Effects of Cholesterol on the Miscibility of Synthetic Glucosamine Diesters in Lipid Bilayers and the Entrapment of Superoxide Dismutase into the Positively Charged Liposomes

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Methyl-β-D-glucosamine-3,6-dilauroyl, dimyristoyl, dipalmitoyl or distearoyl esters were synthesized as positively charged lipids. They were incorporated into phosphatidylcholine liposomal membranes and the entrapment of superoxide dismutase (SOD) into the liposomes was attempted. The efficiency of the SOD-entrainment into the positively charged multilamellar vesicles (MLVs), comprising egg yolk phosphatidylcholine and synthetic glucosamine diesters, was enhanced by the addition of cholesterol to the membranes. A differential scanning calorimetric study showed that the miscibility (solubility) of glucosamine diesters in phosphatidylcholine bilayers increased on the addition of cholesterol to the membranes. Cholesterol assisted in the mixing of phosphatidylcholines with positively charged glucosamine diesters and increased the positive charges on the liposomal membranes. This was confirmed by incremental increases in the zeta-potential of liposomal membranes with an increase in the cholesterol content. Entrapment of SOD thus became more efficient due to the enhanced electrostatic attraction between the positively charged membranes and the negatively charged SOD, and/or the electrostatic repulsive interactions between positively charged membranes; the latter interactions induced a thickening of the water layer in MLVs.

Keywords positively charged liposome; superoxide dismutase; glucosamine; cholesterol; differential scanning calorimetry; dipalmitoyl phosphatidylcholine

Superoxide dismutase (SOD) has been shown to diminish both the permeability changes and lipid peroxidation products associated with membrane free radical attack.1,2) Until recently, SOD was considered limited in its ability to scavenge superoxides generated in the body for two major reasons: 1) its half-life in the circulation is < 6 min3); and 2) its inability to cross cellular membranes or the blood–brain barrier makes it difficult to access the presumed areas of free radical production.4) However, recent studies using polyethylene glycol-conjugated SOD5,6) heparin-associated SOD7) and SOD-entrapped liposomes7,8) have demonstrated positive clinical results, presumably due to an increased uptake of SOD. The positive influence of positively charged liposomes containing SOD in attenuating ischemic brain injury, such as permeability changes and edema formation, has also been demonstrated.7) Such a positively charged liposome has been formed by the incorporation of stearylamine into the otherwise neutrally charged lipid membranes. The administration of liposomes containing stearylamine, however, gives rise to convulsions9); so the stearylamine-containing liposomes are unsuitable for clinical use.

Recently, we synthesized biodegradable and positively charged glucosamine monoesters and found that their incorporation into size-controlled liposomal membranes leads their persistence in the blood circulation for relatively long periods of time.10) But the synthesized glucosamine monoesters can also form micelles in an aqueous solution.10) and their distribution equilibrium among micellar, liposomal and aqueous phases confused the understanding of the role of monoesters in prolonged circulation. In the present study, we synthesized diesters having the same glucosamine head group as the glucosamine monoesters, and they were utilized as novel, positively charged lipids for the preparation of SOD-entrapped liposomes.

In numerous studies on the mixing behavior of glycolipids with phosphatidylcholines, it was found that glycolipids are immiscible with phospholipids at molar fractions greater than 0.1 to 0.2, and that clusters of glycolipids are then formed in host matrices.11-13) This is clearly a disadvantage for their incorporation into liposomal membranes. However, Suzuki et al.11) suggested that the addition of cholesterol to the membranes can lead to a decrease in this cluster phase.

In the present report, the enhancing effect of cholesterol on SOD entrainment into glucosamine diester-incorporated multilamellar vesicles (MLVs) was studied. The effect of cholesterol on the mixing of synthetic glucosamine diesters with phosphatidylcholines was also investigated by differential scanning calorimetry (DSC). Our goal is to elucidate the function of cholesterol in the mixing of glucosamine diesters with phosphatidylcholines and to understand the effects of cholesterol on the efficiency of SOD-entrainment into glucosamine diester-incorporated liposomes.

