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Review

Different stratum corneum lipid liposomes as models to evaluate the effect of the sodium dodecyl sulfate

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Abstract

The stability of stratum corneum (SC) liposomes against the action of surfactants has been revised. To this end, two types of vesicles were used; vesicles formed with the lipid and protein material extracted from SC, and lipid mixtures approximating the SC composition. In this case, the proportion of ceramides (Cer) and cholesteryl sulfate (Chol-sulf) was varied and the relative proportion of the other lipids remained constant. The increasing presence of these two lipids increased the resistance of liposomes against the action of the anionic surfactant sodium dodecyl sulfate (SDS). The rise in the cell-to-cell cohesion that occurred in recessive X-linked ichthyosis due to the accumulation of Chol-sulf could be associated in part to the enhanced stability of (Chol-sulf)-enriched bilayers. It is noteworthy that the surfactant partitioning between bilayers and the aqueous phase increased and decreased, respectively, as the proportion of Cer and Chol-sulf increased. This effect may be attributed to the variations in both the electrostatic interactions lipid–surfactant (electrostatic repulsion between the sulfate groups of both Chol-sulf and SDS), and the hydrophilic lipophilic balance of the lipid mixtures, in which Cer is replaced by the major polar lipid of the mixture (Chol-sulf). The fact that the free surfactant concentration was always smaller than its critical micelle concentration indicates that the permeability alterations were mainly ruled by the action of surfactant monomers, in agreement with the results reported for sublytic interactions of this surfactant with PC liposomes.

Keywords: Stratum corneum lipid liposome; Sodium dodecyl sulfate; Stratum corneum lipid liposome/sodium dodecyl sulfate interaction; Influence of the lipid composition on the liposome stability; Carboxyfluorescein release; Dynamic light scattering; Surfactant/lipid molar ratio; Surfactant partition coefficient

1. Introduction

The stratum corneum (SC) forms a continuous sheath of alternating squamae (protein-enriched corneocytes) embedded in an intercellular matrix enriched in unpolar lipids displayed as lamellar sheets. One of the key functions of SC lipids (SCLs) is to maintain the permeability barrier of the skin [1,2]. It has been established that the perturbations in the organized structure of these lipids affect the skin barrier function [3,4], and changes in the lipid composition are associated with different skin symptoms. Thus, there is a marked decrease in ceramides (Cer) in patients with atopic dermatitis, suggesting that an insufficiency of this lipid is an etiologic factor in atopic dry and barrier-disrupted skin [5–7]. Further-

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more, the level of cholesterol (Chol) and cholesteryl sulfate (Chol-sulf) is claimed to play an important role in the stability properties of the SC (cohesion, desquamation) and in the regulation of the skin barrier function [8–10]. Thus, patients with recessive X-linked ichthyosis show elevated proportions of Chol-sulf due to steroid sulfatase deficiency [11], whereas tissues with extremely tenacious intercellular cohesion also present higher Chol-sulf proportions than that existing in skin lipids [12].

Surfactants have been used for many years in topical skin preparations and are included in standard creams and ointments. Their inclusion is normally based on their effects on the stability or appearance of the product [13,14], and little consideration is generally given to the effect they may have on the activity of compounds contained in the formulation. Surfactants do, however, penetrate the skin and may interact with its components thereby altering its barrier function [15,16]. They have been shown to be capable of both increasing and decreasing the penetration profile of topically administered compounds.

To gain a better understanding of the interaction of surfactants with the SC, the model surfactant sodium dodecyl sulfate (SDS) is frequently used [17–20]. This anionic surfactant induces experimental irritant contact dermatitis in animals and humans [21], and characteristically induces a dose-related increase in transepidermal water loss [22–24]. SDS irritation has also been used to test efficacy and value of barrier creams, moisturizers [25,26] and lipid mixtures [27].

Despite that the use of purebred laboratory animals in current irritation tests seems to ensure a uniformity of response [28] that cannot be expected in humans [29], the degree and the time course of irritation and barrier recovery in each case seem to be different [30]. Therefore, studies have been performed on human skin in vivo [31]. But the situation in humans is complicated given that the factors governing individual responses to irritants are still poorly characterized. Thought also has to be given to the possibility of intrinsic variation in response to irritants [29,32]. It is likely that barrier function, which is mainly related to epidermical lipid composition, plays an important role in the individual's susceptibility and response [29,33].

