# Effect of amphiphilic surfactant LDAO on the solubilization of DOPC vesicles and on the activity of $Ca^{2+}$-ATPase reconstituted in DOPC vesicles

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**Abstract.** Solubilization of large unilamellar 1,2-dioleoylphosphatidylcholine (DOPC) vesicles by *N*-dodecyl-*N*,*N*-dimethylamine-*N*-oxide (LDAO) was studied using turbidimetry. From turbidity data, the LDAO partition coefficient between the aqueous phase and DOPC bilayers was obtained. Using this partition coefficient, the LDAO:DOPC molar ratio in the bilayer was calculated and effects of LDAO on the bilayer stability, bilayer thickness and on the phosphohydrolase activity of sarcoplasmic reticulum $Ca^{2+}$ transporting ATPase (SERCA) reconstituted into DOPC were compared at the same LDAO:DOPC molar ratios in the bilayer. The sequence "bilayers in vesicles – bilayer fragments (flat mixed micelles) – tubular mixed micelles – globular mixed micelles" was suggested for the solubilization mechanism of DOPC vesicles from the combined turbidimetric and small-angle neutron scattering (SANS) results. The effective molecular packing parameter $\delta = 0.5$, corresponding to the mixed bilayer – mixed tubular micelle transition, was calculated from fragmental DOPC and LDAO volumes at the molar ratio LDAO:DOPC = 2.00 in bilayers, in the middle of transition region observed earlier experimentally by small-angle neutron scattering (SANS). The bilayer thickness decrease induced by LDAO in DOPC observed by SANS did not result in the SERCA phosphohydrolase activity decrease and this indicates that some other factors compensated this bilayer effect of LDAO. The ATPase activity decrease at higher LDAO concentrations was caused by the bilayer deformation. This deformation resulted in the formation of non-bilayer aggregates in LDAO+DOPC system.

**Key words:** *N*-dodecyl-*N*,*N*-dimethylamine-*N*-oxide — Dioleoylphosphatidylcholine — Unilamellar vesicles — Solubilization — Partition coefficient

## Introduction

*N*-alkyl-*N*,*N*-dimethylamine-*N*-oxides are non-ionic amphiphilic surfactants at physiological values of pH (Herrman 1964). *N*-dodecyl-*N*,*N*-dimethylamine-*N*-oxide (LDAO) forms spherical micelles in aqueous solutions at concentrations above critical micelle concentration (Timmins et al. 1988) and its ability to form micelles and mixed micelles with phospholipids is widely used for the solubilization of biological membranes, and for the isolation, purification and crystallization of membrane proteins (Rigaud et al. 2000; le Maire et al. 2000). The phosphohydrolase activity of the skeletal sarcoplasmic reticulum $Ca^{2+}$ transporting ATPase (SERCA), a well-known transmembrane protein (Toyoshima et al. 2000; Toyoshima and Nomura 2002; Lee 2002, 2004), is stimulated at low and inhibited at high concentrations of LDAO (Andriamainty et al. 1997; Karlovská et al. 2005, 2006). The LDAO molecules predominantly interact with the lipid component of sarcoplasmic reticulum membranes (Kragh-Hansen et al. 1998) and their membrane concen-

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Dedicated to Doc. RNDr. Daniela Uhríková, CSc. on the occasion of her birthday.

trations at which the SERCA phosphohydrolase activity inhibition occurs seem to correspond to concentrations when the bilayer in model membrane systems is transformed into mixed micelles (Uhríková et al. 2001; Karlovská et al. 2006). To correlate quantitatively the effects of LDAO on the SERCA activity and on the bilayer-micelle transformation, the molar partition coefficient of LDAO between the aqueous phase and the lipid bilayer, $K_p$, is needed. The value of $K_p$ for LDAO was estimated earlier (Hrubšová et al. 2003) in unilamellar vesicles prepared from egg yolk phosphatidylcholine (EYPC). Since the most reliable SERCA activity results were obtained with the isolated SERCA protein reconstituted into 1,2-dioleoylphosphatidylcholine (DOPC) bilayers (Karlovská et al. 2006), in the present paper we estimate the $K_p$ value of LDAO for this lipid using similar approach as in Hrubšová et al. (2003). Using the $K_p$ value obtained, effects of LDAO on DOPC bilayer thickness, bilayer transition into non-bilayer structures and on the SERCA activity found in our previous papers (Uhríková et al. 2001; Karlovská et al. 2006) are recalculated and compared at the same LDAO: DOPC molar ratios in the bilayer. Finally, the experimentally observed effects of LDAO on DOPC bilayers and on the activity of SERCA reconstituted in the same bilayers are compared with the effective molecular packing parameter characterizing the geometry of aggregates consisting of two components (LDAO and DOPC) which is calculated using the approach of Hui and Sen (1989) and Goltsov and Barsukov (2000) and from available fragmental volumes of LDAO and DOPC.

