Permeability Changes Caused by Surfactants in Liposomes That Model the Stratum Corneum Lipid Composition

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ABSTRACT: The alterations caused by different surfactants in the permeability of liposomes formed by a lipid mixture that models the stratum corneum (SC) composition (40% ceramides, 25% cholesterol, 25% palmitic acid, and 10% cholesteryl sulfate) were investigated. The surfactant/lipid molar ratios (*Re*) and the bilayer/aqueous phase surfactant partition coefficients (*K*) were determined at two sublytic levels. The selected surfactants were sodium dodecyl sulfate (SDS); sodium dodecyl ether sulfate (SDES) to assess the influence of the ethylene oxide groups on the anionic surfactant's behavior; Triton X-100 (OP-10EO) and dodecyl betaine (D-Bet) as representatives of nonionic and amphoteric surfactants. Permeability alterations were determined by monitoring the increase in the fluorescence intensity of liposomes due to the 5(6) carboxyfluorescein (CF) released from the interior of vesicles. The SC liposomes/surfactant sublytic interactions were mainly ruled by the action of surfactant monomers. OP-10EO showed the highest ability to alter the permeability of bilayers and the highest affinity with these structures, whereas D-Bet showed the lowest tendencies. Although SDS and SDES exhibited similar activity at 50% CF release (similar *Re* values), SDES appeared to be more active at 100% CF release, its affinity with bilayers being also increased. The different ability exhibited by SDS, SDES, and D-Bet (same alkyl chainlength) to alter the permeability of SC liposomes emphasizes the role played by the polar part of these surfactants in this interaction. Different trends in the evolution of *Re* and *K* were observed when comparing the results with those reported for phosphatidylcholine (PC) liposomes. Thus, whereas SC liposomes appeared to be more resistant to the action of surfactants, the surfactant affinity with SC bilayers was always greater than that reported for PC bilayers.
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KEY WORDS: Carboxyfluorescein release; liposome/surfactant interactions; permeability alteration; stratum corneum liposomes; surfactant partition coefficients; surfactant/stratum corneum lipid molar ratios.

Surfactants are indispensable reagents in the solubilization and reconstitution of membrane proteins (1–3). 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 that govern the interaction of surfactants with simplified membrane models as phospholipid bilayers (4–7). 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 (8), who postulated that the critical effective surfactant/lipid molar ratio (*Re*) producing saturation and solubilization 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 stratum corneum (SC), the outermost layer of mammalian epidermis, consists of flat cells (corneocytes) that are separated by an intercellular matrix mainly composed of lipids. These lipids are organized into bilayers that have been postulated both to account for the permeability properties of SC and to ensure the cohesiveness between corneocytes (9,10). The analytical composition of lipids and proteins that form the SC has been extensively investigated (11–14).

To find out whether SC lipids could form bilayers, Wertz *et al.* (15,16) and Abraham *et al.* (17) prepared liposomes from lipid mixtures that approximate the composition of SC lipids at physiological pH. These authors also investigated the interaction of these bilayer structures with the anionic surfactant sodium dodecyl sulfate (SDS) to study the deleterious effect of this surfactant on human skin (18). Furthermore, Blume *et al*. (19) reported that mixing phosphatidylcholine (PC) liposomes with lipid model mixtures for SC lipids could be one mechanism to contribute to the enhancement of the permeability of the skin to lipid vesicles.

In recent papers, we have studied the interactions of different surfactants and surfactant mixtures with simplified membrane models, such as PC liposomes (20–22), as well as the formation and characterization of liposomes formed with different mixtures of four commercially available synthetic lipids that approximate the composition of SC (23). In the present work, we seek to extend these investigations by char-

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acterizing the surfactant-to-lipid molar ratios and the partition coefficients of different surfactants between these bilayers and the aqueous medium. The selected surfactants were SDS as a typical anionic surfactant; sodium dodecyl ether sulfate (SDES) to assess the influence of the ethylene oxide (EO) groups on the anionic surfactant's behavior; octyl phenol ethoxylated with 10 EO units, Triton X-100, as a representative nonionic surfactant, which is widely used in membrane studies (24–26); and dodecyl betaine as a representative amphoteric surfactant. The results obtained in this study provide new information about the effect caused by these surfactants on the permeability of SC bilayers taking into account the different physicochemical characteristics of the amphiphilic compounds tested.

MATERIALS AND METHODS

SDS was obtained from Merck (Darmstadt, Germany) and further purified by a column chromatographic method (27). SDES was supplied by Tenneco S.A.(Barcelona, Spain). The latter was a commercial-grade product with an active matter of 28.8%, 2.5 average in EO units and the following average in alkyl chain: C-10, 3.9%, C-12, 68.1%; C-14, 22.2%, and C-16, 4.9%. Nonionic surfactant Triton X-100 (OP-10EO), octyl phenol ethoxylated with 10 units of EO, as 100% active matter was purchased from Rohm and Haas (Lyon, France). The amphoteric surfactant *N*-dodecyl-*N,N*-dimethylbetaine (D-Bet) was specially prepared by Albright and Wilson, Ltd. (Warley, West Midlands, United Kingdom); the active matter was 30% in aqueous solution, and the amino free contents was 0.20%. Piperazine-1,4-*bis*(2-ethanesulfonic acid) (PIPES buffer), obtained from Merck was prepared as 20 mM PIPES buffer adjusted to pH 7.20 with NaOH, and contained 110 mM Na_2SO_4. Tris-(hydroxymethyl)aminomethane (TRIS buffer), obtained from Merck, was prepared as 20.0 mM TRIS adjusted to pH values ranging from 5.0 to 7.5 with HCl and contained 110 mM Na_2SO_4.