Taking into account that the intercellular lipid lamellae of the SC provide the major barrier to the percutaneous penetration, it is important to quantitate the partitioning of irritating agents as surfactants into the lipid phase of the SC. These lipid lamellae consist predominantly of Cer, Chol and free fatty acids (FFA) [34,35]. However, SC has been shown to be virtually devoid of phospholipids, as a result of which its ability to form bilayers has proved to be somewhat surprising. In order to establish whether SCLs could form bilayers, Wertz and Abraham [36–38] prepared unilamellar liposomes from lipid mixtures approximating the composition of SCLs at physiological pH. Although these liposomes have not been prepared at pH 5 (surface skin pH), the interest of these structures is their characteristic organization forming lamellae in the same way as the intercellular spaces of the SC. In fact, a recent X-ray diffraction study with SCL mixtures has revealed that the lamellar ordering at pH 5 and 7.4 is similar [39]. Despite the limitations of liposomes, these structures have been described as good models for studying the lipid properties of the SC [40] and the diffusion of molecules in hepatocytes [41]. It would be very advantageous, therefore, to study the interactions of surfactants, or some other skin permeants, with SCL liposomes as an alternative in vitro membrane model for skin permeability studies. This work is a review of our studies, whose purpose is to shed light on the influence of the lipid composition on the resistance of SC liposomes against surfactants. These data could be useful to correlate structural aspects of two types of interactions: surfactant with lipids forming liposomes and with lipids building the SC tissue for the benefit of the scientists whose experience does not include the use of this type of membrane model.

2. Interaction of surfactants with SC liposomes

2.1. Liposomes formed from lipid and protein material extracted from the SC

A mixture of lipids and proteins extracted from the SC using chloroform/methanol mixtures was able to form liposomes when the dried material was hydrated and sonicated at 70°C [42]. These liposomes will be referred to hereafter as 'proteolipo-

somes' given their lipoproteic nature. The experimental evidence of vesicle formation was, in addition to the TEM observations, the fact that these structures showed internal volume (1.2 μl per mg of extracted material). The vesicle size distribution curve after preparation and equilibration for 60 min at 37°C showed a monomodal distribution with a particle size of about 150 nm and a polydispersity index (PI) of 0.240, indicating that the vesicle dispersion was reasonably homogeneous. Both parameters remained constant in the absence of surfactants during the 24 h after preparation with a slight increase in the PI (final value of about 0.280), indicating that this suspension was stable to aggregation during this period.

A kinetic study on the release of the fluorescent agent 5(6)-carboxyfluorescein (CF) encapsulated into proteoliposomes or into PC unilamellar liposomes was carried out to study the permeability changes resulting in the interaction of three surfactants: Triton X-100 (T_{X-100}), SDS and dodecyl betaine (D-Bet) with these vesicles. The permeability alterations were quantitatively determined by monitoring the release of the CF trapped into these vesicles at 25°C [43]. The results are shown in Fig. 1 [42]. The interaction of both types of liposomes with the surfactants showed similar tendencies. Thus, the interaction with the non-ionic surfactant T_{X-100} led in both cases to the largest increase in the percentage of CF released after 50 min of treatment, whereas the amphoteric surfactant D-Bet resulted in the lowest increase. Hence, the T_{X-100} showed the largest capacity for altering liposome permeability and the D-Bet exhibited the smallest. The effect of surfactants on SCL lamellae or even on the viable epidermis is strongly dependent of the concentration used [31]. Taking into account that the three surfactants were used at the same concentration (0.45 mM), the different liposome permeability caused by these surfactants was probably due to their different critical micellar concentrations (T_{X-100} 0.15 mM, SDS 0.50 mM and D-Bet 1.25 mM). For this reason, the SDS that has been extensively used to induce irritant contact dermatitis in animals and humans [21–25] showed an intermediate effect. Although the SDS concentration used in this experiment would be too low to induce a contact dermatitis, it is able to induce changes in the permeability of liposomes.

The proteoliposomes appeared to be particularly resistant to the action of surfactants exhibiting in the initial interaction steps (from 5 to 15 min) low CF release kinetics. The fact that proteoliposomes showed a negligible permeability in the absence of surfactants with respect to that of PC liposomes can be explained bearing in mind the differences in lipid composition and consequently in their membrane organizations. At room temperature, the PC liposomes are in a more fluid phase than the proteoliposomes due to the different phase transition temperatures of egg PC (below 0°C), and pig SCLs building proteoliposomes (between 60 and 65°C) [42]. This fact possibly reduces the spontaneous permeability of proteoliposomes with respect to that of PC liposomes at room temperature. In spite of the different spontaneous permeability of these two liposomes, the presence of identical amounts of different surfactants resulted in both cases in similar changes in the bilayer permeability 50 min after the surfactant addition, although with different CF release kinetics.

