Detergent solubilization of phospholipid vesicles

Effect of electric charge

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In order to explore the effect of electric charge on detergent solubilization of phospholipid bilayers, the interaction of nine electrically charged surfactants with neutral or electrically charged liposomes has been examined. The detergents belonged to the alkyl pyridinium, alkyl trimethylammonium or alkyl sulphate families. Large unilamellar liposomes formed by egg phosphatidylcholine plus or minus stearylamine or dicetyl phosphate were used. Solubilization was assessed as a decrease in light-scattering of the liposome suspensions. The results suggest that electrostatic forces do not play a significant role in the formation of mixed micelles and that hydrophobic interactions are by far the main forces involved in solubilization. In addition, from the study of thirty different liposome–surfactant systems, we have derived a series of empirical rules that may be useful in predicting the behaviour of untested surfactants: (i) the detergent concentration producing the onset of solubilization ($D_{on.}$) decreases as the alkyl chain length increases; the decrease follows a semi-logarithmic pattern in the case of alkyl pyridinium compounds; (ii) for surfactants with critical micellar concentrations (cmc) $< 6 \times 10^{-3}$ M, $D_{on.}$ is independent of the nature of the detergent and the bilayer composition; for detergents having cmc $> 6 \times 10^{-3}$ M, $D_{on.}$ increases linearly with the cmc; and (iii) $D_{on.}$ varies linearly with the surfactant concentration that produces maximum solubilization.

INTRODUCTION

Surfactants are important tools in membrane research (Helenius & Simons, 1975; Helenius et al., 1979). However, one serious drawback in their use is our virtual ignorance of many aspects of their mechanism of action. An improved knowledge in this field would allow, for example, the prediction of the most appropriate surfactant(s) for the solubilization of a given membrane or membrane protein. Meanwhile, the drastic advice of Racker (1985) still holds: "Try them all"!

Among the efforts to rationalize the use of detergents in membrane research, two main lines of approach may be distinguished. In both, phospholipid vesicles (liposomes) have found extensive application. One approach consists of studying in detail relatively simple three-component systems, such as octyl glucoside/phosphatidylcholine (PC)/water (Jackson et al., 1982), or Triton X-100/PC/water (Goñi et al., 1986; Urbaneja et al., 1988). Alternatively, experimental data from one or various laboratories, using many different surfactants, may be collected and compared, with the aim of inferring some semi-empirical laws. Significant contributions of this kind are, for example, those from Lichtenberg (1985), who postulated that the minimum effective detergent/lipid ratio producing solubilization depends on the surfactant critical micellar concentration (cmc) and hydrocarbon/water partition coefficient rather than on the nature of the detergent, and the studies in which homologous series of surfactants are used, leading to generalizations of the kind epitomized by Traube's Rule (Attwood & Florence, 1983).

As a further step in the unravelling of the mechanisms of bilayer solubilization by detergents, we have attempted to characterize the solubilization of neutral or electrically charged lipid bilayers by electrically charged surfactants. In our case, lipid bilayers consisted of PC large unilamellar vesicles, to which 10 mol % of either dicetyl phosphate (DCP) or stearylamine (SA) was added when required to increase the negative or positive surface charge respectively. The surfactants consisted of three series (of three members each), namely dodecyl, tetradecyl and hexadecyl trimethylammonium bromide (DoTAB, TeTAB and HeTAB respectively); decyl, dodecyl and tetradecyl pyridinium bromide (DePB, DoPB and TePB), and sodium decyl, dodecyl and tetradecyl sulphate (NaDeS, NaDoS and NaTeS respectively). From our results, new and interesting generalizations on the interaction of surfactants with lipid bilayers may be derived.