Experimental

Materials Methyl-β-D-glucosamine-3,6-dilauroyl ester (DLGlc, 5a), methyl-β-D-glucosamine-3,6-dimyristoyl ester (DMGlc, 5b), methyl-β-D-glucosamine-3,6-dipalmitoyl ester (DPGlc, 5c) and methyl-β-D-glucosamine-3,6-distearoyl ester (DSGlc, 5d) were synthesized according to the synthesis section and Chart I. For each glucosamine dieste, a single spot was evident on thin layer chromatography using silica gel plates (Merck, Darmstadt, Germany) and a mixture of chloroform and methanol (9:1—8:2 in volume) as the eluting solvent in combination with a chemical detection procedure using molybdenum-H3PO4/ethanol. 1-z-Dipalmitoyl phosphatidylcholine (DPPC), egg yolk phosphatidylcholine (EggPC) and cholesterol were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and no further purifications were performed. SOD from bovine erythrocytes, 3000 units/mg, was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Calcein was obtained from Dojindo Laboratories (Kumamoto,
The total lipid concentrations of liposomal solutions was kept constant at 10 mM.

In the separation of liposomes and untrapped substances, gel permeation chromatography has often been utilized but this method is unsuitable for MLV because the column sometimes clogs. In this study, an ultracentrifugation method was carried out as follows: 3 ml of dispersed solution was ultracentrifuged at about 100000g (63000 rpm) for 1 h at 4°C.

**Determination of Their SOD-Entrapping Efficiency**

The entrapment efficiency of SOD into MLV, %Trap (%), was evaluated using the following equation:

$$\text{%Trap} = 100 \left( \frac{[\text{SOD}]_{\text{inh}} - [\text{SOD}]_{\text{trap}}}{[\text{SOD}]_{\text{inh}}} \right)$$

Here, [SOD]_{inh} is the initial concentration of SOD, being 0.5 μg/ml, [SOD]_{trap} is the concentration (μg/ml) of SOD in the supernatant after ultracentrifugation, which was determined by an assay of SOD activity according to Oyana's nitrite method.\(^{4,5}\)

\(a\) is the volume ratio of the bulk solution to MLV and is described as 

$$a = \frac{V_{\text{sol}}}{V_{\text{MLV}}}$$

where \(V_{\text{sol}}\) and \(V_{\text{MLV}}\) are the volumes of added SOD solution and MLV, respectively. The \(V_{\text{sol}}\) value was calculated from the trapped volume of MLV comprised of EggPC, DPGlc and cholesterol (7:1:2 in molar ratio), the trapped volume value being determined as 4.4 l/mol (total lipid) by using fluorophore calcin as an entrapped substance.\(^{1,5}\) From the above calculation, an \(a\) value of 0.956 was obtained. Of course, the \(V_{\text{sol}}\) values are dependent on the proportion of positively charged lipids in membranes. Under our experimental conditions, however, \(V_{\text{sol}} \gg V_{\text{MLV}}\) and it may be considered that the differences in \(V_{\text{sol}}\) among liposomes is sufficiently small in comparison with the \(a\) value. Therefore, the obtained \(V_{\text{sol}}\) value was used to calculate the %Trap values for other liposomal dispersions.

The ‘entrapped’ SODs measured by this method involve two types of SODs: one SOD is contained in the water layers among the lamellas of MLVs, and the other SOD is stuck on the surface of the outermost lipid layers of MLVs.

**Measurements. Size and Zeta Potential**

Measurements of liposome size were carried out with a Zetazizer 4 photon correlation spectrometer of Malvern Instruments (Worcs., U.K.) at the scattering angle of 90° and at 25°C. The size was calculated with an NEC Power Mate SX/16i computer (Brockbrough, MA, U.S.A.) connected with the photometer. The analysis of size distribution was performed using software supplied by Malvern Instruments, and it contained some automatic correction of the refractive index for layered particles. Average diameters were estimated as the mean values of intensity distribution.

The zeta potential of liposomes was also determined with a Zetazizer 4 using a ZET 5103 small capillary cell and a cross beam mode at 25°C. In this determination, liposomes size-controlled in about 200 nm, prepared by the extrusion method, were used instead of MLV.

**DSC**

Dense lipid solutions without SOD were prepared by the following procedure. Chloroform solutions of condensed lipids were added to aluminum sample pans and the solvent was removed with a stream of dry nitrogen gas. After storage in a vacuum overnight at room temperature, 20 μl of buffer solution were added to pans, which were then sealed. The final lipid concentration was 0.3 μm.