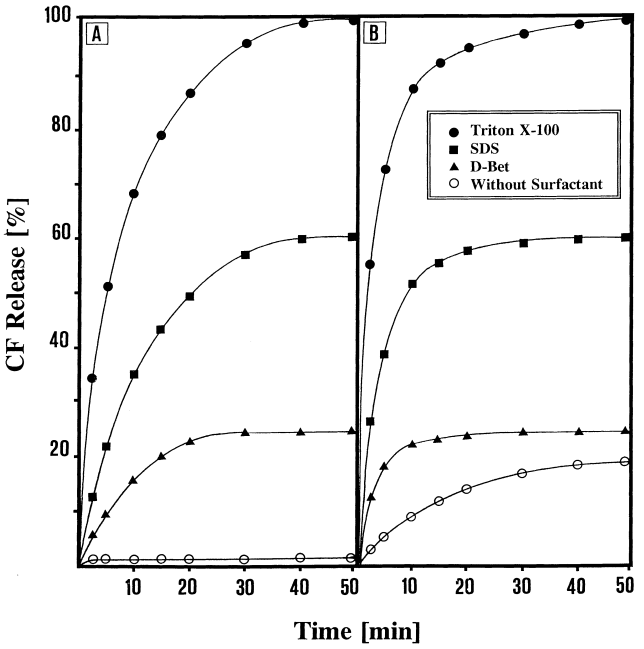

Fig. 1. Variation in the permeability of proteoliposomes (A) and PC liposomes (B) resulting in the interaction with identical amounts (0.45 mM) of T_{X-100} (●), SDS (■) and D-Bet (▲) as a function of time. The spontaneous permeability of liposome suspensions versus time is also indicated in both cases (○).

2.2. Liposomes formed by lipids modeling the SC

Different criteria have been followed to form these type of vesicles. Several authors reported that SCLs including extracted Cer formed stable liposomes [44–48]. These authors demonstrated that SDS in dilute solutions can partition into lipid bilayers so as to affect the properties of the lipid lamellae that constitute the epidermical permeability barrier [48]. A drawback of this model was that the natural SCLs are not available in large quantities. A recent work [49] has described that SCL mixtures containing synthetic Cer from bovine brain are also able to form liposomes, which have been successfully used as model for recessive X-linked ichthyosis. Taking into account these considerations, the Cer used in this work were bovine brain Cer type III (Sigma Chemical, St. Louis, MO, USA) despite the fact that different types of Cer affect the phase behavior of the model system [50]. It is well know that the health SC contains (in addition to Cer and Chol) about 2–5% of Chol-sulf, long-chain FFA (C22, C24) and small amounts of cholesteryl esters and triglycerides [51–53]. The composition used to form these liposomes was similar but not identical to the SC tissue given that we seek to obtain a simple and reproducible model of the SCL structure.

In order to know whether a range of lipid concentrations capable of forming liposomes exists and to characterize these structures, some physico-chemical parameters of these vesicles were determined [54]. To this end, an orthogonal composite factorial design of Box and Behnken [55] was used, the central lipid composition corresponding to that reported by Wertz et al. (40% Cer, 25% Chol, 25% palmitic acid (PA) and 10% Chol-sulf) [36]. A variation of ±15% for the different lipids, except Chol-sulf which was varied ±100%, was considered. The results show that unilamellar bilayer structures are formed in all cases, their physico-chemical properties being correlated with the lipid composition of vesicles. An increase in the proportions of Cer, PA and Chol resulted in a slight decrease in the size and in the internal volume of vesicles, whereas increasing proportions of Chol-sulf led to an opposite effect on these parameters. In addition, other authors have described that the lipid composition of different SC models (such as Langmuir–Blodgett monolayers, lamellar phases, oriented and unoriented multilayers) affected also the lipid organization and the phase behavior [50,56,57].

The mean size and the internal volume of vesicles showed the same behavior versus the changes in the lipid composition, suggesting the formation of the same type of vesicles in all cases. From these mean size and internal volume data and considering the characterization of the geometric properties of PC unilamellar vesicles [58], it is reasonable to assume that unilamellar liposomes were formed throughout the experimental domain studied.

2.2.1. Influence of the lipid composition on the stability of liposomes against SDS

The role played by the Cer and Chol-sulf in the interaction of the anionic surfactant SDS with SC liposomes was investigated. To this end, the surfactant to lipid molar ratios (Re) and the surfactant partitioning between bilayer and the aqueous phase (partition coefficients K) of these interactions were determined. These parameters were determined by monitoring the changes in the fluorescence intensity of liposomes due to the CF released from the interior of vesicles.