5(6)-Carboxyfluorescein (CF) was obtained from Eastman Kodak (Rochester, NY) and further purified by a column chromatographic method (28). Polycarbonate membranes and membrane holders were purchased from Nucleopore (Pleasanton, CA). Reagent-grade organic solvents, ceramides type III (Cer), and cholesterol (Chol) were supplied by Sigma Chemical Co. (St Louis, MO), and palmitic acid (PA) (reagent grade) was purchased from Merck. Cholesteryl sulfate (Chol-sulf) was prepared by reaction of cholesterol with excess chlorosulfonic acid in pyridine and was purified chromatographically. The molecular weight of ceramides type III was determined by low-resolution fast atom bombardment mass spectrometry (FAB–MS) with a Fisons VG Auto Spec Q (Manchester, United Kingdom) and a cesium gun operating at 20 Kv. The lipids of the highest-purity grade available were stored in chloroform/methanol (2:1) under nitrogen at −20°C until use.

Liposome preparation. Liposomes formed by a mixture of lipids to model the composition of SC (40% Cer, 25% Chol, 25% PA, and 10% Chol-sulf) were prepared by following the method described by Wertz *et al.* (15). Individual lipids were dissolved in chloroform/methanol (2:1), and appropriate volumes were combined to obtain the aforementioned mixture. The lipid mixture was then placed in a culture tube, and the solvent was removed with a stream of nitrogen and then under high vacuum at room temperature. Lipid mixture aqueous dispersions were then prepared by suspension in PIPES buffer that contained 110 mM Na_2SO_4 and supplemented with 10 mM 5(6)-carboxyfluorescein to provide a final lipid concentration that ranged between 0.5 mM and 5.0 mM at pH 7.20. The lipids were left to hydrate for 30 min under nitrogen with occasional shaking. The suspensions were then sonicated in a bath sonicator (514 ECT Selecta) at 60ºC for about 15 min until the suspensions became clear (23). Vesicles of defined size (about 200 nm) were obtained by extrusion techniques (VET). To this end, liposome suspensions were extruded through 800-200 nm polycarbonate membranes at 60°C in a thermobarrel extruder equipped with a thermoregulated cell compartment (Lipex, Biomembranes Inc. Vancouver, Canada). The preparations were then annealed at the same temperature for 30 min and incubated at 37ºC under nitrogen atmosphere. Vesicles were freed of unencapsulated fluorescent dye by passage through Sephadex G-50 medium resin (Pharmacia, Uppsala, Sweden) by column chromatography to study the alterations in the bilayer permeability due to the presence of different surfactants.

Analysis and phase transition temperature of bilayer lipid mixture. The bilayer lipid composition after liposome preparation was determined by thin-layer chromatography (TLC) coupled to an automated flame-ionization detection (FID) system (Iatroscan MK-5; Iatron Lab. Inc. Tokyo, Japan) (29). Liposome suspensions were directly spotted onto silica gel-coated chromarods (type S-III) in 0.5-, 1.0-, and 1.5-μL aliquots from a SES 3202/IS-02 semiautomatic sample spotter with a precision two-microliter syringe. The rods were developed for a distance of 10 cm with solvent mixture *n*-hexane/ethyl ether/formic acid (50:20:0.3) to separate the nonpolar lipids PA and Chol from the rest of the compounds. A partial scan of 80% of the rods was performed to quantitate and eliminate them. Redevelopment of the rods with chloroform/methanol/ammonia (58:10:2.5) of 7 cm leads to a good separation of the polar lipids (Cer and Chol-sulf) from the buffer, which remains at the spotting position. A total scan was performed to quantitate Cer and Chol-sulf. The same procedure was applied to different standard solutions of these lipids dissolved in chloroform/methanol (2:1) to obtain the calibration curves for the quantitation of each compound.

To find out whether all mixture lipid components formed liposomes, vesicular dispersions were analyzed for these lipids (29). The dispersions were then spun at 140,000 × *g* at 37°C for 4 h to remove the vesicles (30). The supernatants were tested again for these components. No lipids were detected in any of the supernatants.

Analyses of proton nuclear magnetic resonance (^{1}H NMR) were carried out at temperatures ranging from 25 to 90°C to

determine the phase transition temperature of the lipid mixture that forms liposomes. The ^{1}H NMR spectra were recorded on a Varian Unity of 300 MHz (Palo Alto, CA). The NMR spectra were measured at intervals of 5°C. The line widths of the CH_2 band at 1.3 ppm were measured and 1024 scans were accumulated each time. The different line widths were plotted vs. the temperature, and the inflection point of the curve was taken as a phase transition temperature, which showed a value of 55–56°C.

Vesicle size distribution. The vesicle size distribution and the polydispersity index (PI) of liposomes after preparation were determined with dynamic light-scattering measurements in a photon correlator spectrometer (Malvern Autosizer 4700c PS/MV, Malvern, United Kingdom). The studies were made by particle number measurement. The sample was adjusted to the appropriate concentration range with PIPES buffer, and the measurements were taken at 37°C at a reading angle of 90°. After preparation, the vesicle size distribution varied little (lipid concentration from 0.5 to 5.0 mM) and showed in all samples a similar value of about 200 nm (PI lower than 0.1), thereby indicating that the size distribution was homogeneous. The size of vesicles after the addition of equal volumes of PIPES buffer and equilibration for 60 min showed in all samples values similar to those obtained after preparation, with a slight increase in PI (between 0.10 and 0.12). Hence, the liposome preparations appeared to be reasonably stable in the absence of surfactant under the experimental conditions used in permeability studies.

