Solubilizing Effects Caused by the Nonionic Surfactant Dodecylmaltoside in Phosphatidylcholine Liposomes

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ABSTRACT The interaction of the nonionic surfactant dodecylmaltoside (DM) with phosphatidylcholine liposomes was investigated. Permeability alterations were detected as a change in 5(6)-carboxyfluorescein released from the interior of vesicles and bilayer solubilization as a decrease in the static light scattered by liposome suspensions. This surfactant showed higher capacity to saturate and solubilize PC liposomes and greater affinity with these structures than those reported for the octyl glucoside. At subsolubilizing level an initial maximum in the bilayer/water partitioning (*K*) followed by an abrupt decrease of this parameter occurred as the effective molar ratio of surfactant to phospholipid in bilayers (*Re*) rose. However, at solubilizing level a direct dependence was established between both parameters. A direct correlation took place in the initial interaction steps (*Re* up to 0.28) between the growth of vesicles, their fluidity, and *Re*. A similar direct dependence was established during solubilization (*Re* range from 0.9 to 1.7) between the decrease in both the surfactant-PC aggregate size, the light scattering of the system, and *Re* (composition of aggregates). The fact that the free DM concentration at subsolubilizing and solubilizing levels showed values lower than and similar to its critical micelle concentration indicates that permeability alterations and solubilization were determined, respectively, by the action of surfactant monomer and by the formation of mixed micelles.

INTRODUCTION

Surfactants are indispensable reagents in the solubilization and reconstitution of membrane proteins (Lummis and Martin, 1992; Kerry et al., 1993; Wach et al., 1993). The need to find effective and predictable means to solubilize and reconstitute these membranes, and to scale the reconstitution protocols for biological research or pharmacological applications, is one reason for interest in the nuances of membrane-surfactant interactions. A number of studies have been devoted to the understanding of the principles governing the interaction of surfactants with simplified membrane models as phospholipid bilayers (Almog et al., 1990; Levy et al., 1990; Urbaneja et al., 1990; Partearroyo et al., 1996). This interaction leads to the breakdown of lamellar structures and the formation of lipid-surfactant mixed micelles. A significant contribution in this area has been made by Lichtenberg (Lichtenberg, 1985), who postulated that the critical effective surfactant/lipid molar ratio (*Re*) producing saturation and solubilization of liposomes depends on the surfactant critical micellar concentration (CMC) and on the bilayer/aqueous medium distribution coefficients (*K*) rather than on the nature of the surfactants.

The nonionic surfactant dodecylmaltoside (DM) has been used for solubilization of cytochrome oxidase in active form (Suarez et al., 1984; Bolli et al., 1985) and has been found to have promising properties for solubilization of diverse membrane proteins (Foresta et al., 1992; le Marie et al., 1992; Kragh-Hansen et al., 1993). However, the mechanisms of vesicle-micelle structural transitions involved in its interaction with phospholipid bilayers are far from understood, since a detailed description of the process has yet to be given.

In recent papers, we studied the phase transitions involved in the interaction of the nonionic surfactants Triton X-100 and octyl glucoside (OG) with phosphatidylcholine (PC) unilamellar liposomes (de la Maza and Parra 1994a; 1994b). In the present work we seek to extend these investigations by characterizing the overall interaction of the nonionic surfactant dodecylmaltoside with PC liposomes. Knowledge of the effective surfactant/phospholipid molar ratios and the partition coefficients of this surfactant between lipid bilayers and the aqueous phase could be useful in improving our understanding of the complex phenomenon involved in the lamellar to micelle transitions during the solubilization of PC liposome suspensions by this amphiphilic compound and in establishing a criterion for the evaluation of its activity in biological membranes.

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Abbreviations used: DM, dodecylmaltoside; CF, 5(6)-carboxyfluorescein; CMC, critical micellar concentration; *K*, bilayer/aqueous phase surfactant partition coefficient; K_{SAT}, bilayer/aqueous phase surfactant partition coefficient for bilayer saturation; K_{SOL}, bilayer/aqueous phase surfactant partition coefficient for bilayer solubilization; PC, phosphatidylcholine; PI, polydispersity index; PIPES, piperazine-1,4 bis(2-ethanesulfonic acid); r^2, regression coefficient; *Re*, effective surfactant/lipid molar ratio; Re_{SAT}, effective surfactant/lipid molar ratio for bilayer saturation; Re_{SOL}, effective surfactant/lipid molar ratio for bilayer solubilization; S_B, surfactant concentration in the bilayers; S_W, surfactant concentration in the aqueous medium; SLS, static light scattering; TLC-FID, thin-layer chromatography/flame ionization detection system.