In the analysis of the equilibrium partition model proposed by Schurtenberger et al. [59] for bile salt/lecithin systems, Lichtenberg et al. [60] and Almog et al. [61] have shown that for a mixing of lipids (at a 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:

$$K = \mathrm{Re}/S_W\,(1 + \mathrm{Re}) \quad (1)$$

where Re is the effective surfactant to lipid molar ratio in the bilayers ($\mathrm{Re} = S_B/L$), S_B is the surfactant concentration in bilayers (mM), S_W is the surfactant concentration in the aqueous medium (mM) and L is the total lipid concentration (mM). The validity of this model for the systems investigated has been reported [62,63].

The Re, S_W and K parameters were determined on the basis of the linear dependence between the surfactant concentrations required to achieve 50 and 100% CF release and the SCL concentration, which

is described by the equation:

$$S_T = S_W + Re[SCL] \quad (2)$$

where S_T ($S_{T,50\%CF}$, $S_{T,100\%CF}$) are the total surfactant concentrations. The surfactant to lipid molar ratios Re ($Re_{50\%CF}$, $Re_{100\%CF}$) and the surfactant aqueous concentration S_W ($S_{W,50\%CF}$, $S_{W,100\%CF}$) are in each curve the slope and the ordinate at the origin (zero lipid concentration), respectively. The $K_{50\%CF}$ and $K_{100\%CF}$ parameters (bilayer/aqueous phase partition coefficient for 50 and 100% CF release) were determined from Eq. 1.

2.2.1.1. Influence of the level of Cer. A systematic study of permeability variations of liposomes due to the action of SDS was performed varying the proportion of Cer from 30 to 50% (lower and higher values than that in SCLs, which was 40%), the relative proportion of the other lipids remaining constant (Table 1). To determine the time needed to obtain a constant level of CF release, liposomes at different lipid compositions were treated with SDS and the subsequent CF release changes were studied as a function of time. The curves for 1.0 mM lipid liposomes treated with 0.3 mM SDS are given in Fig. 2. The CF release was in all cases a biphasic process, in which the presence of increasing amounts of Cer in bilayers resulted in an increased period of time to achieve a CF release plateau. Hence, although the liposomes modeling the SCL composition (experiment 3, Table 1) needed about 60 min to achieve this plateau, higher and lower Cer proportions resulted in increased and reduced periods of time to achieve this constant CF release level (from 40 to 80 min). The need of increased periods of time to achieve a constant CF release level in liposomes containing rising Cer amounts may be attributable to the different phase transition temperature of the mixtures of lipids, which depends on chain length, degree of saturation and nature of the polar head [54]. These differences affect both the assembly properties and mobility of the lipids and consequently, their ability to interact with surfactants.

The fact that the curves exhibited always a biphasic behavior may be attributable to the release of the CF encapsulated into the vesicles. In order to explain this release, different arguments have been proposed in the literature. Thus, several authors have considered that the incorporation of surfactant monomers in membranes may induce the formation of hydrophilic pores or merely stabilize transient holes [64–66]. Other authors have described that the lipid mixtures containing Cer form rigid crystalline phases at physiological temperatures [67]. Assuming that the incorporation of SDS in these bilayers could cause a phase transition, the possibility of the release of substances through the phase boundaries should also be considered.

To determine the Re and S_W parameters, the CF release variations were studied for a lipid concentration ranging from 0.5 to 5.0 mM. In order to study the permeability changes at the equilibrium, the CF release changes were measured in each case at the optimum interval, in accordance with the CF release curves of Fig. 2. The release of the fluorescent agent encapsulated into liposomes in the absence of surfactant (spontaneous release) in these intervals was negligible in all cases. In Fig. 3A is plotted the percent-

Table 1
Surfactant to lipid molar ratios (Re), partition coefficients (K) and surfactant concentrations in the aqueous medium (S_W) resulting in the interaction of SDS with SCL liposomes at the two interaction levels investigated (50 and 100% CF release)

Exp. no.	Liposome lipid composition (%)				$S_{W,50\%CF}$ (mM)	$S_{W,100\%CF}$ (mM)	$Re_{50\%CF}$ (mol/mol)	$Re_{100\%CF}$ (mol/mol)	$K_{50\%CF}$ (mM^{-1})	$K_{100\%CF}$ (mM^{-1})	r^2 (50% CF)	r^2 (100% CF)
	Cer	Chol	PA	Chol-sulf								
1	30.0	29.0	29.0	12.0	0.079	0.278	0.12	0.62	1.36	1.37	0.993	0.997
2	35.0	27.0	27.0	11.0	0.081	0.283	0.24	0.84	2.39	1.61	0.994	0.991
3	40.0	25.0	25.0	10.0	0.083	0.289	0.35	1.0	3.12	1.73	0.994	0.996
4	45.0	23.0	23.0	9.0	0.087	0.295	0.39	1.11	3.22	1.78	0.997	0.993
5	50.0	21.0	21.0	8.0	0.091	0.299	0.42	1.17	3.25	1.80	0.993	0.993

The liposome lipid composition, varying the percentage of Cer from 30 to 50% and the relative proportions of the other lipids remaining constant, is also included.