DSC measurements were carried out at the heating rate of 5°C/min with a Shimadzu SC-30 differential scanning calorimeter, equipped with a Shimadzu DT-30 thermal analyzer, using the same volume of buffer solution as a reference. Prior to measurement, each sample was incubated at about 60°C for about 15 min. The temperature of the DSC apparatus was calibrated using the melting point of gallium as a reference. 29.7°C. DSC measurements were performed at least three times, and the reproducibility of DSC curves was good. The transition temperature was described as the temperature at which the DSC curve starts to change.

**Analytical Methods**

The concentration of phosphatidylcholines was determined similarly to that of phosphorus according to Bartlett’s method.\(^{19}\)

The concentration of glucoseamine diesters was obtained using an indole–HCl method (Dische–Borenfreund method)\(^{16}\) as a concentration of glucoseamine after the hydrolysis of glucoseamine diesters. The hydrolysis and the coexistence of phospholipids and/or cholesterol with glucoseamine diesters did not have any influence on the indole–HCl method.

**Results and Discussion**

**Characterization of Glucosamine Diester-Incorporated Liposomes**

The diameter of MLV composed of EggPC, DPGlc and cholesterol (7:1:2 in molar ratio) was

1. Synthesis. 2-(N-Benzoyloxycarbonyl)amino-2-deoxy-D-glucose (2) Benzyl chloroformate (7g) was added dropwise at 5°C to a mixed solution of water (90ml) and methanol (50ml), containing glucosamine 1 (8.3g) and sodium hydroxycarbonate (6g), and the mixture was stirred well for 6 h at room temperature. The solution was washed with water and dichloromethane, followed by crystallization from hot water, to give compound 2 (525g) (step a in Chart 1).

2. Methyl-2-(N-Benzoyloxycarbonyl)amino-2-deoxy-D-glucose (3) A mixture of compound 2 (3.5g) and p-toluenesulfonyl chloride (0.3g) in anhydrous methanol (200ml) was refluxed for 60h. After neutralization with sodium hydroxycarbonate, the mixture was filtered. The filtrate was evaporated and crystallized from ethyl acetate to give compound 3 (3.2g, 87%) (step b in Chart 1).

3. Methyl-2-(N-benzoyloxycarbonyl)amino-2-deoxy-3,6-O-dimethylolylglucose (4c) A mixture of compound 3 (10g) and p-methyl chloride (7.7g) in pyridine (60ml) was stirred overnight. The reaction mixture was poured into 100ml of hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride solution, dried over sodium sulfate, and evaporated to afford a residue, which was chromatographed over silica gel. Elution with chloroform-ethyl acetate (95: 5) gave compound 4c at a 24% yield (step c in Chart 1).

4. Methyl-2-amino-2-deoxy-3,6-dimethylolylglucose (5c) Compound 4 (1.2g) in 30ml of methanol-ethyl acetate (1:1) was stirred with 3% palladium carbon in an atmosphere of hydrogen overnight. After removal of the catalyst by filtration, the solvent was evaporated to leave an oil which was purified by column chromatography over silica gel with chloroform-methanol (99:1:1) as an eluent to give compound 5c (600mg, 62%) (step d in Chart 1).

5. The positions of the amine groups were determined by H-H correlation spectroscopy (COSY) spectrum analysis in CDCl3 using a JEOL Model JMN-GX400 spectrometer. The positions of the esters were also determined by H-H COSY. Anal. Calcd for C24H32NO5: C, 69.91; H, 11.28; N, 2.09. Found: C, 69.76; H, 11.61; N, 2.08.

Additional information on the synthesis and characterization of compounds 2-5 is provided in the supplementary material.
TABLE I. Effects of Cholesterol Content on Entrapping Efficiencies (%Trap) and Zeta Potentials of Liposomes

<table>
<thead>
<tr>
<th>EggPC: DPGlc:cholesterol</th>
<th>%Trap (%)</th>
<th>Zeta potential (mV)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Average ± S.E.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7:1:2</td>
<td>14.02±0.28 (n=3)</td>
<td>+6.0±0.9 (n=7)</td>
</tr>
<tr>
<td>5.7:1:3.3</td>
<td>17.81±0.38 (n=3)</td>
<td>+8.1±1.5 (n=9)</td>
</tr>
</tbody>
</table>

a) Liposomes size: about 200 nm in diameter.