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EXPERIMENTAL

Materials

Phosphatidylcholine (PC) was purified from egg lecithin (Merck, Darmstadt, Germany) according to the method of Singleton (Singleton et al., 1965) and was shown to be pure by thin-layer chromatography (TLC). The nonionic surfactant dodecylmaltoside (DM), (*n*-dodecyl β-D-maltoside), was purchased from Sigma Chemicals Co. Piperazine-1,4 bis(2-ethanesulfonic acid) (PIPES buffer) obtained from Merck was prepared as 20 mM PIPES adjusted to pH 7.20 with NaOH, containing 110 mM Na_2SO_4. The starting material, 5(6)-carboxyfluorescein (CF), was obtained from Eastman Kodak (Rochester, NY) and further purified by a column chromatographic method (Weinstein et al., 1986).

Methods

Unilamellar liposomes of a defined size (~200 nm) and PC concentration ranging from 0.5 to 5.0 mM were prepared by extrusion of large unilamellar vesicles previously obtained by reverse phase evaporation (de la Maza and Parra, 1994b). A lipidic film was formed by removing the organic solvent by rotatory evaporation from a chloroform solution of PC. The lipid was then redissolved in diethyl ether, and PIPES buffer (supplemented with 110 mM CF when studied liposome permeability changes) was added to the solution of phospholipid. Gentle sonication led to the formation of a W/O-type emulsion. After evaporation of the ethyl ether under reduced pressure a viscous gel was formed. Elimination of the final traces of the organic solvent at high vacuum transformed the gel into a liposome suspension in which no traces of ether were detectable by NMR (Allen, 1986). Unilamellar vesicles were obtained by extrusion of vesicle suspensions through 800-200 nm polycarbonate membranes to achieve a uniform size distribution (Mayer et al., 1986).

To study the bilayer permeability changes, vesicles containing CF were freed of unencapsulated fluorescent dye by passage through Sephadex G-50 medium resin (Pharmacia, Uppsala, Sweden) by column chromatography. The PC concentration in liposomes was determined using thin-layer chromatography (TLC) coupled to an automated flame ionization detection (FID) system (Iatroscan MK-5, Iatron Lab. Inc., Tokyo, Japan) (Ackman et al., 1990).

The size distribution and the polydispersity index (PI) of liposomes and surfactant-PC aggregates resulting in the interaction investigated were determined with a photon correlator spectrometer (Malvern Autosizer 4700c PS/MV) at 25°C at a reading angle of 90° (de la Maza and Parra, 1996).

The internal volume (volume enclosed by a given amount of lipid, expressed in ml/mmol), and the encapsulation efficiency (fraction of the aqueous compartment sequestered by bilayers, expressed in percent with respect to the original volume), of liposome suspensions were determined by measuring the concentration of CF encapsulated into liposomes after chromatographic separation of unencapsulated material (Sephadex G-50 medium resin) and subsequent destruction of liposomes by addition of 10% (v/v) Triton X-100 aqueous solution (Deamer and Uster, 1983; Weinstein et al., 1986).

The surface tensions of buffered solutions containing increasing concentrations of DM were measured by the ring method using a Krüss tensiometer. The surfactant critical micelle concentration (CMC) was determined from the abrupt change in the slope of the surface tension values versus surfactant concentration (Lunkenheimer and Wantke, 1981), showing a value of 0.125 mM.

Solubilizing parameters

In the analysis of the equilibrium partition model proposed by Schurtenberger (Schurtenberger et al., 1985) for bile salt/lecithin systems, Lichtenberg (Lichtenberg, 1985) and Almog et al. (Almog et al., 1990) have shown that for a mixing of lipids [at a concentration PL (mM)] and surfactant [at a concentration S_T (mM)], in dilute aqueous media, the distribution of surfactant between lipid bilayers and aqueous media obeys a partition coefficient K, given (in mM^{-1}) by:

$$K = S_B/[(PL + S_B)S_W] \quad (1)$$

where S_B is the concentration of surfactant in the bilayers (mM) and S_W is the surfactant concentration in the aqueous medium (mM). For $PL \gg S_B$, the definition of K, as given by Schurtenberger, applies:

$$K = S_B/(PL \cdot S_W) = Re/S_W \quad (2)$$

where Re is the effective molar ratio of surfactant to phospholipid in the bilayer: ($Re = S_B/PL$). Under any other conditions, Eq. 1 has to be employed to define K; this yields:

$$K = Re/[S_W(1 + Re)] \quad (3)$$

This approach is consistent with the experimental data offered by Lichtenberg (Lichtenberg, 1985) and Almog et al. (Almog et al., 1990) for different surfactant phospholipid mixtures over wide ranges of Re values. Given that the range of phospholipid concentrations used in our investigation is similar to that used by Almog to test his equilibrium partition model, the K parameter has been determined using this equation. The determination of these parameters can be carried out on the basis of the linear dependence existing between the surfactant concentrations required to achieve these parameters and the phospholipid concentration in liposomes, which can be described by the equation:

$$S_T = S_W + Re \cdot PL \quad (4)$$

where Re and the aqueous surfactant concentration (S_W) are in each curve, respectively, the slope and the ordinate at the origin (zero phospholipid concentration).