Parameters involved in the interaction of surfactants with SC liposomes. In the analysis of the equilibrium partition model proposed by Schurtenberger *et al.* (31) for bile salt/lecithin systems, Lichtenberg (8) and Almog *et al*. (30) have shown that, for a mixing of lipids [at a lipid concentration L (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

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

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 $L \gg S_B$, the definition of K, as given by Schurtenberger *et al.* (31), applies:

$$K = S_B/(L \cdot S_W) = Re/S_W \quad [2]$$

where Re is the effective molar ratio of surfactant to lipid in the bilayers ($Re = S_B/L$). Under any other conditions, Equation 2 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 (8) and Almog (30) for different surfactant–lipid mixtures over wide ranges of Re values. Given that the range of lipid concentrations used in the mixture is similar to that used by Almog *et al.* (30) to test his equilibrium partition model, the K parameter has been determined from this equation.

The determination of these parameters can be carried out on the basis of the linear dependence that exists between the surfactant concentrations required to achieve 50 and 100% of CF release and the SC lipid concentration (SCL), which can be described by the equations:

$$S_T = S_{W,50\%CF} + Re_{50\%CF} \cdot [SCL] \quad [4]$$

$$S_{T'} = S_{W,100\%CF} + Re_{100\%CF} \cdot [SCL] \quad [5]$$

where $Re_{50\%CF}$, $Re_{100\%CF}$, and the aqueous concentrations of surfactant $S_{W,50\%CF}$ and $S_{W,100\%CF}$ are in each curve, the slope and the ordinate at the origin (zero lipid concentration), respectively.

Surfactant CMC. The surface tensions of buffered solutions with increasing concentrations of surfactants were measured by the ring method (32) with a Krüss tensiometer. The CMC of the different surfactants was determined from the abrupt change in the slope of the surface tension value vs. surfactant concentration. The CMC values obtained for each surfactant tested are given in Table 1.

Permeability alterations. The permeability changes caused by the presence of different surfactants in SC liposomes were determined quantitatively 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 (22). Fluorescence measurements were made with a spectrofluorophotometer, Shimadzu RF-540 (Kyoto, Japan), equipped with a thermoregulated cell compartment. On excitation at 495 nm, a fluorescence maximum emission of CF was obtained at 515.4 nm. The fluorescence intensity mea-

TABLE 1
Surfactant-to-Lipid Molar Ratios (Re), Partition Coefficients (K), and Surfactant Concentrations in the Aqueous Medium (S_W), Resulting in the Subsolubilizing Interaction (50 and 100% of 5(6)-carboxyfluorescein release) of Triton X-100, Sodium Dodecyl Sulfate, Sodium Dodecyl Ether Sulfate, and Dodecyl Betaine Surfactants with Stratum Corneum Liposomes[a]

	CMC (mM)	$S_{w,50\%CF}$ (mM)	$S_{W,100\%FC}$ (mM)	$Re_{50\%CF}$ mole/mole	$Re_{100\%CF}$ mole/mole	$K_{50\%CF}$ (mM^{-1})	$K_{100\%CF}$ (mM^{-1})	r^2 (50%CF)	r^2 (100%CF)
OP-10EO	0.15	0.039	0.089	0.190	0.448	4.09	3.47	0.993	0.994
SDS	0.50	0.083	0.289	0.350	1.0	3.12	1.70	0.994	0.996
SDES	0.12	0.086	0.105	0.351	0.733	3.02	3.99	0.995	0.993
D-Bet	1.25	0.418	0.838	0.653	0.756	0.95	0.51	0.997	0.995

[a]The regression coefficients of the straight lines obtained are also included.

surements were taken at 37ºC. The percentage of CF released was calculated by means of the equation:

$$\%\text{CF release} = (I_T - I_0)/(I_\infty - I_0) \cdot 100 \quad [6]$$

where I_0 is the initial fluorescence intensity of CF-loaded liposome suspensions in the absence of surfactant, I_t is the fluorescence intensity measured 60 min after adding the surfactant solution to liposome suspensions. This interval was chosen as the minimum period of time needed to achieve a constant level of CF release for the lipid concentration range used. The experimental determination of this interval is indicated in the Results and Discussion section. I_∞ corresponds to the fluorescence intensity that remains after the complete destruction of liposomes by the addition of OP-10EO [60 µL of 10% (vol/vol) aqueous solution] (22).

RESULTS AND DISCUSSION

Permeability studies. The ability of the SC lipids to form bilayers has been reported by Wertz *et al.* (15), who demonstrated that these lipids form liposomes when hydrated at 80°C. The Cer type III used in this work is composed primarily of simple sphingosines linked to largely monounsaturated fatty acids. Therefore, it has a much lower bulk melting temperature than SC ceramides, which contain only saturated fatty acids, including hydroxy acids. In preliminary experiments, we determined the suitable sonication temperature of the investigated lipid mixture by preparing liposomes at temperatures that approximated their phase transition temperature (55–56°C). It was found that temperatures exceeding this temperature by more than 10°C caused noticeable alterations in Cer and Chol-sulf. As a consequence, the lipid mixture was sonicated at 60ºC.