MATERIALS AND METHODS

DoTAB, TeTAB and HeTAB were purchased from Sigma and used without further purification and tetradecyl pyridinium bromides DePB, DoPB and TePB were synthesized as follows: 0.1 mol of alkyl bromide was dissolved in 1 mol of pyridine; after boiling for 2 h under reflux, the mixture was left at 4 °C overnight, which produced crystallization of the product. Crystals were vacuum-filtered and twice recrystallized from anhydrous methanol/acetone. NaDeS, NaDoS and NaTes were also

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Abbreviations used: cmc, critical micellar concentration; $D_{on.}$, D_{50} and D_{100}, detergent concentrations producing respectively the onset of solubilization, 50% and 100% solubilization; $(D/L)_{on.}$, $(D/L)_{50}$ and $(D/L)_{100}$, detergent/lipid molar ratios producing respectively the onset of solubilization, 50% and 100% solubilization; DePB, DoPB and TePB, decyl, dodecyl and tetradecyl pyridinium bromide respectively; DoTAB, TeTAB and HeTAB, dodecyl, tetradecyl and hexadecyl trimethylammonium bromide respectively; NaDeS, NaDoS and NaTeS, sodium decyl, dodecyl and tetradecyl sulphate respectively; PC, phosphatidylcholine; DCP, dicetyl phosphate; SA, stearylamine; LUV, large unilamellar vesicles.

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obtained from Sigma. Egg PC was grade I from Lipid Products (South Nutfield, Redhill, Surrey, U.K.). DCP and SA were supplied by Sigma. Double-distilled water was used throughout this study.

Large unilamellar vesicles (LUV) were prepared as follows. Lipids were mixed as required in chloroform solution; solvent was evaporated and the dry samples left in a vacuum extractor for at least 2 h before use. Liposomes were formed by addition of buffer (50 mM-Tris/HCl, pH 7.0) and vortex-mixing. LUV about 100 nm in diameter were obtained by the extrusion procedure described by Mayer et al. (1986).

LUV suspensions were adjusted to 1 mg of lipid/ml. To these, equal volumes of the appropriate detergent solutions were added, and the resulting mixtures were left to equilibrate. Preliminary experiments showed that equilibrium is reached in most cases after a few minutes; however, measurements were routinely made after 24 h.

Liposome solubilization was monitored as a decrease in light scattered by the suspension (Goñi et al., 1986). Light-scattering was measured at 90 °C in a Shimadzu RF540 spectrofluorimeter, with both monochromators adjusted at 500 nm. All procedures were carried out at 25 °C, above the critical micellar temperature of the various systems under study. Lipid phosphorus was assayed according to Bartlett (1959).

RESULTS

Solubilization parameters

A typical example of a solubilization curve is shown in Fig. 1; the change in light-scattering of an egg PC liposome suspension is plotted as a function of surfactant (NaDoS) concentration. In order to rationalize the results obtained with the various detergents and bilayer compositions, three parameters have been established, corresponding to the molar detergent concentrations at which: (i) light-scattering starts to decrease, $D_{on.}$; (ii) light-scattering reaches 50 % of the original value, D_{50}; (iii) no further decrease in light-scattering is observed, D_{100}. These parameters are obtained by graphical methods, as shown also in Fig. 1. Note that all our studies have been carried out at a constant lipid concentration (0.5 mg/ml), so that the above parameters may be used for comparative purposes. Sometimes the corresponding detergent/lipid molar ratios $(D/L)_{on.}$, $(D/L)_{50}$ and $(D/L)_{100}$ are used instead.
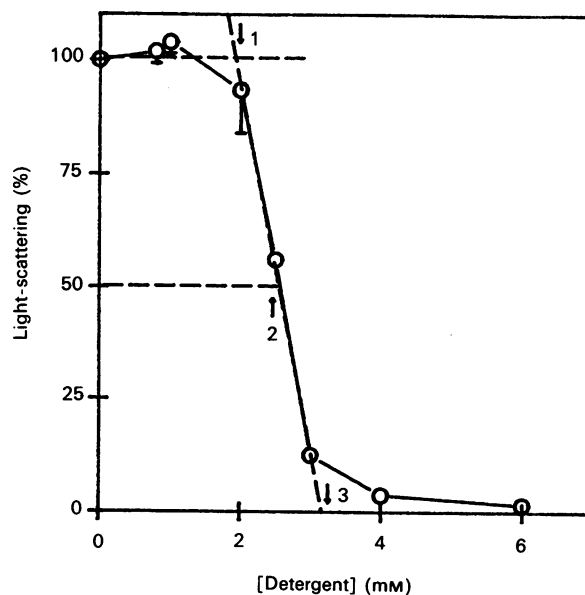

Fig. 1. Percentage change in light-scattering of an egg PC/liposome suspension in the presence of various amounts of NaDoS

The plot is intended to exemplify the graphical methods for estimating $D_{on.}$, D_{50} and D_{100} (see the Results section). The arrows, numbered 1, 2 and 3, correspond respectively to those three parameters.