## Materials and Methods

Synthetic DOPC was purchased from Avanti Polar Lipids, Inc. (Alabaster, USA), and methanol from Slavus (Bratislava, Slovakia). LDAO was prepared from *N*-dodecyl-*N*,*N*-dimethylamine by hydrogen peroxide oxidation as described by Devínsky et al. (1978). A weighted amount of DOPC was dispersed in the redistilled water by hand shaking, vortexing and a brief sonication in the UC405 BJ-1 bath sonicator (Tesla Vráble, Slovakia) at room temperature. This dispersion was extruded through one polycarbonate filter (Nuclepore, Plesanton, USA) with pores of diameter 100 nm, using the LiposoFast Basic extruder (Avestin, Ottawa, Canada) fitted with two gas-tight Hamilton syringes (Hamilton, Reno, USA) as described in (MacDonald et al. 1991). The samples were subjected to 51 passes through the filter at room temperature. An odd number of passes were performed to avoid contamination of the sample by large and multilamellar vesicles, which might not have passed through the filter. The whole procedure described results in case of DOPC in the dispersion of unilamellar vesicles with a small amount of admixtures of oligolamellar vesicles as confirmed by small-angle X-ray and neutron scattering and dynamic light scattering (Kučerka et al. 2007). The DOPC concentration in the prepared dispersion of unilamellar vesicles was estimated by spectrophotometry. Briefly, the dispersion was diluted in redistilled water and 10 $\mu$l portions of the diluted dispersion were added into 2.6 ml of methanol in the spectrophotometric quartz cell. The absorbance $A(\lambda)$ at $\lambda$ = 210 nm of the sample thus prepared was compared with the absorbance $A(210)$ of a series of DOPC solutions prepared by a direct dissolution of known amounts of DOPC in methanol. The dispersion of DOPC unilamellar vesicles with known amount of DOPC was diluted serially in the redistilled water to reach the needed DOPC concentration. To 2.6 ml of the diluted DOPC dispersion placed in the thermostated spectrophotometric cell, small amounts (20 $\mu$l) of LDAO solution in the redistilled water were added stepwise at 37 ± 1°C. Before, during and after each LDAO addition, the dispersions were stirred using a magnetic stirrer placed on the bottom of the cell and their turbidity $A_T(\lambda)$ at $\lambda$ = 500 nm and 37°C was measured. Shortly after LDAO addition (~15 min), the turbidity readings equilibrated and were recorded. The spectrophotometric and turbidimetric measurements were performed with a HP 8452A diode-array spectrophotometer (Hewlett Packard, Palo Alto, USA). For the evaluation of experimental data, the freeware least squares curve fitter program written by Pezzullo (2007) and the commercial software Origin® (Microcal Software, Northampton, USA) were used.

## Results and Discussion

Typical dependencies of equilibrated values of turbidity $A_T$ on the LDAO concentration in the sample are shown in Fig. 1. It is seen that the turbidity slightly decreases at lower LDAO concentrations (stage I). After this stage I, the turbididy decreases sharply (stage II) and, finally, reaches low values (stage III). The turbidimetric observation of three stages in the unilamellar DOPC vesicles – LDAO interaction is in agreement with the phase behaviour of bilayer-forming lipid – micelle-forming surfactant mixed systems in an excess of water, as reviewed by Lichtenberg et al. (2000): in the stage I, the vesicles with surfactant intercalated into lipid bilayers (mixed bilayers) are in equilibrium with free surfactant molecules in the aqueous phase; in the stage II, the mixed bilayers coexist with mixed lipid – surfactant micelles and free surfactant molecules in the aqueous phase; in the stage III, the mixed lipid – surfactant micelles are in equilibrium with free surfactant molecules in the aqueous phase. Because the diameter of extruded vesicles is minimally one order of magnitude larger than the diameter of LDAO micelles (Timmins et al. 1988; Kučerka et al. 2007), the bilayer-micelle transformation, also termed solubilization,

results in drastic turbidity changes as observed in Fig. 1. The small drop in turbidity observed after the first LDAO addition to vesicle dispersions could be caused by the dissociation of oligolamellar vesicles which are present as an admixture in the dispersion of unilamellar vesicles when the DOPC multilamellar vesicles are extruded through filters with pore diameters larger than 50 nm (Kučerka et al. 2007).