It is known that, in surfactant–lipid systems, complete equilibrium may take several hours (6,8). However, in subsolubilizing interactions, a substantial part of the surfactant effect takes place within approximately 30 min after its addition to the liposomes (26). To determine the time needed to obtain a constant level of CF release of liposomes in the lipid concentration range investigated, a kinetic study of the interaction of various surfactants with SC liposomes was carried out. Liposomes were treated with a constant subsolubilizing surfactant concentration (0.5 mM), and subsequent changes in permeability were studied as a function of time. The results obtained for two lipid concentrations (5.0 and 1.0 mM) are shown in Figure 1, A and B, respectively. About 60 min was needed to achieve a constant level of CF release in both samples. Hence, changes in permeability were studied 60 min after addition of surfactants to the liposome suspensions at 37°C. This finding contrasts with that reported for the interaction of these surfactants with PC liposomes, where the time needed to obtain a constant level of CF release was always clearly lower (33). The CF release of SC liposomes in the absence of surfactant in this period of time was negligible.

To determine the *Re* and S_W parameters at two sublytic levels (50 and 100% CF release), a systematic investigation of

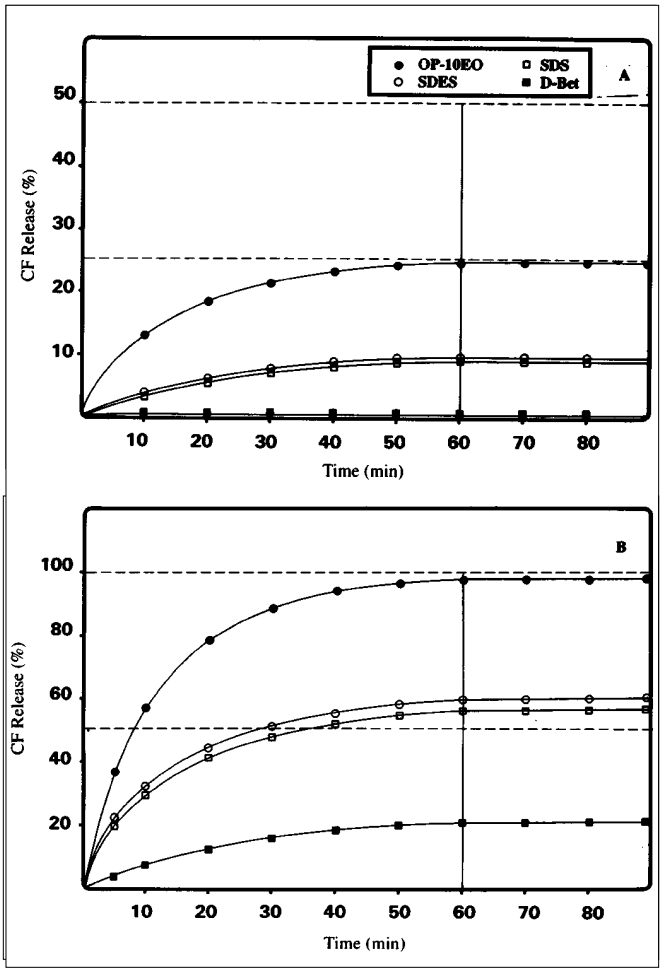

FIG. 1. Time curves of the release of 5(6)-carboxyfluorescein (CF) trapped into stratum corneum liposomes, caused by the addition of a constant concentration (0.5 mM) of Triton X-100 (OP-10EO) (●), sodium dodecyl sulfate (SDS) (□), sodium dodecyl ether sulfate (SDES) (○), and dodecyl betaine (D-Bet) (■). The lipid concentration was (A) 5.0 mM and (B) 1.0 mM.

permeability changes, caused by the addition of different surfactants, was carried out for various SC lipid concentrations (from 0.5 to 5.0 mM). The curves obtained for the anionic surfactant SDS are given in Figure 2. The surfactant concentrations resulting in 50 and 100% of CF release for each surfactant tested were graphically obtained and plotted vs. lipid concentration. An acceptable linear relationship was established in each case. These results are plotted in Figure 3A (50% CF release) and 3B (100% CF release), respectively. The straight lines obtained corresponded to the aforementioned Equations 4 and 5 from which *Re* and S_W were determined. These parameters, including the regression coefficients (r^2) of the straight lines, are also given in Table 1.

The S_W values increased as the CF release percentage rose, although the values were smaller than those corresponding to the surfactant CMC in all cases. This finding suggests that the surfactant–liposome interaction is ruled mainly by the action of surfactant monomers, unlike the behavior of surfactants in solubilization of phospholipid bilayers, where micelle forma-

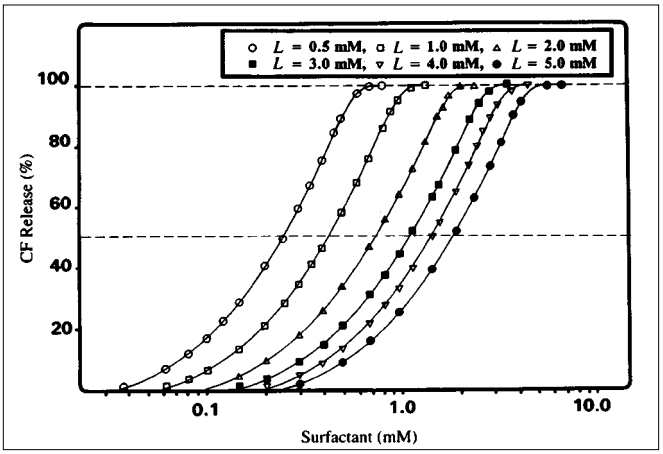

FIG. 2. Percentage changes in CF release of stratum corneum liposomes, (lipid concentration ranging from 0.5 to 5.0 mM), induced by the presence of increasing concentrations of SDS. Lipid concentrations: 0.5 mM (○), 1.0 mM (□), 2.0 mM (△), 3.0 mM (■), 4.0 mM (▽), 5.0 mM (●). See Figure 1 for other abbreviations.

