., Bryant G., Lynch D. V., *Biochim. Biophys. Acta*, **1193**, 143—150 (1994).
 6) Sun W. Q., Leopold A. C., Crowe L. M., Crowe J. H., *Biophys. J.*, **70**, 1769—1776 (1996).
 7) Crowe J. H., Hoekstra F. A., Nguyen K. H. N., Crowe L. M., *Biochim. Biophys. Acta*, **1280**, 187—196 (1996).
 8) Crowe L. M., Crowe J. H., *Biochim. Biophys. Acta*, **946**, 193—201 (1988).
 9) Ozaki K., Hayashi M., *Chem. Pharm. Bull.*, **45**, 165—170 (1997).
10) Crowe L. M., Crowe J. H., *Biochim. Biophys. Acta*, **1064**, 267—274 (1991).
11) Arakawa T., Timasheff S. N., *Biochemistry*, **21**, 6536—6544 (1982).
12) Nagase H., Ueda H., Nakagaki M., *Biochim. Biophys. Acta*, **1328**, 197—206 (1997).
13) Nagase H., Ueda H., Nakagaki M., *Biochim. Biophys. Acta*, **1371**, 223—321 (1998).
14) Lee C. W. B., Gupta S. K. D., Mattai J., Shipley G. G., Abdel-Mageed O. H., Makriyannis A., Griffin R. G., *Biochemistry*, **28**, 5000—5009 (1989).
15) Rudolph B. R., Chandrasekhar I., Gaber B. P., Nagumo M., *Chem. Phys. Lipids*, **53**, 243—261 (1990).
16) Miyajima K., Machida K., Taga T., Komatsu H., Nakagaki M., *J. Chem. Soc., Faraday Trans. 1*, **84**, 2537—2544 (1988).
17) Yano Y., Tanaka K., Doi Y., Janado M., *J. Sol. Chem.*, **17**, 347—358 (1988).
18) Crowe L. M., Crowe J. H., Chapman D., *Arch. Biochem. Biophys.*, **236**, 289—296 (1985).
19) Tanaka K., Takeda T., Miyajima K., *Chem. Pharm. Bull.*, **39**, 1917—1921 (1991).
20) Nakagaki M., Handa T., Ohashi K., *Yakugaku Zasshi*, **99**, 1—7 (1979).
21) Kodama M., Kuwabara M., Seki S., *Biochim. Biophys. Acta*, **689**, 567—570 (1982).
22) Nakagaki M., Nagase H., Ueda H., *J. Mem. Sci.*, **73**, 173—180 (1992).
23) Chu S. S. C., Jeffrey G. A., *Acta Cryst.*, **B24**, 830—838 (1968).
24) Ruocco M. J., Shipley G. G., *Biochim. Biophys. Acta*, **691**, 309—320 (1982).
25) Uedaira H., Ikura M., Uedaira H., *Bull. Chem. Soc. Jpn.*, **62**, 1—4 (1989).
26) Uedaira H., Ishimura M., Tsuda S., Uedaira H., *Bull. Chem. Soc. Jpn.*, **63**, 3376—3379 (1990).