We fitted the experimental $A_T$ *vs.* LDAO concentration ($c_{LDAO}$) data with a sum of linear functions (see Fig. 1). This procedure is frequently used to find the solubilizing concentration of surfactant, $c_S$, as the onset concentration corresponding to the intersection of lines approximating the data in the first and second stage (Lichtenberg 1985; de la Maza and Parra 1995; Ribosa et al. 1997). The $c_S$ values obtained from the simultaneous fit of the data (excluding the values at $c_{LDAO} = 0$) with linear functions at different DOPC concentrations ($c_{DOPC}$), are shown in Fig. 2. As derived in the Appendix, the molar partition coefficient $K_P$ can be obtained from the linear $c_S$ *vs.* $c_{DOPC}$ function

$$c_S = \frac{n_{L,S}}{n_{DOPC}} \cdot \left( \frac{1}{N_A V_{DOPC}} \cdot \frac{1}{K_P} + c_{DOPC} \right) \quad (1)$$

where the constant $n_{L,S}$:$n_{DOPC}$ is the molar ratio of LDAO located in the lipid phase and DOPC at the concentration $c_S$, $N_A = 6.0221 \times 10^{23}$ mol$^{-1}$ is the Avogadro constant and $V_{DOPC} = 1.3067$ nm$^3$ the molecular volume of DOPC in vesicles at 37°C (Uhríková et al. 2007). The error weighted fitting of experimental $c_S$ *vs.* $c_{DOPC}$ data in Fig. 2 by using the function above gave $n_{L,S}$:$n_{DOPC} = 0.76 \pm 0.08$ and $K_P = 683 \pm 130$ with the correlation coefficient of linear fit $R = 0.983$. The partition coefficient found for DOPC is within error margins equal to the partition coefficient $K_P = 507 \pm 215$ for 100 nm extruded EYPC vesicles at room temperature (Hrubšová et al. 2003). It is interesting why the partition coefficients for two different lipid bilayers (DOPC and EYPC) at two different temperatures (RT and 37°C) are so close. The first factor is probably the composition – the averaged length of the EYPC acyl chains is 17.8 carbon atoms with 1.2 double bonds (Filípek et al. 1993), which is close to the composition of DOPC acyl chains (18 carbon atoms, 1 double bond). For two similar lipid bilayers one would expect that the difference in temperatures influences the partition equilibrium, but this is not the case – the effect, if any, is smaller than the error margins. Our results support therefore the conclusion, that the amphiphile partitioning between the aqueous phase and phosphatidylcholine bilayers is rather insensitive to temperature changes provided that bilayers are in the fluid state (Fraser et al. 1991; Middleton et al. 2004) like DOPC and EYPC bilayers.

As follows from the Eq. (A6) in the Appendix, the partition coefficient can be used for the calculation of the molar ratio of LDAO located in the lipid phase and DOPC, $n_L$:$n_{DOPC}$, at any $c_{LDAO}$ and $c_{DOPC}$ concentrations in the sample:

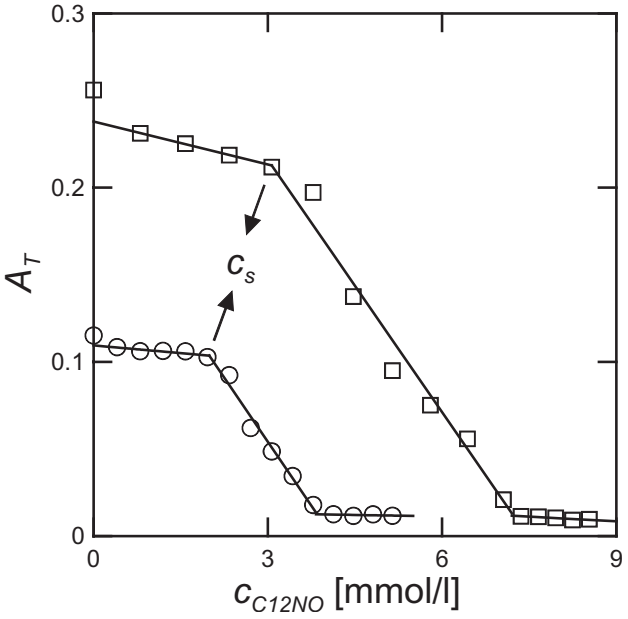


**Figure 1.** Dependence of the turbidity $A_T$ of the dispersion of DOPC unilamellar vesicles on the LDAO concentration $c_{LDAO}$ at DOPC concentration $c_{DOPC} = 0.926$ mmol/l (circles) and 2.469 mmol/l (squares).