2109 ± 123 nm (average ± S.D., n = 5). However, the measurable range in our apparatus is from 5 nm to 5 μm in diameter, and the MLV probably contains particles over 5 μm. Therefore, the actual mean diameter may be slightly higher than the measured value.

Zeta potential values of liposomes comprised of EggPC, DPGlc and cholesterol (7:1:2 and 5.7:1:3.3 in molar ratio) were +6.0 and +8.1 mV, respectively, in a buffer solution at 25 °C, as shown in Table I. The glucosamine group of our synthetic diesters is positively charged due to the +NH₃ group in our buffer solution (pH = 7.2), and their incorporation into liposomal bilayers led to the positive charging of the liposomal membranes. An incremental increase in the cholesterol content while the concentration of glucosamine diester was kept resulted in an increase in the positive charge of liposomal membranes.

Entrapping Efficiency of SOD in MLVs In preparations of liposomes containing glucosamine diesters above a molar fraction (X_Glc) of 0.25, the lipid thin-film could not be dispersed with a buffer solution and all the lipids remained on the wall of a round-bottomed flask, even if the temperature was raised to about 60 °C. This was confirmed by the finding that there was no detectable amount of EggPC in the solution. When the cholesterol molar fraction (X_Chol) was above 0.33 or below 0.20, not all MLVs were precipitated after ultracentrifugation and the %Trap could not be determined.

Figure 1 illustrates the effects of the DPGlc molar ratio on %Trap of SOD in EggPC MLVs at X_Chol = 0.20 and 0.33. Table I also shows the %Trap values at X_Glc = 0.1. The %Trap increased with an increase in the amount of DPGlc (the total lipid concentrations were constant). Up to X_Glc = 0.20, the entrapment was more efficient at X_Chol = 0.33 than at X_Chol = 0.20.

Figure 2 depicts the influence of the acyl chain length of glucosamine diesters on the %Trap of SOD in EggPC MLVs. Though the entrapment was most efficient with DMGlc and DPGlc, the differences in the %Trap values among all glucosamine diesters were trivial, and the efficiencies were essentially independent of acyl chain length. However, the cholesterol content greatly affected the entrapment efficiency of SOD. In all cases, higher entrapment values were observed with X_Chol = 0.33 than at X_Chol = 0.20.

Recovery of Glucosamine Diesters from Liposomal Solutions It is very important to confirm whether or not all lipids, which form a thin-film on the wall of flask in the preparation of liposomes, are dispersed by the vortex.

Figure 3 demonstrates data on the recovery of DSGlc and EggPC from dispersed liposomal solutions. In the absence of cholesterol, as shown in the lower part of this figure, the amount of DSGlc in the dispersed liposomal solution continued to increase, but not in direct proportion to the concentration of DSGlc taken in the preparations, in spite of the complete recovery of DSGlc and EggPC from both the liposomal solution and the undispersed lipids on the flask wall (in Fig. 3A). This indicates that a part of the DSGlc remains on the wall of flask without being dispersed. A similar remainder was also observed in DPPC/DSGlc liposomes, as depicted in Fig. 4.

On the other hand, in the presence of cholesterol, as shown in Fig. 4B, most of the DSGlc was withdrawn from the liposomal solution at X_Chol = 0.20, indicating that the used lipids in the preparation were dispersed by the vortex and that the DSGlc molecules were almost incorporated.

Fig. 1. Effects of DPGlc Molar Ratio on the Entrapment Efficiency (%Trap) of SOD into EggPC MLVs at the Cholesterol Molar Fraction (X_Chol) of 0.2 (●) and 0.33 (▲).

The results are expressed as the mean ± S.E. of 3–6 determinations from three different MLV preparations. The concentrations of total lipids are 10 mg.

Fig. 2. Effects of Methyl Constituent Number in the Acyl Chains of Synthetic Glucosamine Diesters on the Entrapment Efficiency (%Trap) of SOD into EggPC MLVs at Cholesterol Molar Fractions (X_Chol) of 0.2 (●) and 0.33 (▲).

The results are expressed as the mean ± S.E. of 3–6 determinations from three different MLV preparations. The concentrations of total lipids are 10 mg.