Effect of electric charge and chain length

The results obtained with the various surfactants and liposome compositions are summarized in Table 1; $(D/L)_{on.}$, $(D/L)_{50}$ and $(D/L)_{100}$ are shown for each case. In addition to the electrically charged detergents, the non-ionic amphiphile Triton X-100 has been included for comparative purposes. The most striking result is the observation that, in the vast majority of cases, solubilization of electrically charged bilayers by electrically charged surfactants is virtually unaffected by the sign of the electric charge, that is, positively or negatively charged bilayers are solubilized at the same detergent concentrations. Three main exceptions are detected, all for cationic detergents: negatively charged bilayers appear to be particularly resistant to solubilization by DoPB and HeTAB; also positively charged liposomes require for

Table 1. Solubilization parameters

Liposomes (1 mg of lipid/ml) were incubated with the appropriate surfactant solutions for 24 h, and the change in light-scattering of the resulting suspensions was recorded. The solubilization parameters were derived from the light-scattering-versus-detergent-concentration plots shown in Fig. 1. Errors were less than 5 % of the tabulated values. Cmc values are mostly taken from Mukerjee & Mysels (1971). See the Materials and methods section for additional details.

| Detergent	Cmc (M)	Egg PC			Egg PC + SA			Egg PC + DCP		
		$(D/L)_{on.}$	$(D/L)_{50}$	$(D/L)_{100}$	$(D/L)_{on.}$	$(D/L)_{50}$	$(D/L)_{100}$	$(D/L)_{on.}$	$(D/L)_{50}$	$(D/L)_{100}$
Triton X-100	3.5×10^{-4}	1.4	2.2	4.0	1.4	2.2	3.0	1.6	2.2	4.0
DePB	4.2×10^{-2}	29.5	34.1	40.0	30.9	37.0	40.0	32.4	36.3	40.0
DoPB	1.1×10^{-2}	6.0	6.9	8.0	6.0	7.4	9.0	12.1	14.0	16.0
TePB	3.0×10^{-3}	1.4	2.0	2.5	1.8	2.3	3.4	2.2	3.0	5.0
DoTAB	1.4×10^{-2}	14.9	17.2	20.0	15.2	17.4	20.0	15.5	19.5	24.0
TeTAB	3.5×10^{-3}	1.9	3.1	5.0	10.5	13.8	18.0	2.0	3.3	5.0
HeTAB	8.7×10^{-4}	1.7	2.7	4.0	1.5	2.5	4.0	2.3	8.7	40.0
NaDeS	3.3×10^{-2}	30.0	36.0	50.0	27.4	34.6	50.0	22.4	33.2	40.0
NaDoS	8.5×10^{-2}	3.9	4.9	6.0	3.9	5.4	8.0	3.9	5.4	8.0
NaTeS	2.1×10^{-3}	2.0	3.6	6.0	2.0	3.0	6.0	1.7	3.0	4.0
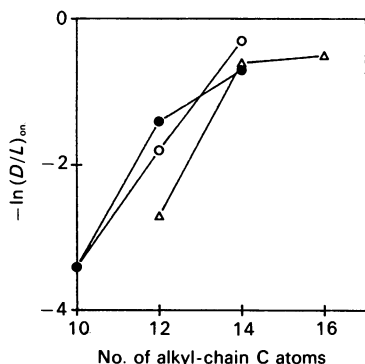
Fig. 2. Detergent/lipid molar ratio at the onset of solubilization, $(D/L)_{on.}$, as a function of surfactant alkyl chain length

O, Alkyl pyridinium bromides; △, alkyl trimethylammonium bromides; ●, sodium alkyl sulphates.

solubilization higher TeTAB concentrations than their neutral or negatively charged counterparts.