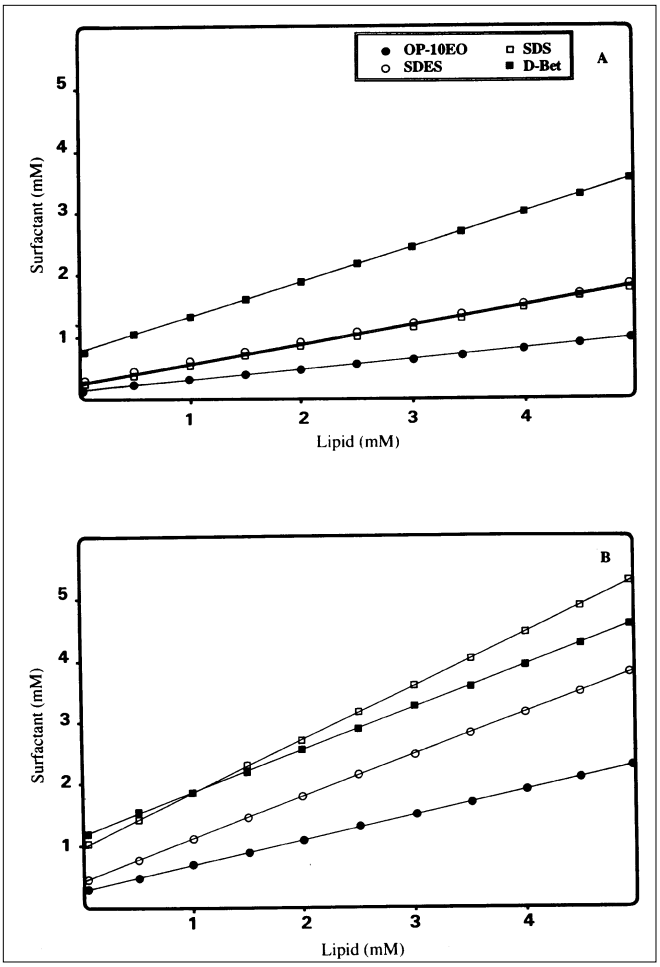

FIG. 3. Surfactant concentrations resulting in (A) 50% of CF release and (B) 100% of CF release vs. lipid concentration of liposome suspensions. OP-10EO (●), SDS (□), SDES (○), and D-Bet (■). See Figure 1 for abbreviations.

tion plays an important role (8). These findings are in agreement with those reported for subsolubilizing and solubilizing interactions of these surfactants with PC unilamellar liposomes in the same buffered working medium (33,34).

As for the *Re* parameter, this value increased as the CF release percentage rose, regardless of the chemical structure of the surfactant tested. Bearing in mind that the surfactant capacity to alter the permeability of bilayers is inversely related to the *Re* parameter, the maximum activity at 50% of CF release corresponded to the nonionic surfactant OP-10EO (lowest *Re* values), and the minimum to the amphoteric D-Bet (highest *Re* values), with the anionics SDS and SDES exhibiting intermediate values. Similar behavior was detected at 100% CF release except for SDES, which exhibited higher bilayer activity than SDS. This effect may be attributed to the presence of 2.5 EO units in the SDES molecular structure, given that this is the unique structural difference between both anionic surfactants. The different activity of SDS, SDES, and D-Bet, which contain the same alkyl chainlength (same hydrophobicity), emphasizes the role played by the polar part of these surfactants in their abilities to alter the permeability of SC liposomes at the two investigated interaction levels.

Comparison of the *Re* values with those reported for the interaction of these surfactants with PC unilamellar liposomes (33) reveals that ability of these surfactants to alter the permeability of SC bilayers (50% CF release) appeared to be less (higher *Re* values) than that reported for PC unilamellar liposomes in all cases. Thus, SC bilayer structures appeared to be more resistant to the surfactant perturbations at the sublytic level.

The surfactant partition coefficients between SC bilayers and aqueous medium, both at 50 and 100% of CF release, indicate that OP-10EO molecules had the highest affinity with bilayers (maximum *K* values), whereas the amphoteric surfactant D-Bet showed the lowest (minimum *K* values). As for the nonionic surfactants, although at 50% of CF release SDS showed higher affinity with bilayers than SDES, at 100% CF release the affinity of these surfactants with SC liposomes exhibited opposite tendencies. As discussed above, this effect may be attributed to the presence of 2.5 EO units in the SDES molecular structure. The different affinity of SDS, SDES, and D-Bet (same hydrophobicity) with SC liposomes (premicellar association leading to formation of mixed liposomes) at the two interaction levels investigated also underlines the role played by the polar part of these surfactants in these interactions.

The fact that OP-10EO, SDS, and D-Bet showed lower *K* values at 100% CF release than at 50% could be explained by assuming that, at low *Re* (*ca*. in the interval of CF release between 30 and 60%), only the outer vesicle leaflet was available for interaction with surfactant molecules, while the binding of additional molecules to bilayers is being hampered at slightly higher *Re* values. These findings are in agreement with those reported by Schubert *et al*. (35) for sodium cholate and with our previous investigations that involved the overall interaction of OP-10EO and SDS with PC liposomes (20,22).