CH₂ number in the acyl chains of synthetic glucosamine diesters
Fig. 3. Recovery of DSGlc and EggPC from Dispersed Liposomal Solutions and Undispersed Lipid Films on the Walls of Round-Bottomed Flasks

A, the total recovery % of DSGlc (△) and EggPC (●) from the dispersed liposomal solution and the undispersed lipids on the flask wall; B, the relationship of DSGlc molar fractions (●) between the dispersed liposomal solutions and the taken lipids in the preparations. The concentration of total lipids is 10 mM.

Fig. 4. Recovery of DSGlc and DPPC from Dispersed Liposomal Solutions and Undispersed Lipid Films on the Walls of Round-Bottomed Flasks with and without Cholesterol

A, the total recoveries % of DSGlc (△) and DPPC (●) from the liposomal solution and the remaining lipids on the flask wall without cholesterol; B, the relationship between the DSGlc molar fractions with (○) and without (●) cholesterol in the dispersed liposomal solutions and the taken lipids in the preparations. The concentration of total lipids is 10 mM.

Fig. 5. Differential Scanning Calorimetry (DSC) Heating Curves of Mixtures of Phosphatidylcholines with/without Synthetic Glucosamine Diesters and/or Cholesterol in Excess Water

A and B, EggPC/DSGlc (X_Glc = 0.15 and 0.20); C, DPPC alone; D, E, F and G, DPPC/DSGlc (X_Glc = 0.10, 0.15, 0.25 and 0.5); H, DSGlc alone; I and J, DPPC/DSGlc/cholesterol (X_Glc = 0.2, X_Glc = 0.15 and 0.25). Heating rate, 5°C/min. The concentration of total lipids is 0.3 M.

...function of cholesterol on the mixing of glucosamine diesters with EggPC, because DPPC has a "gel to liquid-crystalline" phase transition in the temperature region where such an approach is easily applicable.

In the following measurements, DSGlc and DPPC are used as glucosamine diesters because the %Trap is essentially independent of acyl chain length, as shown in Fig. 2.

EggPC/DSGlc Mixtures When DSC was performed with pure EggPC alone in excess water, no thermal transitions were detected over the entire temperature range of these studies (25–60°C). The reason is that the temperature of the main transition from a gel to the liquid-crystalline phase of phosphatidylcholine (T_m) is below −15 to −7°C,¹⁸ whereas the membrane was in the liquid-crystal phase when the DSC measurements were carried out. In the case of pure DPGlc alone in excess water, one thermal transition was detected at 46.5°C. This was ascribed to the transition of DPGlc from a solid to a liquid.

As DPGlc was added in increasing amounts to pure EggPC, no thermal transition could be detected up to X_Glc = 0.15 (curve A in Fig. 5). Above X_Glc = 0.20, however, a new transition appeared and about 46°C, which agreed with the transition temperature of DPGlc alone in excess water (curve B in Fig. 5), and the transition remained up to X_Glc = 1.

DPPC/DSGlc Mixtures Calorimetric heating scans obtained for pure DSGlc, pure DPPC and DPPC/DSGlc mixtures in excess water are shown in Figs. 5C–H. The DSC for pure DPPC is characteristic of its multilamellar dispersions, with a pretransition at about 33°C and a main transition at 41.4°C, as shown in Fig. 5C. The enthalpy of the main transition was 37.1 kJ/mol, in good agreement with the reported value of 36.6 kJ/mol by Mabrey and Sturtevant.¹⁸ In the case of pure DSGlc alone in excess water, one thermal transition was detected at 51.4°C, as shown in Fig. 5H, this melting point being slightly higher...
than that of DPGlc.

As DSGlc was added in increasing amounts to pure DPPC, the $T_m$ of the DPPC membrane gradually decreased from 41.4°C to 38.4°C up to $X_{Glc} = 0.15$. When $X_{Glc}$ was increased beyond 0.15, a new transition appeared at about 51°C and remained up to $X_{Glc} = 1$. The new transition peak appeared at the same temperature as the peak in the case of pure DSGlc alone in excess water. The temperature of the original main transition remained constant or perhaps decreased slightly, as shown in Fig. 5, curves D-G. The appearance of the new transition peak indicates that DSGlc can no longer mix with phospholipid molecules and the excess glucosamine diesters remain in a solid-like phase at $X_{Glc}$ values above 0.15.