Permeability alterations and solubilization of liposomes

The permeability alterations caused by DM in CF-containing liposomes (PC concentration ranging from 0.5 to 5.0 mM) were determined by monitoring the increase in the fluorescence intensity of the liposome suspensions due to the CF released from the interior of vesicles to the bulk aqueous phase (Weinstein et al., 1986; de la Maza and Parra, 1994b). Fluorescence measurements were made with a Shimadzu RF-540 spectrofluorophotometer. On excitation at 495 nm, a fluorescence maximum emission of CF was obtained at 515.4 nm. The fluorescence intensity measurements were taken at 25°C. The percentage of CF released was calculated by means of the equation:

$$\%\ \text{CF release} = (I_T - I_0)/(I_\infty - I_0) \cdot 100 \quad (5)$$

where I_0 is the initial fluorescence intensity of CF-loaded liposomes in the absence of DM and I_T is the fluorescence intensity measured 40 min after adding equal volumes of appropriate surfactant solutions to liposome suspensions (PC conc. 1.0–10.0 mM). I_∞ corresponds to the fluorescence intensity resulting after the complete destruction of liposomes by the addition of Triton X-100 aqueous solution (Weinstein et al., 1986).

With regard to liposome solubilization, it has been previously demonstrated that static light scattering (SLS) constituted a very convenient technique for the quantitative study of the bilayer solubilization by surfactants (Urbaneja et al., 1990; Ruiz et al., 1994; Partearroyo et al., 1996). Accordingly, the solubilizing perturbation produced by DM in PC liposomes was monitored using this technique. The overall solubilization can be mainly characterized by two parameters termed Re_{SAT} and Re_{SOL}, according to the nomenclature adopted by Lichtenberg (Lichtenberg, 1985) corresponding to the Re ratios at which static light scattering starts to decrease with respect to the initial value and shows no further decrease. These parameters corresponded to the DM/PC molar ratios at which the surfactant: 1) saturated liposomes and 2) led to a complete solubilization of these structures.

Liposomes were adjusted to the appropriate PC concentration (from 1.0 to 10.0 mM). Equal volumes of the appropriate surfactant solutions were added to these liposomes and the resulting mixtures were left to equilibrate for 24 h. This time was chosen as the optimum period needed to achieve a complete equilibrium surfactant/liposome for the lipid concentration range used. Light-scattering measurements were made using the spectrofluorophotometer Shimadzu RF-540 at 25°C with both monochromators adjusted to 500 nm. The assays were carried out in triplicate and the results given are the average of those obtained (de la Maza and Parra, 1994b).

RESULTS AND DISCUSSION

Characterization and stability of liposome suspensions

The mean vesicle size of liposome suspensions after preparation varied little (~200 nm). The internal volume of these vesicles was ~6.2 ml/mmol. The percentage of CF encapsulated into liposomes [encapsulation efficiency (%), directly proportional to PC conc.] showed values ranging from 0.31% to 3.10% (PC concentration ranging from 0.5 to 5.0 mM). The polydispersity index (PI), defined as a measure of the width of the particle size distribution obtained from the "cumulants analysis" (based on the distribution of cumulative frequencies) remained in all cases <0.1, indicating that the liposome suspensions showed a homogeneous size distribution in all cases. The size of vesicles after the addition of equal volumes of PIPES buffer and equilibration for 24 h showed in all cases values similar to those obtained after preparation, with a slight increase in the PI (between 0.12 and 0.14). Hence, the liposomes appeared to be reasonably stable in the absence of DM under the experimental conditions used in solubilization studies.

Interaction of DM with liposomes

To determine the time needed to obtain a constant level of CF release of liposomes in the range of PC concentration investigated (0.5 and 5.0 mM), a kinetic study of the interaction of DM with liposomes was carried out. Liposome suspensions were treated with this surfactant at subsolubilizing concentrations (affecting only the permeability of bilayers) and subsequent changes in permeability were studied as a function of time. The CF release was in all cases a biphasic process in which ~40 min was needed to achieve CF release plateaus and ~80% of CF release took place during the initial 10 min. This biphasic behavior suggests that the release of the fluorescent dye encapsulated into the vesicles was produced through holes, or channels, created in the membrane and not to bilayer fusion, in agreement with the concept of transient channels suggested by Edwards et al. in the surfactant-mediated increase in phospholipid membrane permeability for different nonionic and anionic surfactants (Edwards and Almgren, 1990; 1992; Silvander et al., 1996). The incorporation of surfactant monomers to membranes may directly induce the formation of hydrophilic pores in these structures or merely stabilize transient holes. The long time course required to reach a constant CF release could also be related to permeation or flip-flop of surfactant across the vesicle membrane. A similar biphasic behavior has been reported for the interaction of Triton X-100 and OG with PC liposomes (de la Maza and Parra, 1994a,b). Bearing in mind these findings, changes in permeability were studied 40 min after addition of surfactant to the liposomes at 25°C. The CF release in liposome suspensions in the absence of DM in this period of time was negligible.