This behavior contrasts with the increased bilayer affinity exhibited by SDES at 100% CF release. This effect may be attributed to the specific structure of this anionic surfactant, which could affect the rate of flip-flop of surfactant molecules (or permeabilization of the bilayers to surfactants), thus making the inner monolayer available for the interaction of added surfactant. Comparison of the *K* values obtained with those reported for the interaction of these surfactants with PC liposomes indicates that the surfactant affinity with SC bilayers appeared to be greater than that for PC ones (20,22,33).

It is known that betaines (internally compensated quaternary ammonium compounds) with a carboxylate ion form external salts with acids, such as hydrochloric acid, which confers to these type of surfactants a certain cationic character at acidic pH. This characteristic may allow us to elucidate the role played by the polar part of D-Bet in its low ability to alter the permeability of SC bilayers with respect to that exhibited by the surfactant SDS and SDES with the same alkyl chain-length.

To clarify this aspect, a systematic investigation of permeability changes, caused by D-Bet on SC liposomes (SC lipid concentration also ranging from 0.5 to 5.0 mM), was carried out at various pH constant values. To this end, a 5.0 mM TRIS buffer solution, containing 100 mM NaCl and adjusted to different pH (pH values ranging from 5.0 to 7.5) with HCl, was used (36). The results obtained (Table 2) showed that the ability of D-Bet to alter the permeability of SC liposomes at 50% CF release increased as the pH of the buffered medium decreased (*Re* values diminished from 0.68 at pH 7.5 to 0.43 at pH 5.0). A similar tendency was observed at the interaction step of 100% CF release (*Re* values decreased from 0.79 at pH 7.5 to 0.55 at pH 5.0). The free surfactant concentrations ($S_{W,50\%CF}$, $S_{W,100\%CF}$) also decreased as the pH of the buffered solution diminished.

As for the partition coefficients, these parameters also diminished as the pH of the buffered solution decreased (from 0.968 at pH 7.5 to 0.802 at pH 5.0, and from 0.526 at pH 7.5 to 0.473 at pH 5.0 for 50% and 100% of CF release, respectively). This means that the affinity of this surfactant with the SC lipids decreased with the pH in spite of the increased ability of this surfactant to alter the permeability of these bilayer structures at the two interaction levels investigated under the same pH conditions. The relative cationic character of this compound at acid pH appears to be responsible for these two opposite tendencies, which may be attributed to electrostatic interactions between the surfactant and the polar groups of the lipids building the bilayer structures.

These findings explain the relative low activity detected for D-Bet on SC liposomes with respect to the other compounds tested at pH 7.20 (PIPES buffer) and underline the influence of the polar part of this surfactant in its capacity to alter the permeability of SC liposomes. These results are also in accordance with the more active properties reported for the external salts of betaines that contain a carboxylate ion at slightly acid pH solutions (37). It is interesting that the activity and affinity of SDS and SDES with respect to SC liposomes was not significantly affected by the changes in the pH of the buffered medium (TRIS buffer) in the same pH range investigated for D-Bet.

In general terms, different trends in the interaction of these surfactant with SC and PC liposomes may be observed at subsolubilizing levels. Thus, whereas SC liposomes appeared to be more resistant to the action of surfactant monomers the affinity of these compounds with SC structures appeared to be greater than with PC. Thus, although a greater number of surfactant molecules was needed to produce alterations in SC bilayers, these molecules showed increased affinity with these structures. This behavior is directly correlated with the lesser free surfactant concentrations ($S_{W,50\%CF}$ and $S_{W,100\%CF}$) obtained in the interaction of these surfactants with SC liposomes (20,22,33).

We are aware of the fact that the lipids used in this work are not exactly the same as those existing in the SC. Nevertheless, our approach may be useful for studying the interaction of different surfactants with these lipid structures and comparing the surfactant–lipid molar ratios for different sublytic interaction steps with those obtained for phospholipid liposomes. This comparison also could be useful in establishing a criterion for the evaluation of the activity of these surfactants in human skin.

From these findings, we may conclude that surfactant–SC liposome subsolubilizing interactions are mainly ruled by the

TABLE 2
Surfactant-to-Lipid Molar Ratios (*Re*), Partition Coefficients (*K*) and Surfactant Concentrations in the Aqueous Medium (S_W), Resulting in the Subsolubilizing Interaction (50 and 100% of 5(6)-carboxyfluorescein release) of Dodecyl Betaine Surfactants with Stratum Corneum Liposomes at Different pH[a]

	$S_{w,50\%CF}$ (mM)	$S_{W,100\%FC}$ (mM)	$Re_{50\%CF}$ mole/mole	$Re_{100\%CF}$ mole/mole	$K_{50\%CF}$ (mM^{-1})	$K_{100\%CF}$ (mM^{-1})	r^2 (50%CF)	r^2 (100%CF)
7.5	0.418	0.838	0.68	0.79	0.968	0.526	0.994	0.994
7.0	0.411	0.810	0.61	0.72	0.921	0.516	0.996	0.992
6.5	0.405	0.785	0.54	0.66	0.865	0.506	0.992	0.998
6.0	0.395	0.760	0.49	0.61	0.832	0.498	0.996	0.994
5.5	0.385	0.753	0.45	0.57	0.806	0.482	0.994	0.996
5.0	0.375	0.750	0.43	0.55	0.802	0.473	0.997	0.995

[a]The regression coefficients of the straight lines obtained are also included.