The data in Table 1 also reveal a gradual change of the solubilizing properties of the detergents as a function of alkyl chain length. These alkyl-chain-length-dependent effects are depicted in Fig. 2 for pure EYL bilayers, so that they are not obscured by charge effects. A linear dependence of $\ln(D/L)_{on.}$ on chain length is observed for the alkyl pyridinium detergents, but not for the other two families. Essentially similar results are obtained for bilayers containing SA or DCP (results not shown), thus confirming the small influence of electrostatic factors on solubilization.

Intra-surfactant relationships

As a further step in the search for regularities in the behaviour of the surfactants under study, we considered the possible relationships among the parameters described above, or between them and other detergent properties, for a given detergent. Two of these relationships appear to be particularly relevant, one of them being shown by the linear plot of $\ln D_{100}$ against $\ln D_{on.}$ (Fig. 3).

All detergent–liposome systems tested (30 in all) appear to follow this linear relationship, whose equation is:

$$\ln D_{100} = -0.5 + 0.81 \ln D_{on.}$$

and allows the estimation of the surfactant concentration producing 100% solubilization from data on the start of solubilization and vice versa. Another important relationship is shown in Fig. 4, which shows a plot of $\ln D_{on.}$ against $\ln$ cmc. Two regions may be distinguished in this plot, above and below $\ln$ cmc ≈ -5. Below this point, i.e. for surfactants having cmc $< 6 \times 10^{-3}$ M, $\ln D_{on.}$ is independent of the nature of the detergent and the bilayer composition; in our case (final lipid concentration $= 0.5$ mg/ml) solubilization starts in all cases at about 5×10^{-4} M-surfactant. For detergents having cmc $> 6 \times 10^{-3}$ M, the concentration at which solubilization starts does vary with the cmc, the empirical equation being in this case:

$$\ln D_{on.} = 0.4 + 1.4 \ln \text{cmc}$$

Just one surfactant, TeTAB, fails to obey the above rules. Again this plot is of practical importance, since cmc is a frequently tabulated parameter, and may be used, according to Fig. 4, to predict the surfactant concentrations above which solubilization will occur.
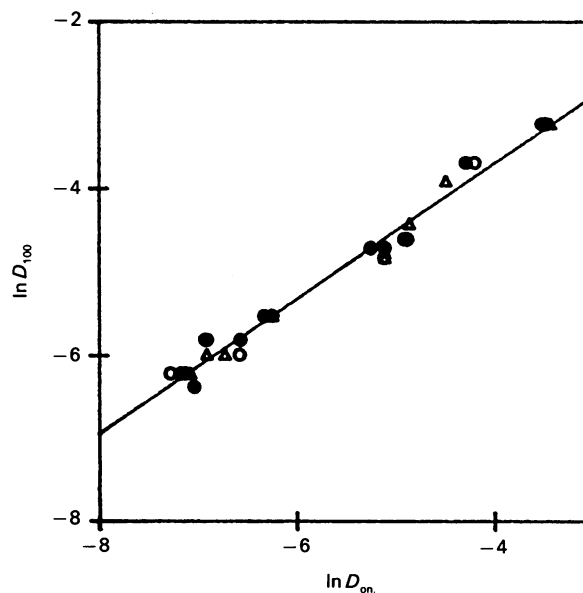

Fig. 3. Detergent concentration producing maximum solubilization, D_{100}, as a function of the detergent concentration at the onset of solubilization, $D_{on.}$
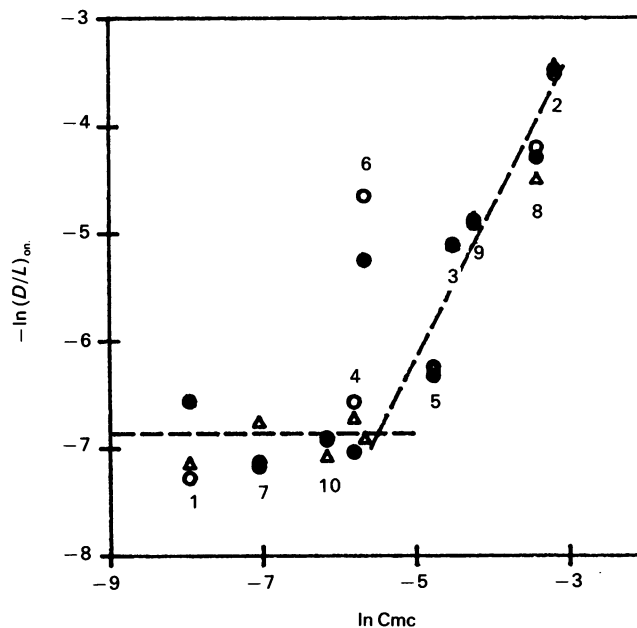
O, PC; ●, PC/SA; △, PC/DCP.