To determine K at subsolubilizing level a systematic study of permeability changes caused by the addition of DM to liposomes was carried out for different PC concentration. The results obtained are plotted in Fig. 1 *A*. The DM concentrations resulting in different percentages of CF release were graphically obtained and plotted versus the PC concentration (Fig. 1 *B*). An acceptable linear relationship was established in each case. The straight lines obtained

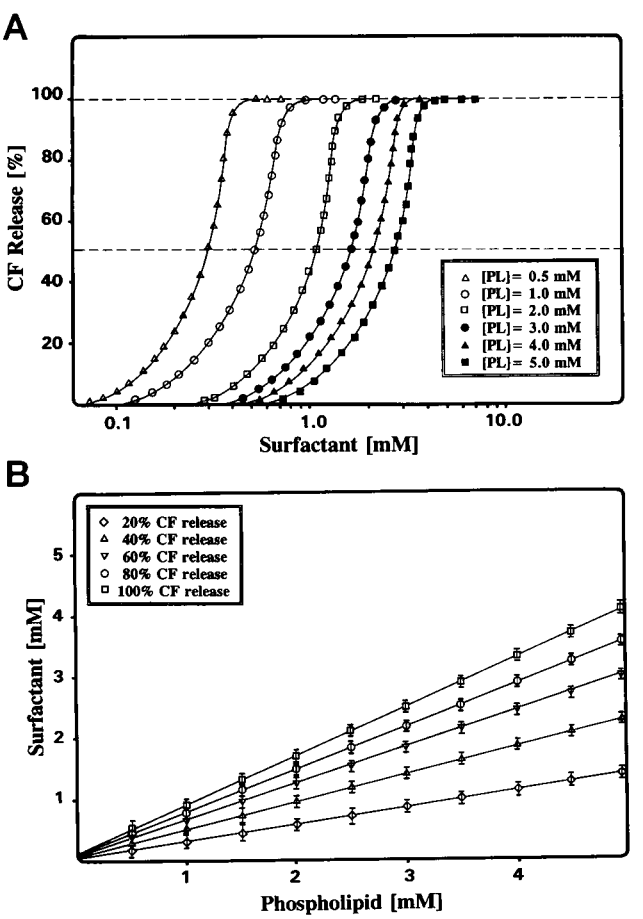

FIGURE 1 (*A*) Percentage change in CF release from unilamellar liposomes, (lipid concentration ranging from 0.5 to 5.0 mM), induced by the presence of increasing concentrations of dodecyl maltoside. [PL] = 0.5 mM (△); [PL] = 1.0 mM (○); [PL] = 2.0 mM (□); [PL] = 3.0 mM (●); [PL] = 4.0 mM (▲); [PL] = 5.0 mM (■). (*B*) Surfactant concentrations resulting in different percentages of CF release versus phospholipid concentration in liposome suspensions. Percentages of CF release: 20% (◇); 40% (△); 60% (▽); 80% (○); 100% (□).

correspond to Eq. 4 from which the *Re* and *K* parameters were determined. The results obtained including the free surfactant concentration (S_W) and the regression coefficient of each straight line (r^2) are given in Table 1.

Different trends in the evolution of *Re* and *K* were observed as the percentage of CF rose. Thus, whereas *Re* progressively increased, the *K* values showed a maximum for 20% CF release followed by an abrupt decrease up to the complete release of the encapsulated dye. Furthermore, S_W increased as the percentage of CF rose. Given that the CMC experimentally obtained for DM was 0.125 mM, S_W showed at subsolubilizing level always lower values than the surfactant CMC (0.094 mM for 100% CF release), thereby confirming the generally admitted assumption that permeability alterations were determined by the action of surfactant monomers (Lichtenberg, 1985).

The solubilizing interaction of DM with liposomes was studied through the changes in the static light scattered by these systems 24 h after the addition of surfactant (Urbaneja et al., 1990; Ruiz et al., 1994; Partearroyo et al., 1996). Fig. 2 *A* shows the solubilization curves of liposomes (PC concentration ranging from 0.5 mM to 5.0 mM). An initial increase in the scattered intensity of the system was always observed due to the surfactant incorporation into bilayers. Additional surfactant amounts resulted in a fall in this intensity until a low constant value for bilayer solubilization. A similar behavior has been reported by different authors when studying the solubilizing interactions of various surfactants with neutral or electrically charged PC liposomes (Urbaneja et al., 1990; Ruiz et al., 1994; Partearroyo et al., 1996; Silvander et al., 1996). The surfactant concentrations for different static light scattering percentages were also graphically obtained and plotted versus PC concentration (Fig. 2 *B*). An acceptable linear relationship was also established in each case. The corresponding *Re* and *K* parameters were determined from these straight lines (Eq. 4) and are also given together with their regression coefficients (r^2) in Table 1.

It should be noted that both the *Re* and *K* parameters increased as the percentage in static light scattering decreased. It may also be assumed that an increasing degree of partitioning of surfactant molecules into the liposomes governs their association with the PC building these structures to form mixed micelles. The fact that S_W at solubilizing level was always comparable to the DM CMC (0.125 mM)

TABLE 1 Surfactant to phospholipid molar ratios (*Re*), partition coefficients (*K*), and surfactant concentrations in the aqueous medium (S_W) resulting in the overall interaction of dodecylmaltoside with PC liposomes. The regression coefficients of the straight lines obtained are also included.