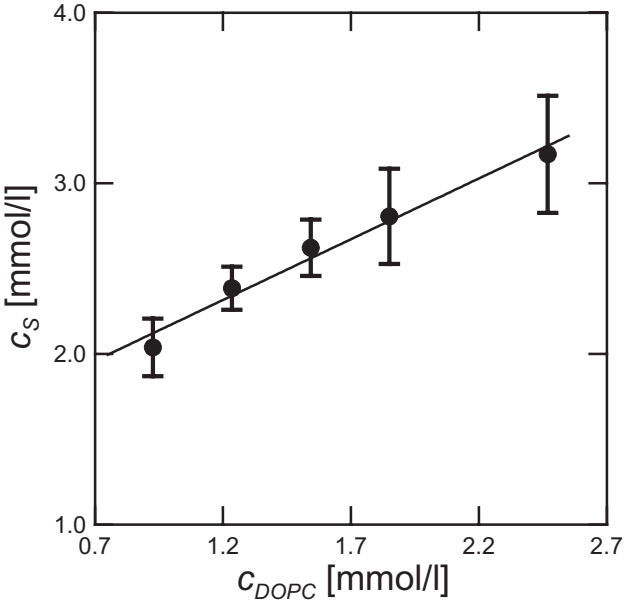


**Figure 2.** Dependence of the solubilization concentration $c_S$ on the DOPC concentration $c_{DOPC}$.

$$\frac{n_L}{n_{DOPC}} = c_{C12NO} : \left( \frac{1}{N_A V_{DOPC}} \cdot \frac{1}{K_P} + c_{DOPC} \right) \quad (2)$$

Using this equation, we have recalculated the turbidity data at three different $c_{DOPC}$ concentrations. The normalized turbidity $A_T/A_{T,0}$, where $A_{T,0}$ is the turbidity at $c_{LDAO} = 0$ , is plotted in Fig. 3 as a function of $n_L$:$n_{DOPC}$ molar ratio. The horizontal error bars are the $n_L$:$n_{DOPC}$ uncertainties propagating from the uncertainty of $K_P$ and the vertical dashed lines delimit the $n_{L,S}$:$n_{DOPC}$ molar ratio where the onset of solubilization occurs. These data can be compared with the data from small-angle neutron scattering (SANS) experiments of Uhríková et al. (2001). In these SANS experiments, the solubilization of unilamellar DOPC vesicles induced by LDAO was studied as a function of $c_{LDAO}$ concentration at a constant $c_{DOPC}$ = 12.72 mmol/l. From the scattering curves, the shape parameter $r$ was obtained. The dependence of shape parameter $r$ on the $n_L$:$n_{DOPC}$ molar ratio obtained by using Eq. (2) is shown in Fig. 3.

As discussed extensively by Uhríková et al. (2001), the shape parameter $r$ = 1, 2, and 3 holds for infinite sheet-like objects like flat (discoid) micelles with the lateral dimension larger than the transversal one (e.g. bilayer fragments), for rod-like objects of infinite length and constant cross section like tubular micelles, and for globular objects like spherical micelles, respectively; $r$ = 1 is a good approximation also for polydisperse hollow spheres having a constant shell thickness like unilamellar vesicles. It is evident from SANS data in Fig. 3, that bilayers ($r \approx 1$) are present in the DOPC+LDAO mixture up to $n_L$:$n_{DOPC}$ = 1.3 molar ratio, well above the solubilizing $n_{L,S}$:$n_{DOPC}$ molar ratio and that tubular micelles predominate in the DOPC+LDAO mixture between $n_L$:$n_{DOPC}$ = 2.6 and 3.0 molar ratios. Using the quasielastic light scattering, Kragh-Hansen et al. (1998) detected the presence of 470 nm particles toward the end of stage II of the solubilization of DOPC vesicles and suggested that these particles could be large bilayer sheets. Our results support this suggestion. It is also evident that in the stage III of solubilization, the turbidity measurements are not sensitive enough to detect shape transformations of mixed micelles. At higher molar ratios ($n_L$:$n_{DOPC}$ > 3), a transition of tubular mixed micelles into globular mixed micelles occurs (Fig. 3). The comparison of turbidity and SANS data in Fig. 3 suggests the sequence "bilayers in vesicles – bilayer fragments (flat mixed micelles) – tubular mixed micelles – globular mixed micelles" for the solubilization mechanism of DOPC vesicles.