Fig. 4. Detergent/lipid molar ratio at the onset of solubilization $(D/L)_{on.}$, as a function of the cmc of the detergent

1, Triton X-100; 2, DePB; 3, DoPB; 4, TePB; 5, DoTAB; 6, TeTAB; 7, HeTAB; 8, SDeS; 9, SDoS; 10, STeS.

DISCUSSION

The experiments described above provide useful information on two important points of bilayer–surfactant interaction, namely the nature of the main forces involved and the possibility of predicting some aspects of detergent behaviour. Each of these two problems will be separately discussed now.

Forces involved in bilayer solubilization

It is now well established that membrane–surfactant interaction consists, initially, of the incorporation of detergent monomers into the bilayer; eventually, a local surfactant con-

centration is reached that allows bilayer solubilization, i.e. its conversion into lipid/detergent mixed micelles (Helenius & Simons, 1975; Lichtenberg et al., 1983; Goñi et al., 1986). Inoue et al. (1986) have studied, by differential scanning calorimetry, the incorporation of surfactant molecules, either charged or neutral, into phospholipid bilayers. Their results convincingly demonstrate that the main forces involved are hydrophobic in nature.

Our experimental observations deal primarily with the stage of bilayer solubilization, or micellization. Tanford (1980) describes the "principle of opposing forces", according to which micellization would occur as the combined result of (i) the hydrophobic effect, responsible for the self-association of amphiphiles into micellar aggregates, and (ii) repulsive forces, coming primarily from the head group, which would impose a limit on micelle size, thus preventing separation of the amphiphile into an entirely separate phase. More detailed treatments of the repulsive forces involved are also available (Israelachvili et al., 1980; Cevc & Marsh, 1987). The above principle applies to pure as well as mixed micelles. For the latter case, Tanford (1980) points out that the main condition for micelle formation would be that the interaction between head groups be repulsive. Given the above rules, one would expect some difference in $(D/L)_{on.}$ and/or $(D/L)_{100}$ for the solubilization of positively or negatively charged bilayers by any electrically charged surfactant. However, in most cases, no difference is observed (Table 1). This raises questions about the nature of the repulsive forces; for example, our results would not agree with forces of an essentially electrostatic nature, and confirm the role of the hydrophobic effect in micelle formation. N.m.r. data from various laboratories (Beyer, 1986; Scherer & Seelig, 1989) indicate that ionic detergents produce very large perturbations of the phosphocholine head group, whereas the hydrophobic region of the membrane is almost unaffected. This would appear to be at odds with our conclusion of the essential role of the hydrophobic effect in bilayer solubilization, were it not for the classical observation of Hartley (1936), that hydrophobic forces do not rely on any strong attraction of paraffin chains for one another, but rather on "a very strong attraction of water molecules for one another in comparison with which the paraffin–paraffin or paraffin–water attractions are very slight". In turn, the observed deviations from the general rule, pointed out in the Results section, might be attributed to peculiarities of specific surfactants, e.g. certain molecular geometries could either facilitate or hinder micelle formation, thus modifying the entropic component of the process. Factors such as hydrophobic or electrostatic interactions may hardly be invoked to explain those anomalies, since surfactant molecules with similar polar head groups or hydrocarbon tails do conform to the general behaviour. However, it is clear that a positive explanation to the observed anomalies requires further experimentation.