	S_W [mM]	*Re* mol/mol	r^2	*K* [mM^{-1}]
CF release (%)				
10	0.009	0.14	0.997	13.72
20	0.015	0.28	0.993	14.58
30	0.025	0.37	0.998	10.81
40	0.034	0.455	0.994	9.21
50	0.044	0.53	0.992	7.87
60	0.053	0.60	0.997	7.07
70	0.062	0.65	0.995	6.35
80	0.073	0.70	0.991	5.64
90	0.084	0.755	0.996	5.12
100	0.094	0.80	0.998	4.72
Light-Scattering (%)				
100	0.123	0.90	0.998	3.85
90	0.123	1.03	0.996	4.12
80	0.124	1.16	0.994	4.33
70	0.124	1.29	0.995	4.54
60	0.125	1.42	0.999	4.69
50	0.125	1.55	0.994	4.86
40	0.126	1.68	0.994	4.97
30	0.126	1.82	0.997	5.11
20	0.126	1.94	0.993	5.23
10	0.127	2.07	0.995	5.31
0	0.127	2.23	0.993	5.43

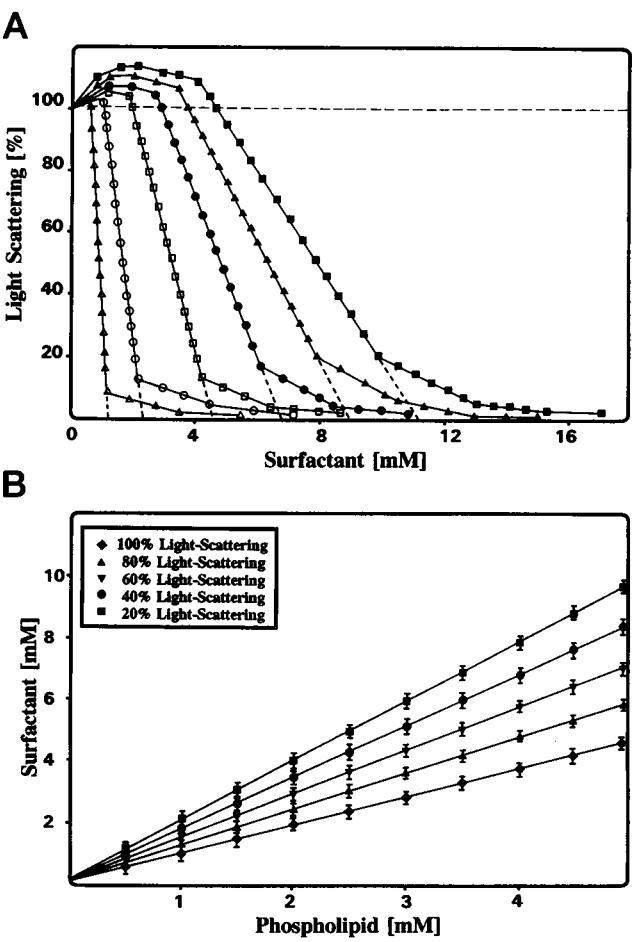

FIGURE 2 (*A*) Percentage change in static light scattering of unilamellar liposomes, (lipid concentration ranging between 0.5 and 5.0 mM), induced by the presence of increasing concentrations of dodecyl maltoside. [PL] = 0.5 mM (△); [PL] = 1.0 mM (○); [PL] = 2.0 mM (□); [PL] = 3.0 mM (●); [PL] = 4.0 mM (▲); [PL] = 5.0 mM (■). (*B*) Surfactant concentrations resulting in different percentages of static light scattering versus the phospholipid concentration of liposome suspensions. Percentages of static light scattering: 100% (♦); 80% (▲); 60% (▼); 40% (●); 20% (■).

supports the generally admitted assumption that the concentration of free surfactant must reach the CMC for solubilization to occur (Lichtenberg, 1985).

Relationship between the Re *parameter,* S_W, *and* K

Fig. 3 *A* shows the variation of S_W with *Re* throughout the interaction of DM-liposomes (vesicles or mixed micelles). A marked increase in S_W occurred as *Re* rising up to *Re* = 0.80 (100% CF release). The extrapolation of the curve (*shaded area*) led approximately to the initial S_W value for solubilization (Re_{SAT} = 0.90 for 100% light scattering), which corresponded approximately to the surfactant CMC (S_W = 0.123 mM and DM CMC = 0.125 mM). The increase in *Re* resulted in a slight increase in S_W up to Re_{SOL}, which corresponded to the complete solubilization of liposomes through the formation of mixed micelles (*Re* = 2.23, for 0% SLS).