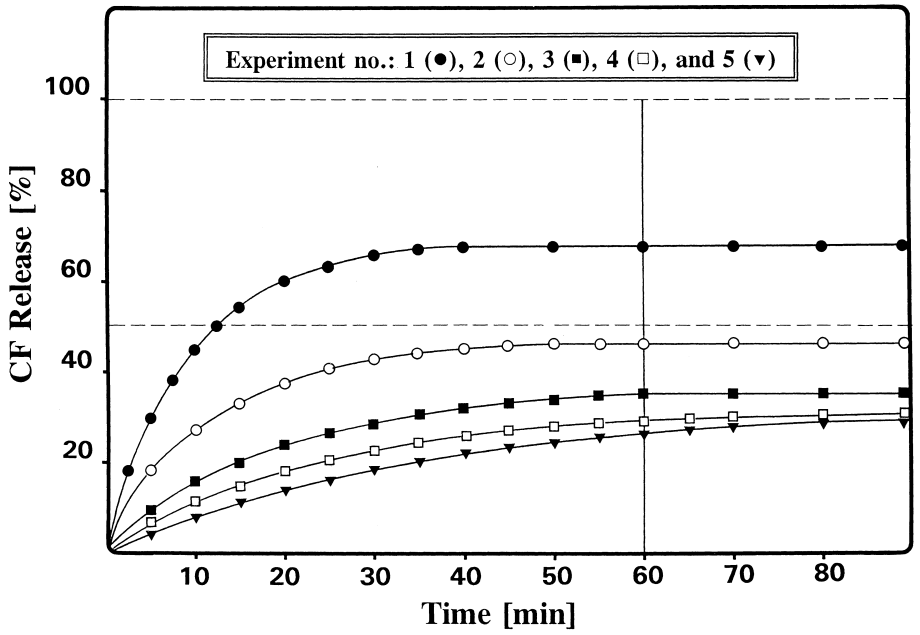

Fig. 2. Time curves of the release of CF trapped into SCL liposomes at the five lipid compositions indicated in Table 1, and caused by the addition of SDS. Lipid concentration in liposomes 1.0 mM and SDS concentration 0.3 mM. Experiment symbols 1 (●), 2 (○), 3 (■), 4 (□) and 5 (▼).

age of CF release as a function of the SDS concentration (resulting in 50% and 100% CF release) for a lipid concentration (ranging from 0.5 to 5.0 mM) corresponding to experiment no. 3 (40% Cer, 25% Chol; 25% PA, 10% Chol-sulf). In Fig. 3A, all the CF release data were measured when a constant release level was achieved (40–80 min after SDS addition, as it is noted in Fig. 2). A linear relationship was established in each case. The results corresponding to experiment no. 3 are plotted in Fig. 3B. The error bars given in this figure are S.D. and represent the error of three replicates. The straight lines obtained corresponded to Eq. 2 from which Re and S_W were determined. The Re, K and S_W parameters for the five experiments investigated including the regression coefficients (r^2) of the straight lines are given in Table 1.

Re is the effective surfactant to lipid molar ratio in the bilayer that induces 50 or 100% of the encapsulated agent release ($Re_{50\%CF}$ and $Re_{100\%CF}$, respectively). This parameter is inversely related to the surfactant activity, i.e. the higher Re value the higher surfactant concentration needed to induce a given permeability alteration in bilayers. The variations of the $Re_{50\%CF}$ and $Re_{100\%CF}$ versus the proportion of Cer are plotted in Fig. 4A. A progressive increase in Re (lower SDS ability to alter the permeability of liposomes) occurred as the percentage of Cer in bilayers rose, this increase being more pronounced at low Cer proportions especially for $Re_{100\%CF}$.

K is the partition coefficient of surfactant between the lipid bilayer and the aqueous media. The $K_{50\%CF}$ and $K_{100\%CF}$ correspond with this coefficient when 50 or 100% of encapsulated agent is released from the liposomes. The K parameters are directly related to the ability of the surfactant to be incorporated on the bilayers (surfactant affinity). The variations of K (50% and 100% of CF release) versus the percentage of Cer are plotted in Fig. 4B. Both the $K_{50\%CF}$ and $K_{100\%CF}$ parameters increased with Cer concentration in bilayers up to achieve almost constant values for about 45% of Cer. The $K_{100\%CF}$ showed lower values than those for $K_{50\%CF}$. This indicates that the affinity of surfactant for the bilayers decreases as the number of SDS molecules incorporated in bilayers increases (Re increase). This fact could be explained assuming that at low Re only the outer vesicle leaflet was available by the surfactant molecules, the binding of additional SDS molecules to bilayers being hampered at slightly higher Re values. These findings are in agreement with those reported by Schubert et al. [68] for the interaction sodium cholate/PC liposomes. The