Predicting detergent properties

Another important conclusion of our work is that, after studying an extended number of liposome–detergent systems, some regularities have been observed (Figs. 2–4) that would allow the prediction of a number of surfactant properties. For instance, Fig. 2 reflects the changes in detergent/lipid ratio producing the onset of solubilization $(L/D)_{on.}$ as a function of surfactant alkyl chain length. Isomaa (1979) published similar data for the haemolytic effects of alkyl trimethylammonium salts, and the effect of alkyl chain length was also examined for membrane intermixing catalysed by alkylamines in acidic liposomes (Eytan et al., 1984). In our case (Fig. 2), the semi-

logarithmic presentation is intended to facilitate comparison with the 'ln cmc versus no. of alkyl-chain C atoms' plots often used for two-component (surfactant + water) systems (Tanford, 1980). Those are usually straight lines; in particular, linearity has been shown for alkyl trimethylammonium bromides (Geer et al., 1971) and alkyl sulphates (Evans, 1956). The fact that linearity is lost, at least for these two families, when $\ln(D/L)_{on.}$ is represented instead of ln cmc, may reflect differences in the thermodynamics of micelle formation when more than one amphiphile is involved. [ln Cmc is related to the free energy of micelle formation, and $\ln(D/L)_{on.}$ could, by analogy, have a similar meaning in the case of mixed micelles.]

The relationships shown in Figs. 3 and 4 are empirical in origin, and their predictive properties will have to be tested in the light of measurements from the various laboratories. In recent years, Lichtenberg and his co-workers (Lichtenberg et al., 1983; Lichtenberg, 1985) have advocated the use of such generalizations; in their review (Lichtenberg et al., 1983), they express the necessity of experimental data correlating the cmc of a detergent with its solubilizing power, as we have done in Fig. 4. In general, we have used 'total' (bilayer + solution) rather than 'effective' (in the bilayer) detergent concentrations for the sake of simplicity; this should not influence the comparison of various detergents acting on similar lipid systems, as in our case.

REFERENCES

Attwood, D. & Florence, A. T. (1983) Surfactant Systems, Chapman and Hall, London

Bartlett, G. R. (1959) J. Biol. Chem. 234, 466–568

Beyer, K. (1986) Biochim. Biophys. Acta 855, 365–374

Cevc, G. & Marsh, D. (1987) Phospholipid Bilayers, John Wiley and Sons, New York

Evans, H. C. (1956) J. Chem. Soc. 579–585

Eytan, G. D., Mary, T., Broza, R. & Shaltin, Y. (1984) Biochim. Biophys. Acta 778, 38–48

Geer, R. D., Eylar, E. H. & Anacker, E. W. (1971) J. Phys. Chem. 75, 369–373

Goñi, F. M., Urbaneja, M. A., Arrondo, J. L. R., Alonso, A., Durrani, A. A. & Chapman, D. (1986) Eur. J. Biochem. 160, 659–665

Hartley, G. S. (1936) Aqueous Solutions of Paraffin-Chain Salts, Hermann, Paris

Helenius, A. & Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79

Helenius, A., McCaslin, D. R., Fries, E. & Tanford, C. (1979) Methods Enzymol. 56, 734–749

Inoue, T., Miyakawa, K. & Shimozawa, R. (1986) Chem. Phys. Lipids 42, 261–270

Isomaa, B. (1979) Biochem. Pharmacol. 28, 975–980

Israelachvili, J. N., Marcelja, S. & Horn, R. G. (1980) Q. Rev. Biophys. 13, 121–200

Jackson, M. L., Schmidt, C. F., Lichtenberg, D., Litman, B. J. & Albert, A. D. (1985) Biochemistry 21, 4576–4582

Lichtenberg, D. (1985) Biochim. Biophys. Acta 821, 470–478

Lichtenberg, D., Robson, R. J. & Dennis, E. A. (1983) Biochim. Biophys. Acta 737, 285–304

Mayer, L. D., Hope, M. J. & Cullis, P. R. (1986) Biochim. Biophys. Acta 858, 161–168

Mukerjee, P. & Mysels, K. J. (1971) Critical Micelle Concentrations of Aqueous Surfactant Systems, NSRDS–NBS 36, U.S. Government Printing Office, Washington, DC

Racker, E. (1985) Reconstitution of Transporters, Receptors and Pathological States, Academic Press, Orlando

Scherer, P. G. & Seelig, J. (1989) Biochemistry 28, 7720–7728

Tanford, C. (1980) The Hydrophobic Effect, 2nd edn., Wiley-Interscience, New York

Urbaneja, M. A., Goñi, F. M. & Alonso, A. (1988) Eur. J. Biochem. 173, 585–588