Fig. 3 *B* shows the variation in *K* versus *Re* during the overall DM-liposome interaction. An initial increase in *K* was observed as *Re* rose, reaching a maximum (*K* = 14.58) for *Re* = 0.28 (20% CF release). Increasing *Re* values resulted in a fall in *K* values up to *Re* = 0.80 (100% CF release), this decrease being more pronounced in the *Re* interval 0.28–0.53. Thus, the increase in *Re* resulted in two opposite effects on the bilayer/water partitioning of DM. At low *Re*, *K* first increased possibly because only the outer vesicle leaflet was available for interaction with surfactant molecules, the binding of additional surfactant to the bilayer being hampered up to *Re* = 0.53 (abrupt fall in *K*). The *Re* = 0.28 (corresponding to 20% CF release) may be correlated with the saturation of the outer vesicle leaflet by the surfactant. Increasing *Re* values (*Re* between 0.53 and 0.80, corresponding to a low decrease in *K*), led to an increased rate of flip-flop of the surfactant molecules (or permeabilization of the bilayers to surfactant), thus also making the inner monolayer available for interaction with added surfactant. The aforementioned long time course required to reach a constant CF release, especially noticeable in this CF release interval, (from 50% to 100% CF release, see Table 1) could be also correlated with this behavior. These findings are in agreement with the results reported by Schubert et al. for the interaction of sodium cholate/PC liposomes (Schubert et al., 1986). The extrapolation of the curve (*shaded area*) led approximately to the initial *K* value for solubilization (Re_{SAT} = 0.90 for 100% SLS). The subsequent increase in *Re* resulted again in a rise in *K* up to Re_{SOL} = 2.23, which corresponded to the solubilization of liposomes via mixed micelles formation.

Comparison of the *Re* and *K* values obtained for DM with those reported for subsolubilizing interactions of OG with PC liposomes (de la Maza and Parra, 1994b) reveals that although the *Re* parameter showed similar values (0.14–0.80 for DM versus 0.20–1.18 for OG) the *K* values for DM were greatly increased (13.72–4.72 for DM versus 0.092–0.038 for OG). Thus, although the ability of these two

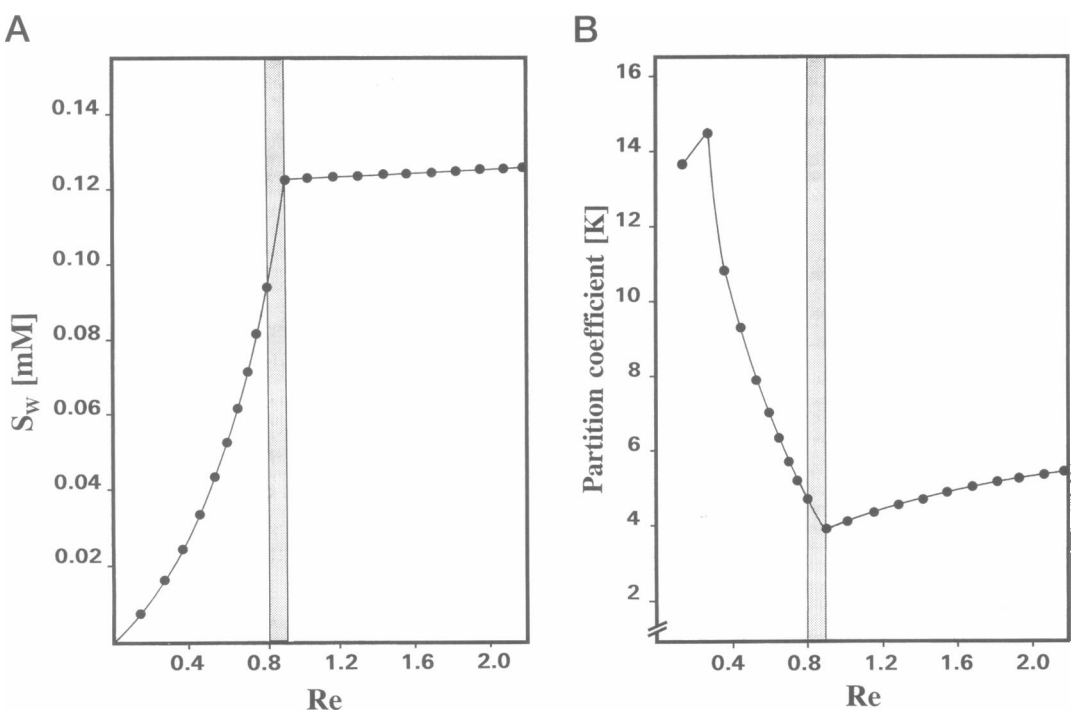

FIGURE 3 (*A*) Variation in the free surfactant concentration versus the effective surfactant to phospholipid molar ratio (*Re*) during the overall interaction between the dodecylmaltoside surfactant and PC liposomes. (*B*) Variation in the partition coefficients (*K*) versus the effective surfactant to phospholipid molar ratio (*Re*) during the overall interaction between the dodecylmaltoside and PC liposomes.

surfactants to alter the permeability of PC bilayers appeared to be similar (slightly higher for DM) the affinity of DM with bilayers was more than 100× higher than that reported for OG. At solubilizing level the DM showed an increased ability to solubilize bilayers (~2× higher) and a very increased affinity with these structures (also >100 times higher) than that reported for OG (de la Maza and Parra, 1994b). This increased activity and affinity could be explained bearing in mind that the molecular structure of DM appears to be more equilibrated in terms of hydrophilic-lipophilic balance (HLB) than that of the OG given the greater length of its hydrophobic tail. In fact, the adsorption of surfactant molecules on the outer leaflet of liposomes and their subsequent incorporation into bilayers (association with PC molecules) are correlated with the HLB of each surfactant as well as with the composition and physicochemical characteristics of the bilayer structure.