The geometry of surfactant + phospholipid aggregates consisting of two components depends on the effective molecular packing parameter (Israelachvili et al. 1976; Hui and Sen 1989; Goltsov and Barsukov 2000)

$$\delta = X_L V_{HL}/A_L L_{HL} + X_S V_{HS}/A_S L_{HS} \quad (3)$$

where

$$X_S = n_L/(n_L + n_{DOPC}) \quad (4)$$

is the molar fraction of surfactant LDAO in the aggregate of surfactant (LDAO) and phospholipid (DOPC), $X_L = 1 - X_S$ is the molar fraction of phospholipid DOPC in the aggregate, $V_{Hi}$ is the hydrophobic volume, $L_{Hi}$ the effective length of the hydrocarbon chain and $A_i$ the surface area at the interface with the aqueous phase, and the indices $i = S$ denote the surfactant (LDAO) and $i = L$ the phospholipid (DOPC). The theory predicts that molecules with the packing parameter $\delta < 0.3$ will form

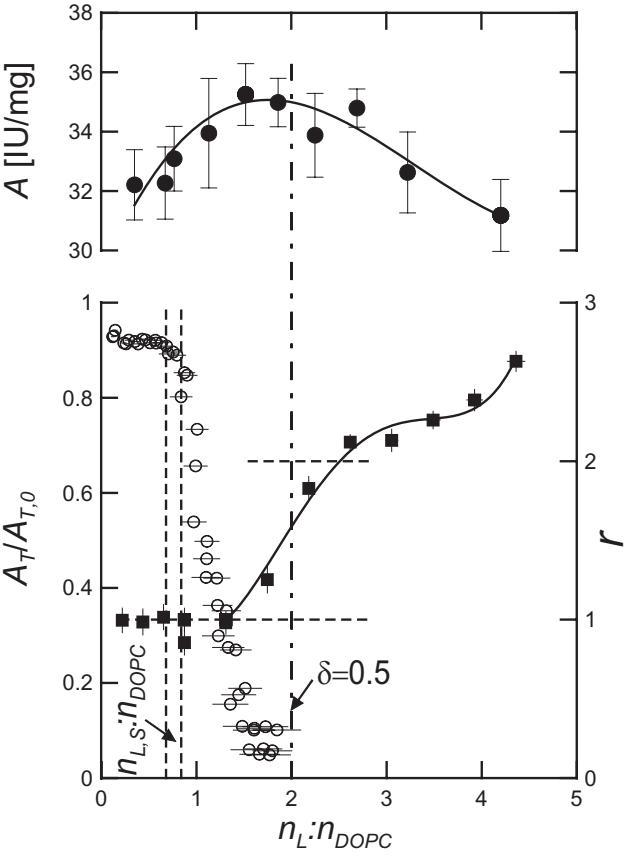


**Figure 3.** Comparison of the normalized turbidity $A_T/A_{T,0}$ (○) at $c_{DOPC}$ = 0.926 mmol/l, 1.235 mmol/l and 1.543 mmol/l and SANS shape parameter $r$ (■) at $c_{DOPC}$ = 12.72 mmol/l of the dispersion of DOPC unilamellar vesicles with LDAO on the molar ratio $n_L$:$n_{DOPC}$ in the lipid phase and the ATPase activity $A$ of SERCA protein reconstituted into DOPC at different $n_L$:$n_{DOPC}$ molar ratios in the lipid phase (●). The activity data were taken from Karlovská et al. (2006) and the $r$ data from Uhríková et al. (2001); the $n_L$:$n_{DOPC}$ cordinates for these data were calculated in the present work using the partition coefficent $K_P$.