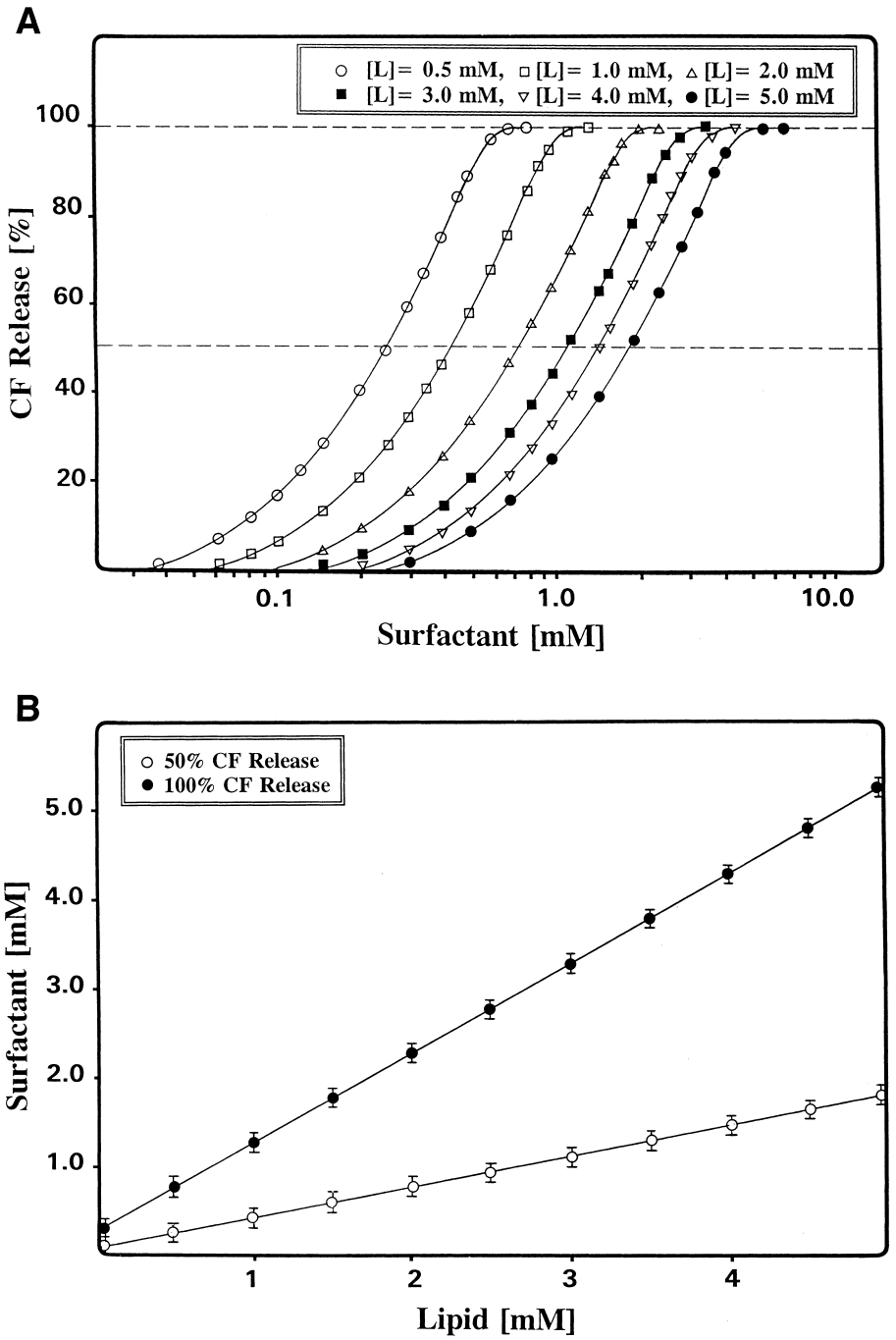

Fig. 3. A: Percentage changes in CF release of SCL liposomes (lipid composition for the experiment no. 3, Table 1), the lipid concentration ranging from 0.5 to 5.0 mM, induced by the presence of increasing concentrations of SDS. Lipid concentrations: 0.5 mM (○), 1.0 mM (□), 2.0 mM (△), 3.0 mM (■), 4.0 mM (▽), 5.0 mM (●). B: Surfactant concentrations resulting in 50 and 100% CF release versus lipid concentration of SCL liposomes for the experiment no. 3 of Table 1. 50% CF release (○) and 100% CF release (●).