Comparison of the *Re* and *K* values obtained for DM with those reported for Triton X-100 (de la Maza and Parra, 1994a) shows that although Triton X-100 had at subsolubilizing level higher ability to alter the permeability of PC bilayers than DM (low *Re* values), the DM showed higher capacity to solubilize these structures. In addition to that, the affinity of DM with bilayers was clearly increased during the overall interaction with PC liposomes.

Dependence of the surfactant-PC aggregate size, CF release, and static light scattering on Re

A systematic investigation based on dynamic light-scattering measurements of surfactant-PC aggregates was carried out throughout the process to elucidate the dependencies of the size of these aggregates (vesicles or micelles), the changes in the percentages of both the CF release and the static light scattering of the system, on *Re*. The values obtained for 5.0 mM PC concentration are given in Table 2. A progressive growth of vesicles was detected as the percentage of CF release increased (formation of mixed vesicles upon addition of small amounts of DM); the maximum increase was reached in the interval of CF release between 70% and 100%. The growth of vesicles occurred in a few seconds with little change over a period of hours. As for static light scattering variations, the 100% corresponding to the Re_{SAT} produced a slight fall in the vesicle size, albeit with a monomodal distribution. When the light scattered by the system decreased, a sharp distribution curve appeared at ~56 nm, which corresponded to a new particle size distribution (PC-surfactant mixed micelles). The curve for these small particles rose until 20% of scattered light exhibited a monomodal distribution again at this point, which corresponded to the surfactant/PC mixed micelles (particles of 56 nm). These findings are in agreement with those reported by

TABLE 2 Mean size distributions (nm) and polydispersity indexes of surfactant-PC aggregates (vesicles or micelles) resulting in the overall interaction of dodecylmaltoside with PC liposomes.

	Curve distribution (particle number)						
		1st peak		2nd peak		Average	Polydispersity
	Type	nm	%	nm	%	(nm)	Index
CF release (%)							
0	M*	—	—	200	100	200	0.100
10	M	—	—	268	100	268	0.120
20	M	—	—	330	100	330	0.129
30	M	—	—	364	100	364	0.136
40	M	—	—	380	100	380	0.140
50	M	—	—	391	100	391	0.149
60	M	—	—	398	100	398	0.158
70	M	—	—	402	100	402	0.166
80	M	—	—	408	100	408	0.170
90	M	—	—	409	100	409	0.176
100	M	—	—	408	100	408	0.181
Light-Scattering %							
100	M	—	—	400	100	400	0.204
90	B#	56	8.4	369	91.6	343	0.237
80	B	56	14.7	330	85.3	290	0.250
70	B	56	18.9	268	81.1	228	0.241
60	B	56	24.3	229	75.7	187	0.231
50	B	56	31.1	160	68.9	128	0.225
40	B	56	36.4	103	63.6	86	0.217
30	B	56	46.1	86	53.9	72	0.210
20	B	56	66.7	74	33.3	62	0.204
10	M	56	100	—	—	56	0.188
0	M	56	100	—	—	56	0.153

*M, monomodal.
#B, bimodal.

Almog et al. for OG/PC liposomes interaction, who demonstrated that in the corresponding *Re* range (between ~90–20% SLS), vesicles and micelles coexisted (Almog et al., 1990).

Fig. 4 *A* shows the variation in both the percentage of CF release and vesicle size of liposomes versus *Re* at subsolubilizing level. The increase in *Re* led initially to a linear increase in both the percentage of CF release and the size of vesicles. However, *Re* values exceeding 0.28 resulted in a lower growth of vesicles, (maximum for *Re* = 0.75, 90% of CF release), which coexisted with a progressive increase in the release of the CF trapped into liposomes (bilayer permeability). Given that ~80% of the permeability changes occurred in the initial interaction steps (initial 10 min) and that the vesicle growth took place in a few seconds after DM addition, we may assume that the growth of vesicles was related to the leakage of entrapped CF for *Re* values <0.28. These two physicochemical properties were, as a consequence, dependent on the composition of mixed vesicles (*Re*). These findings are in agreement with the results reported by Almog et al. for OG (Almog et al., 1990). From these findings we may assume that the initial linear dependence between the percentage of CF release, the size of vesicles, and *Re* could be correlated with the surfactant saturation of the outer vesicle leaflet of liposomes (*Re* values ranging from 0.14 to 0.28). The subsequent coexistence of slight changes in the vesicle size and the progressive increase in the fluidity of bilayers (*Re* values ranging from 0.30 to 0.80) could also be correlated with the increase in the rate of flip-flop of the surfactant molecules into bilayers and with the aforementioned long time period needed to reach a constant CF release.