action of surfactant monomers, in agreement with the behavior reported for the interaction of these surfactants with PC unilamellar liposomes (33). The nonionic surfactant OP-10EO showed the highest ability to alter the permeability of SC bilayers (lowest *Re* values) and the highest affinity with these structures (highest *K* values), whereas the amphoteric surfactant D-Bet showed the lowest tendencies. The low activity of D-Bet at pH 7.20 in PIPES buffer may be attributed to the fact that, at this pH, this surfactant was structured as a internally compensated quaternary ammonium compound instead of a external salt with some cationic character. This fact emphasizes the role played by the polar part of this surfactants in its ability to alter the permeability of SC liposomes. Although the anionic surfactants SDS and SDES showed, at 50% CF release, similar capacities to alter the permeability of SC bilayer, the SDES appeared to be more active at 100% CF release (low *Re* value), and the affinity of these surfactants with SC bilayers follow similar tendencies. This effect can be attributed to the presence of 2.5 EO units in the SDES molecular structure. Different trends in the evolution of the interaction of surfactant–SC liposomes with respect to those for surfactant–PC liposomes may be observed when comparing the present *Re* and *K* parameters with those reported for the interaction of these surfactants with PC liposomes. Thus, whereas SC liposomes appeared to be more resistant to the action of surfactant monomers, the affinity of these compounds with SC structures was always greater than with PC structures. This finding is closely connected with the fact that the free surfactant concentration for SC liposomes ($S_{W,50\%CF}$ and $S_{W,100\%CF}$) was always smaller than that reported for PC unilamellar liposomes in the same interaction steps.

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REFERENCES

1. Foresta, B., F. Henao, and P. Champeil, Kinetic Characterization of the Perturbation by Dodecylmaltoside of Sarcoplastic Reticulum Ca^{2+}-ATPase, *Eur. J. Biochem. 209*:1023–1034 (1992).
2. Wach, A., N.A. Dencher, and P. Gräber, Co-reconstitution of Plasma Membranes H^+-ATPase from Yeast and Bacteriorhodopsin into Liposomes. ATP Hydrolysis as a Function of External and Internal pH, *Eur. J. Biochem. 214*:563–568 (1993).
3. Kerry, C.J., H.L. Sudan, K. Abutidze, I.R. Mellor, E.A. Barnard, and P.N.R. Usherwood, Reconstitution of Glutamate Receptor Proteins Purified from Xenopus Central Nervous System into Artificial Bilayers, *Mol. Pharmacol. 44*:142–152 (1993).
4. Partearroyo, M.A., M.A. Urbaneja, and F.M. Goñi, Effective Detergent/Lipid Ratios in the Solubilization of Phosphatidylcholine Vesicles by Triton X-100, *FEBS Lett 302*:138–140 (1992).
5. Levy, D., A. Gulik, M. Seigneuret, and J.L. Rigaud, Phospholipid Vesicle Solubilization and Reconstitution by Detergents. Symmetrical Analysis of the Two Processes Using Octaethylene Glycol Mono-*n*-dodecyl Ether, *Biochemistry 29*:9480–9488 (1990).
6. Urbaneja, M.A., A. Alonso, J.M. González-Mañas, F.M. Goñi, M.A. Partearroyo, M. Tribout, and S. Paredes, Detergent Solubilization of Phospholipid Vesicles. Effect of Electric Charge, *Biochem. J. 270*:305–308 (1990).
7. Kragh-Hansen, U., M. le Marie, J.P. Nöel, T. Gulik-Krzywicki, and J.V. Møller, Transition Steps in the Solubilization of Protein-Containing Membranes and Liposomes by Nonionic Detergent, *Biochemistry 32*:1648–1656 (1993).
8. Lichtenberg, D., Characterization of the Solubilization of Lipid Bilayers by Surfactants, *Biochim. Biophys. Acta 821*:470–478 (1985).
9. Friberg, S.E., L.B. Goldsmith, I. Kayali, and H. Suhaimi, in I*nterfacial Phenomena in Biological Systems,* Surfactant Science Series, Vol. 39, edited by M. Bender, Marcel Dekker, Inc., New York, 1991, pp. 3–32.
10. Bouwstra, J.A., G.S. Gooris, W. Bras, and D.T. Downing, Lipid Organization in Pig Stratum Corneum, *J. Lipid Res. 36*:685–695 (1995).
11. Abraham, W., P.W. Wertz, and D.T. Downing, Linoleate-Rich Acylglucosylceramides of Pig Epidermis: Structure Determination by Proton Magnetic Resonance. *Ibid. 26*:761–766 (1985).
12. Ranasingle, A.W., P.W. Wertz, D.T. Downing, and J.C.Mackeine, Lipid Composition of Cohesive and Desquamated Corneocytes from Mouse Ear Skin, *J. Invest. Dermatol. 86*:187–190 (1986).
13. Imokawa, G., A. Abe, K. Jin, Y. Higaki, M. Kamashima, and A. Hidano, Decreased Level of Ceramides in Stratum Corneum of Atopic Dermatitis: An Etiologic Factor in Atopic Dry Skin? *J. Invest. Dermatol. 96*:523–526 (1991).
14. Wertz, P.W., and D.T. Downing, Stratum Corneum: Biological an Biochemical Considerations, in *Transdermal Drug Delivery. Developmental Issues and Research Initiatives,* edited by J. Hadgraft and R.H Guy, Marcel Dekker, Inc. New York, 1989, pp. 1–22.
15. Wertz, P.W., W. Abraham, L. Landman, and D.T. Downing, Preparation of Liposomes from Stratum Corneum Lipids, *J. Invest. Dermatol. 87*:582–584 (1986).
16. Wertz, P.W., Liposome Dermatics, Chemical Aspects of the Skin Lipid Approach, in *Liposome Dermatics (Griesbach Conference),* edited by O. Braun-Falco, H.C. Korting, and H. Maibach, Springer-Verlag, Berlin, Heidelberg, 1992, pp. 38–43.
17. Abraham, W., P.W. Wertz, L. Landman, and D.T. Downing, Stratum Corneum Lipid Liposomes: Calcium-Induced Transformation into Lamellar Sheets, *J. Invest. Dermatol. 88*:212–214 (1987).
18. Downing, D.T., W. Abraham, B.K. Wegner, K.W. Willman, and J.M. Marshall, Partition of Sodium Dodecyl Sulfate into Stratum Corneum Lipid Liposomes, *Arch. Dermatol. Res. 285*:151–157 (1993).
19. Blume, A., M. Jansen, M. Ghyczy, and J. Gareiss, Interaction of Phospholipid Liposomes with Lipid Model Mixtures for Stratum Corneum Lipids, *Int. J. Pharm. 99*:219–228 (1993).
20. de la Maza, A., and J.L. Parra, Vesicle-Micelle Structural Transitions of Phosphatidylcholine Bilayers and Triton X-100, *Biochem. J. 303*:907–914 (1994).
21. de la Maza, A., and J.L. Parra, Structural Phase Transitions Involved in the Interaction of Phospholipid Bilayers with Octyl Glucoside, *Eur. J. Biochem. 226*:1029–1038 (1994).
22. de la Maza, A., and J.L. Parra, Solubilization of Unilamellar Liposomes by Betaine-type Zwitterionic/Anionic Surfactant Systems, *J. Am. Oil Chem. Soc. 72*:131–136 (1995).
23. de la Maza, A., A.M. Manich, L. Coderch, P. Bosch, and J.L. Parra, The Formation of Liposomes *in vitro* by Mixtures of Lipids Modeling the Composition of the Stratum Corneum, *Colloids and Surfaces A: Physicochem. Eng. Aspects 101*:9–19 (1995).