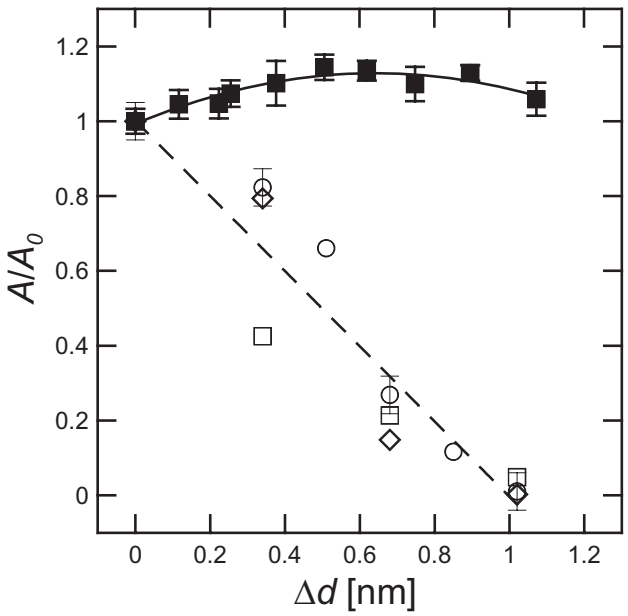


**Figure 4.** Comparison of the normalized ATPase activity of SERCA protein $A/A_0$ on the decrease in lipid bilayer thickness $\Delta d$. The protein was reconstituted into LDAO+DOPC mixtures (■) or into monounsaturated diacylphosphatidylcholine bilayers (◇○□), the activity data were taken from Karlovská et al. (2006) (■□), Lee et al. (1991) (◇) and Johannsson et al. (1981) (○), the bilayer thickness data were taken from Karlovská et al. (2006) (◇○□) and Uhríková et al. (2001, 2005) (■).

spherical micelles, with $0.3 < \delta < 0.5$ cylindrical micelles, with $0.5 < \delta \leq 1$ bilayers, and with $\delta > 1$ inverted micelles. To compare predictions of the theory with experimental data in Fig. 3, the molar ratio LDAO:DOPC = $n_L$:$n_{DOPC}$ in the aggregate corresponding to the transition from bilayers and bilayer sheets ($r = 1$) to cylindrical micelles ($r = 2$), i.e. to the molecular packing parameter $\delta = 0.5$, will be calculated in the following. For this calculation, the values of $V_{HL}$, $A_L$, $L_{HL}$, $V_{HS}$, $A_S$, and $L_{HS}$ are needed. These values can be extracted from published experimental data: Using SANS, Timmins et al. (1988) have found that LDAO forms spherical micelles with radius $R_{M1} = 2.07$ nm and aggregation number (number of LDAO molecules in the micelle) $N_M = 69$ at $t_1 = 20$°C. Since our experiments were performed at $t_2 = 37$°C, we corrected these data for the temperature effect using the isobaric thermal volume expansion coefficient $\alpha = V_M^{-1}(\partial V_M/\partial T)_P$, where $V_M$ is the micelle volume and $T$ the absolute temperature. For micelles and fluid bilayers, its value is in the range: $\alpha \approx 0.0008 \div 0.0010$ K$^{-1}$ (Heerklotz et al. 2004; Uhríková et al. 2007). For spherical LDAO micelles, it follows that their radius $R_{M2}$ at $t_2 = 37$°C should be $R_{M2} = R_{M1}\cdot\exp[\alpha\cdot(t_2 - t_1)]^{1/3} = 2.08$ nm. This gives the effective LDAO surface area $A_S = 4\pi R_{M2}^2/N_M = 0.79$ nm$^2$ at 37°C. Supposing that the hydrophobic volumes of the methylene and methyl groups in the LDAO dodecyl chain at 37°C are the same as in liquid alkanes $V_{CH2} = 0.027$ nm$^3$ and $V_{CH3} = 0.056$ nm$^3$, respectively (Bánó and Balgavý 1996; Koenig and Gawrisch 2005), one calculates $V_{HS} = 11V_{CH2} + V_{CH3} = 0.353$ nm$^3$. Since the methylene and methyl groups of LDAO dodecyl chains fill-in the spherical hydrophobic core of micelles, the effective length of the dodecyl chain can be obtained by using simple formula as $L_{HS} = (3V_{HS}N_M/4\pi)^{1/3} = 1.788$ nm. The molecular volume of DOPC in bilayers at 37°C is $V_{DOPC} = 1.3067$ nm$^3$ and the DOPC headgroup volume, which includes glycerol and carbonyl groups, can be set to $V_H = 0.3239 \div 0.3294$ nm$^3$ (Uhríková et al. 2007). This gives $V_{HL} = V_{DOPC} - V_H = 0.9773 \div 0.9828$ nm$^3$. The DOPC surface area in bilayers at 37°C is $A_L = 0.73$ nm$^2$ (Karlovská et al. 2006). Using the data above, one obtains $L_{HL} = V_{HL}/A_L = 1.339 \div 1.346$ nm. Substituting the values above into Eqs. (3) and (4) and solving for the molar ratio $n_L$:$n_{DOPC}$, one obtains $n_L$:$n_{DOPC} = 1.999 \div 2.000 = 2.00$ for the effective molecular packing parameter $\delta = 0.5$. The vertical dashed-dotted line in Fig. 3 shows this $n_L$:$n_{DOPC}$ value corresponding to $\delta = 0.5$. It is seen that the $n_L$:$n_{DOPC}$ value predicted from the theoretical model for the borderline between bilayers and cylindrical micelles ($\delta = 0.5$) is located in the middle of transition region from bilayers and bilayer sheets ($r = 1$) to cylindrical micelles ($r = 2$) observed in SANS experiments. One can conclude that the experimental SANS $r$ data and the theoretical $\delta$ value for the surfactant (LDAO) and phospholipid (DOPC) are in accord.