Fig. 4 *B* shows the variation in the percentage of static light scattering and the surfactant-PC aggregate size (average mean) versus *Re* at solubilizing level. The increase in *Re* produced a linear decrease in both parameters, except for the surfactant-PC aggregate size in the range of static light scattering values between 30% and 10% (*Re* values >1.7). This means that, in the range of *Re* values between 0.9 and 1.70 (corresponding to the mixed micelle formation), a direct correlation between both parameters was established as well as with the composition of the surfactant-PC aggregates and, consequently, with the *Re* parameter. This linear dependence, together with the progressive increase of the distribution curve corresponding to the PC-surfactant mixed micelles at 56 nm (Table 2) in the last interaction steps, emphasizes the suitability of this surfactant in the solubilization of PC liposomes.

CONCLUSIONS

The experimental results provide some new insights concerning the aggregative states of DM with phosphatidylcholine liposomes. This surfactant showed during the overall process a clear increased affinity with bilayers and higher ability to saturate and solubilize PC liposomes than that reported for the nonionic surfactant OG (de la Maza and Parra, 1994b). Furthermore, DM had a higher solubilizing capacity of PC liposomes (lower Re_{SOL}) than that reported for Triton X-100 (de la Maza and Parra, 1994a). At subsolubilizing level, a maximum bilayer/water partitioning of DM (*K*) appeared at *Re* value of 0.28 (20% CF release), whereas at solubilizing level *K* progressively increased with *Re*.

When *Re* was <0.28 the growth of vesicles was directly correlated with the leakage of entrapped CF, both parameters being dependent on the bilayer composition (*Re*). This

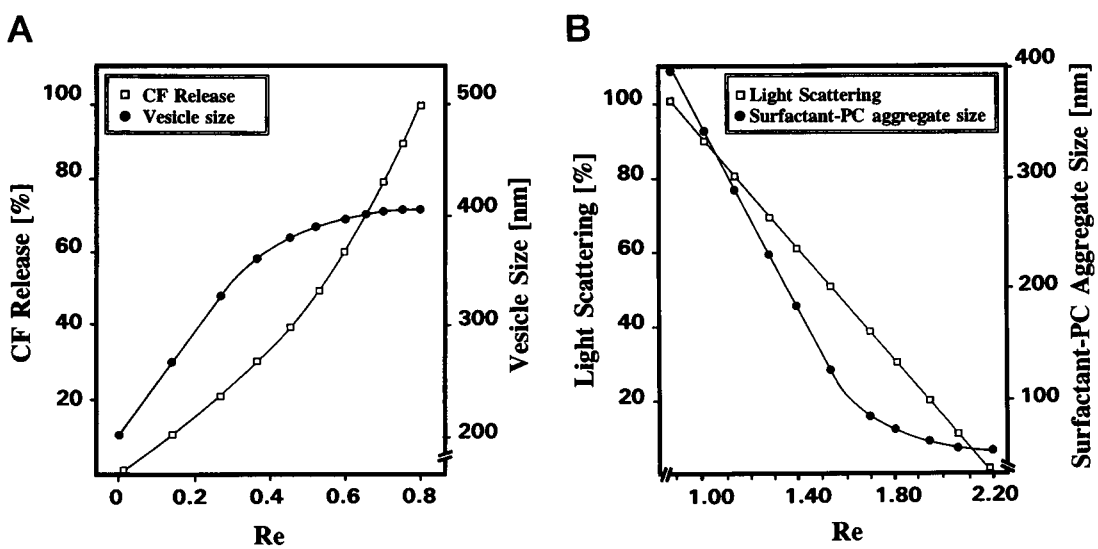

FIGURE 4 (*A*) Variation in the percentage of CF release (□) and vesicle size (●) of liposomes treated with dodecylmaltoside versus *Re* at subsolubilizing level. (*B*) Variation in the percentage of static light scattering (□) and surfactant-PC aggregate size (●) of surfactant-PC aggregates versus *Re* at solubilizing level.

dependence may be correlated with the increasing presence of surfactant molecules in the outer vesicle leaflet and its saturation (maximum K value at $Re = 0.28$). However, in the interval of CF release percentages between 60 and 100 (Re values between 0.60 and 0.80), a progressive increase in bilayer permeability occurs with a slight change in the size of vesicles, which may be related to the increased rate of flip-flop of the surfactant molecules into bilayers (low decrease in K).

A linear dependence was also established at solubilizing level in the range of Re values between 0.9 and 1.70 between the decrease in both the surfactant-PC aggregate size and the static light scattering of the system, and the composition of these aggregates (Re). The fact that the free surfactant concentration at subsolubilizing and solubilizing levels showed values lower than and similar to its CMC indicates that permeability alterations and solubilization were determined respectively by the action of surfactant monomer and by the formation of mixed micelles.

The present work open up new avenues in the potential application of DM in biological domains given its specific characteristics in solubilization of PC liposomes.

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