24. Edwards, K., M. Almgren, J. Bellare, and W. Brown, Effects of Triton X-100 on Sonicated Lecithin Vesicles, *Langmuir 5*:473–478 (1989).
25. Paternostre, M.T., M. Roux, and J.L. Rigaud, Mechanisms of Membrane Protein Insertion into Liposomes During Reconstitution Procedures Involving the Use of Detergents. 1. Solubilization of Large Unilamellar Liposomes (Prepared by Reverse Phase Evaporation) by Triton X-100, Octyl Glucoside and Sodium Cholate, *Biochemistry 27*:2668–2677 (1988).
26. Ruiz, J., F.M. Goñi, and A. Alonso, Surfactant-Induced Release of Liposomal Contents. A Survey of Methods and Results, *Biochim. Biophys. Acta 937*:127–134 (1988).
27. Rosen, M.J., Purification of Surfactants for Studies of Their Fundamental Surface Properties, *J. Colloid Interface Sci. 79*:587–588 (1981).
28. Weinstein, J.N., E. Ralston, L.D. Leserman, R.D. Klausner, P. Dragsten, P. Henkart, and R. Blumenthal, Self-Quenching of Carboxyfluorescein Fluorescence: Uses in Studying Liposome Stability and Liposome Cell Interaction, in *Liposome Technology,* edited by G. Gregoriadis, CRC Press, Boca Raton, Vol. III, 1986, pp. 183–204.
29. Ackman. R.G., C.A. Mc Leod, and A.K. Banerjee, An Overview of Analyses by Chromarod-Iatroscan TLC-FID, *J. Planar Chrom. 3*:450–490 (1990).
30. Almog, S., B.J. Litman, W. Wimley, J. Cohen, E.J. Wachtel, Y. Barenholz, A. Ben-Shaul, and D. Lichtenberg, States of Aggregation and Phase Transformations in Mixtures of Phosphatidylcholine and Octyl Glucoside. *Biochemistry 29*:4582–4592 (1990).
31. Schurtenberger, P., N. Mazer, and W. Känzig, Micelle to Vesicle Transition in Aqueous Solutions of Bile Salt and Lecithin, *J. Phys. Chem. 89*:1042–1049 (1985).
32. Lunkenheimer, K., and D. Wantke, Determination of the Surface Tension of Surfactant Solutions Applying the Method of Lecomte du Noüy (Ring Tensiometer), *Colloid and Polymer Sci. 259*:354–366 (1981).
33. de la Maza, A., J. Sanchez, J.L. Parra, M.T. Garcia, and I. Ribosa, Permeability Changes of Phospholipid Bilayers Caused by Surfactants, *J. Amer. Oil Chem. Soc. 68*:315–319 (1991).
34. de la Maza, A., and J.L. Parra, Solubilization of Phospholipid Bilayers Caused by Surfactants, *Ibid. 70*:699–706 (1993).
35. Schubert, R., K. Beyer, H. Wolburg, and K.H. Schmidt, Structural Changes in Membranes of Large Unilamellar Vesicles After Adding of Sodium Cholate, *Biochemistry 25*:5263–5269 (1986).
36. de la Maza, A., and J.L. Parra, Alterations in Phospholipid Bilayers Caused by Oxyethylenated Nonylphenol Surfactants, *Arch. Biochem. Biophys. 329*:1–8 (1996).
37. Ernst, R., and E.J. Miller, in *Amphoteric Surfactants,* Surfactant Science Series, Vol. 12, edited by B.R. Bluestein and C.L. Hilton, Marcel Dekker, Inc., New York, 1982, pp. 71–174.

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