fact that the difference between $K_{50\%CF}$ and $K_{100\%CF}$ increased with the concentration of Cer in bilayers (up to achieve the maximum difference for a Cer proportion of about 45%) suggests that the increasing presence of this lipid progressively hampers the incorporation of surfactant monomers to the outer vesicle leaflet, in agreement with the results given in Fig. 2. The more pronounced increase occurred for

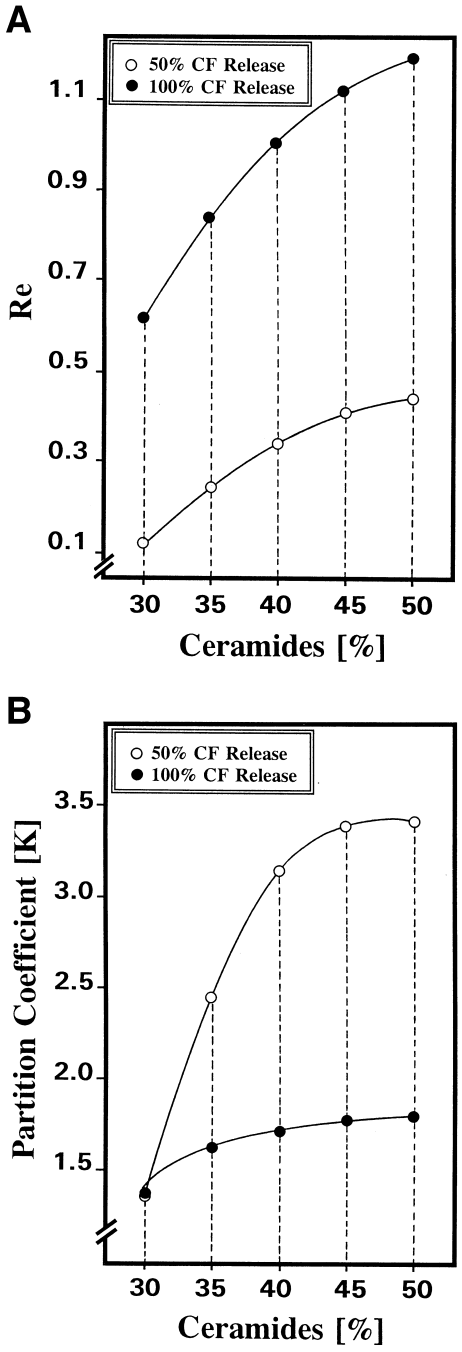

Fig. 4. A: Effective surfactant to lipid molar ratios ($Re_{50\%CF}$ and $Re_{100\%CF}$) in SCL liposomes for SDS versus the percentage of Cer in liposomes. $Re_{50\%CF}$ (○) and $Re_{100\%CF}$ (●). B: Partition coefficients ($K_{50\%CF}$ and $K_{100\%CF}$) in SCL liposomes for SDS versus the percentage of Cer in liposomes. $K_{50\%CF}$ (○) and $K_{100\%CF}$ (●).

$K_{50\%CF}$ in the range of Cer concentrations from 30 to 40%. Hence, despite the reduced partitioning of SDS molecules in liposomes containing a low proportion of Cer (low affinity with these bilayer structures), their ability to alter these structures was higher than that for bilayers approximating the SCL composition (percentage of Cer of about 40%). This finding emphasizes the low resistance of these bilayers against the action of SDS. Inversely, the high K and Re values at Cer levels approximating that in the SCLs reveals that although an increased number of surfactant molecules were incorporated into bilayers, these molecules were less able to alter their permeability.

As for the free surfactant concentrations (S_W) the rise in the Cer percent in liposomes resulted in a slight increase in both $S_{W,50\%CF}$ and $S_{W,100\%CF}$, although showing smaller values than that of the surfactant critical micellar concentration in all cases (0.50 mM) [69]. This finding indicates that the permeability alterations were mainly ruled by the action of surfactant monomers, in agreement with the results reported for sublytic interactions of this surfactant with PC liposomes [69].

2.2.1.2. Influence of Chol-sulf. In parallel to Section 2.2.1.1, a systematic CF release study of liposomes varying the level of Chol-sulf and due to the action of SDS was performed [63]. To this end, the Chol-sulf percent was varied from 1 to 25% (lower and higher values than in SCLs, which was about 2% in human skin), the relative proportion of the other lipids remained constant (Table 2). In parallel to the preceding experiments, the CF release was in all cases a biphasic process, in which increasing Chol-sulf amounts led to an increased period of time to achieve a CF release plateau. This increase may be due, in addition to the reasons indicated in the case of the Cer, to the increasing electrostatic repulsion between the electronegative charges (sulfate groups) of both Chol-sulf and SDS. This repulsion may progressively hinder the incorporation of SDS monomers into the membranes.