Figure 6 illustrates the transition temperatures evaluated from the DSC measurements at various $X_{Glc}$ in DPPC/DSGlc mixtures. The addition of DSGlc to DPPC decreased the $T_m$ up to $X_{Glc} = 0.15$. This corresponds to a depression effect of DSGlc for the freezing point of DPPC host matrix, indicating that DSGlc is freely soluble in DPPC up to $X_{Glc} = 0.15$.

The appearance of the new transition, which is the same peak as pure DPGlc alone, when the amount of glucosamine diesters was increased, was also observed in the DPGlc/EggPC mixture (curve B in Fig. 5), suggesting that the mixing behavior of DPGlc with EggPC is similar to that of DSGlc with DPPC.

The $T_m$ depression effect of glucosamine diesters are in contrast to those reported for myristic acid,13 spingomyelin,19 gangliosides11 and other glycolipids,12,20 in which cases their addition to DPPC increased $T_m$ and stabilized the DPPC gel phase. Such stabilization is due partly to the shielding of phospholipid head groups from each other, reducing their repulsive interaction.21 Stabilizing interactions are also thought to take place directly between the ganglioside head groups.22

Phosphatidylcholine/|Glucosamine Diesters/Cholesterol Mixtures Curves I and J in Fig. 5 show the calorimetric heating scans for DPPC/DSGlc/cholesterol mixtures. At $X_{Glc} = 0.25$, as shown in Fig. 5, curve J, a peak starting at 51°C, corresponding with the transition of DSGlc from solid to liquid, decreased in size, and the main transition of DPPC became much broader after the addition of 20 mol% cholesterol in comparison with that in the absence of cholesterol (curve F in Fig. 5). The broadening effects of cholesterol on the main transition of DPPC have already been observed and discussed.23 At $X_{Glc} = 0.15$, a very small transition peak was detected at about 51°C without cholesterol (Fig. 5E) but the addition of 20 mol% cholesterol led to the disappearance of the peak. When cholesterol was added to EggPC/DPGlc mixtures, the disappearance of the peak starting at about 46°C was dependent on $X_{Chol}$ (the DSC charts were not shown but $X_{Chol}$ and $X_{Glc}$, at which the peak starting at about 46°C disappeared on the DSC curve, will be shown on the diagram in Fig. 7B). These results indicate that the addition of cholesterol to the membrane enhances the solubilization of glucosamine diesters into the phosphatidylcholine membranes.

Figure 7A shows a diagram of the composition of a three-component system involving a variety of compositions of DSGlc, DPPC and cholesterol in excess water, and it was evaluated according to the molar fractions at which the peak starting at about 51°C disappeared on the DSC curves. The addition of cholesterol to the components lead to an increase in the mixing phase, shown at the top left in the three-component diagram. This is also a clear indication that cholesterol assists in the mixing of DSGlc with DPPC. An equivalent function of cholesterol was also observed when it was mixed with DPGlc and EggPC, as shown in Fig. 7B, which evaluated from the molar fraction at the disappearance of the peak starting at about 46°C. These results agreed well with the finding that an incremental increase in cholesterol content while the concentration of glucosamine diester remained constant led to an increase in the zeta-potential of liposomal membranes (Table I).

Enhancing SOD Entrapments by the Addition of Cholesterol An increase in the amount of cholesterol in EggPC liposomal membranes resulted in an incremental increase in the entrapment of SOD into MLVs. From the above discussion, we propose that cholesterol serves to aid the mixing of glucosamine diesters with EggPC and thereby enhances their solubility into the EggPC membrane; therefore, the entrapment of SOD becomes more efficient.
due to electrostatic interactions involving two phenomena: 1) the attractive interaction between the negatively charged SOD and the positively charged lipid membrane surface, and 2) the repulsive interaction among the positively charged membranes. The latter results in an incremental increase of the distance between multilamellar membranes and induces a thickening of the water layer in MLVs.

In conclusion, cholesterol enhanced the mixing of positively charged glucosamine diesters with phosphatidylcholines and, as a result, increased the positive charge on liposomal membranes. The entrapment of SOD thus became more efficient due to enhanced electrostatic interactions. Thus, cholesterol plays an important role in mixing phosphatidylcholines with particular lipids, which are otherwise scarcely soluble in phosphatidylcholine membranes.

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References