We have used the Eq. (2) to calculate the $n_L$:$n_{DOPC}$ molar ratio in the lipid phase of SERCA protein reconstituted into DOPC with LDAO. For this we have used the experimental data published recently by Karlovská et al. (2006). The dependence of the specific SERCA phosphohydrolase activity, presented in Fig. 4 in the paper of Karlovská et al. (2006), on the $n_L$:$n_{DOPC}$ molar ratio calculated in the present work is shown in Fig. 3. It is seen that the decreasing part of the activity curve is observed at $n_L$:$n_{DOPC}$ molar ratios where cylindrical and globular mixed micelles form, in isolation, from DOPC vesicles under influence of LDAO admixtures. This correlation does not necessarily mean that the SERCA protein is located in mixed micelles at these $n_L$:$n_{DOPC}$ molar ratios; it is possible that the lipid surrounding is bilayer-like, but the value of $\delta < 0.5$ indicates a substantial change of the lateral pressure profile resulting in a change of the protein conformation, and thus in the activity decrease of the protein.

From the SANS data obtained with unilamellar vesicles and bilayer sheets ($r = 1$), the bilayer thickness $d$ has been obtained as a function of $c_{LDAO}$ concentration at a fixed $c_{DOPC}$ concentration in the sample (Uhríková et al. 2001,

2005). Using the partition coefficient $K_P$ obtained in the present study and the Eq. (2), the $c_{LDAO}$ and $c_{DOPC}$ values were recalculated into $n_L$:$n_{DOPC}$ values. Since the SERCA phosphohydrolase specific activity $A$ is known as a function of $n_L$:$n_{DOPC}$ molar ratio too (Fig. 3), the activity can be then replotted as a function of bilayer thickness. Fig. 4 shows the normalized activity $A/A_0$ as a function of bilayer thickness decrease $\Delta d$, where $A$ is the activity measured at the bilayer thickness $d$ and $A_0$ the activity measured at the thickness $d_0$, the thickness $d_0$ being the thickness of neat DOPC bilayers and the thickness decrease is defined as $\Delta d = d - d_0$. The plot of $A/A_0$ *vs*. $\Delta d$ is convenient for a comparison of activity data at the same $\Delta d$ but induced by different mechanisms. In Fig. 4, we compare the $A/A_0$ data obtained with the SERCA protein reconstituted into DOPC+LDAO mixtures (Karlovská et al. 2006) with those obtained with the SERCA protein reconstituted into fluid bilayers from diacylphosphatidylcholines with monounsaturated acyl chains (Johannsson et al. 1981; Lee et al. 1991; Karlovská et al. 2006).

It is well known that the ATPase activity of SERCA depends on phase states, hydrocarbon chain lengths, structure and charges of polar head groups of annular lipids surrounding the protein (Lee 1991, 1998, 2002, 2003, 2004): a) the activity is practically zero in the solid-like (gel phase) bilayer and high in the fluid (liquid crystalline) bilayer, but the particular value of fluidity in the fluid state has no effect; b) for high activity, a fluid bilayer from lipids with zwitterionic head groups is required – charged lipids support low activities; c) lower activity is observed in lipids under conditions when they form non-bilayer aggregates in isolation; d) the activity in (zwitterionic) diacylphosphatidylcholines is highest in the fluid bilayer of DOPC, but lower in fluid bilayers with shorter or longer acyl chains. The data in Fig. 4 clearly demonstrate that the decrease in bilayer thickness results in the drastic reduction of ATPase activity when the SERCA protein is reconstituted into fluid diacylphosphatidylcholine bilayers (without any admixtures) with acyl chains shorter than DOPC, however, the equivalent thickness decrease due to LDAO admixtures in DOPC bilayers slightly stimulates the activity. It is possible that the influence of thickness decrease can be compensated in case of LDAO by some other factors, e.g. the lateral bilayer expansion, changes in the conformation and/or hydration of bilayer polar region, bilayer dipole potential, direct interaction of LDAO with SERCA protein, etc. The role of these factors must be studied in future.

In conclusion, from the turbidity studies of unilamellar DOPC vesicle solubilization induced by LDAO, the partition coefficient between aqueous phase and DOPC bilayer was obtained. Using this partition coefficient, the LDAO: DOPC molar ratio in the bilayer was calculated and the effects of LDAO on the bilayer stability, bilayer thickness and ATPase activity of SERCA protein reconstituted into DOPC bilayers could be compared at the same LDAO: DOPC molar ratios in the bilayer. The sequence "bilayers in vesicles – bilayer fragments (flat mixed micelles) – tubular mixed micelles – globular mixed micelles" was suggested for the solubilization mechanism of DOPC vesicles. The ATPase activity decrease due to LDAO is caused by the bilayer deformation resulting in the formation of non-bilayer aggregates in LDAO+DOPC system in isolation. The bilayer thickness decrease induced by LDAO in DOPC does not result in the ATPase activity decrease and this indicates that some other factors must compensate this effect of LDAO.

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## Appendix

In equilibrium, the Nernstian molar partition coefficient $K_P$ of an amphiphile between lipid and aquoeus phase is defined as the ratio of amphiphile molar concentration in the lipid phase ($c_L$) and that in the aqueous phase ($c_W$):

$$K_P = \frac{c_L}{c_W} \tag{A1}$$

In the present paper, $c_L$ is the LDAO molar concentration in DOPC bilayers and $c_W$ the LDAO molar concentration in the aqueous phase. Evidently, the Eq. (A1) can be rewritten as

$$K_P = \frac{n_L \cdot V_W}{n_W \cdot V_L} \tag{A2}$$

where $n_L$ and $n_W$ is the number of LDAO moles in the lipid phase and in the aqueous phase, respectively, and $V_W$ and $V_L$ is the volume of the aqueous phase and the lipid phase, respectively. Since the total amount of LDAO in the sample is

$$n_T = n_L + n_W \tag{A3}$$

the Eq. (A2) can be rearranged as

$$\frac{n_L}{V_L} = n_T \frac{K_P}{V_W + V_L K_P} = \frac{n_T}{V_T} \cdot \frac{K_P}{\frac{V_W}{V_T} + \frac{V_L}{V_T} K_P} \tag{A4}$$

where $n_T/V_T = c_{LDAO}$ is the molar concentration of LDAO in the sample and $V_T = V_L + V_W$ the total volume of the sample. The volume of the lipid phase can be written as

$$V_L = n_{DOPC} N_A V_{DOPC} = c_{DOPC} V_T N_A V_{DOPC} \tag{A5}$$

where $n_{DOPC}$ is the number of moles of DOPC in the sample, $N_A = 6.0221 \times 10^{23}$ mol$^{-1}$ is the Avogadro constant, $V_{DOPC} = 1.3067$ nm$^3$ is the molecular volume of DOPC in vesicles at 37°C calculated from volumetric data of Uhríková et al. (2007), and $n_{DOPC}/V_T = c_{DOPC}$ the molar concentration of DOPC in the sample. For concentrations $c_{DOPC} = 0.7 \div 2.7$ mmol/l used in experiments in the present paper (see Fig. 2), the ratio $V_W/V_T$ is

$$\frac{V_W}{V_T} = 1 - \frac{V_L}{V_T} = 1 - c_{DOPC} N_A V_{DOPC} =$$
$$= 1 - (0.0006 \div 0.0021) \cong 1$$

i.e. the total volume of the sample is within very small error margins equal to the aqueous phase volume. After setting $V_W/V_T = 1$ and substituting $n_T/V_T = c_{LDAO}$, $n_{DOPC}/V_T = c_{DOPC}$, and $V_L = n_{DOPC} N_A V_{DOPC}$, the Eq. (A4) can be rearranged as

$$c_{C12NO} = \frac{n_L}{n_{DOPC}} \cdot \left( \frac{1}{N_A V_{DOPC}} \cdot \frac{1}{K_P} + c_{DOPC} \right) \quad \text{(A6)}$$

According the principle of isoeffective dose (de Paula and Schreier 1995; Šeršeň 1995), the admixture effect occurs in a lipid membrane at a constant concentration of the admixture in the membrane independently of the membrane concentration in the sample. Applying this principle to experiments decribed in the present paper, the bilayer solubilization observed at solubilizing LDAO concentration $c_S$ occurs at a constant solubilizing molar ratio of LDAO located in the lipid phase and DOPC, $n_{L,S}$:$n_{DOPC}$ = const, independently of DOPC concetration $c_{DOPC}$. The Eq. (A6) can be therefore rewritten as the Eq. (1) and used for the estimation of partition coefficient $K_P$ and $n_{L,S}$:$n_{DOPC}$ molar ratio from experimental $c_S$ *vs.* $c_{DOPC}$ data.

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