To determine the Re and S_W parameters, the CF release changes were studied following the same experimental procedure described for Cer (lipid concentration ranging from 0.5 to 5.0 mM). The values for the six experiments investigated, including the regression coefficients (r^2) of the straight lines, are also given in Table 2. The increase in the proportion of Chol-sulf resulted in a rise in the Re values (spe-

cially pronounced for $Re_{100\%CF}$) up to a maximum was reached for Chol-sulf ranging from 10 to 15%. This indicates a decrease in the surfactant activity in the bilayers. Thus, the lowest SDS activity (maximum Re value) corresponded to the lipid mixtures containing 10–15% Chol-sulf. The increased SDS activity at low Chol-sulf proportions is surprising taking into account that the weight percentages of Chol-sulf in porcine and human SC are 3.9 and 1.9%, respectively [53]. In any case, these findings are in accordance with the recent study reported by Hatfield et al. [49], who demonstrated using a model system of SCL liposomes that vesicles with no Chol-sulf and 30% Chol were less stable against fusogenic agents such as Ca^{2+} and H^+ than liposomes containing 15% Chol-sulf and 15% Chol.

The SDS partitioning between bilayers and water sharply decreased as the percent of Chol-sulf in liposomes rose (decrease in the K values) up to almost a constant value was reached. This indicates a decrease in the surfactant affinity. As aforementioned, the increasing electrostatic repulsion between the negative groups of Chol-sulf and SDS may hinder the SDS incorporation into membranes, progressively decreasing the surfactant partitioning into bilayers. In the experimental conditions (20 mM PIPES buffer containing 110 mM Na_2SO_4 adjusted to pH 7.20), the FFA could be partially dissociated and hinder the SDS incorporation. However, this possible effect induced by the FFA charges would result constant so the FFA proportion remains unaltered in all these experiments. As a result, the progressive decrease of the SDS affinity is a consequence of the increasing amounts of Chol-sulf. The SDS also showed at 100% CF release lower K values than those for 50% possibly because at low Re only the outer vesicle leaflet was available for the interaction with surfactant molecules as occurred when varying the proportion of Cer [70]. The increasing presence of Chol-sulf in bilayers resulted in a clear increase in both $S_{W,50\%CF}$ and $S_{W,100\%CF}$, being this rise specially pronounced at low Chol-sulf proportions (from 1 to 10%). This effect is in accordance with the decrease in the surfactant partitioning in this range of Chol-sulf proportions. This finding, which is in agreement with the results reported for sublytic interactions of this surfactant with PC liposomes [66] and with SC liposomes varying the Cer proportions [62], indicates that the permeability alterations were in this case also ruled by the action of surfactant monomers.

It is believed that the ratio Chol/Chol-sulf in SCLs may be regulated by inserting exogenous Chol. Thus, it appears that the degree of scaling in patients suffering from X-linked ichthyosis (associated to excess of Chol-sulf) can be reduced by Chol replacement therapy. In fact, Rodrigueza et al. demonstrated using a PC liposome model that the rate of intermembrane exchange for Chol-sulf was approximately 10-fold faster than for Chol [71]. The stability of liposomes containing Chol-sulf concentrations from 15 to 25% (high and low Re and K values, respectively, Table 2) may be possibly attributed to the following reasons: first, a suitable equilibrium Chol/Chol-sulf in the range of molar ratios from 1.24 to 0.65 (experiments 4–6, Table 2). Second, the hydrophilic nature of Chol-sulf could affect the Ninham packing ratio of these lipid structures, which is mainly correlated with the volume of the molecule and its polar

Table 2
Surfactant to lipid molar ratios (Re), partition coefficients (K) and surfactant concentrations in the aqueous medium (S_W) resulting in the interaction of SDS with SCL liposomes at the two interaction levels investigated (50 and 100% CF release)

Exp. no.	Liposome lipid composition (%)				$S_{W,50\%CF}$ (mM)	$S_{W,100\%CF}$ (mM)	$Re_{50\%CF}$ (mol/mol)	$Re_{100\%CF}$ (mol/mol)	$K_{50\%CF}$ (mM^{-1})	$K_{100\%CF}$ (mM^{-1})	r^2 (50% CF)	r^2 (100% CF)
	Cer	Chol	PA	Chol-sulf								
1	44.0	27.5	27.5	1.0	0.023	0.120	0.11	0.41	4.31	2.42	0.992	0.993
2	42.2	26.4	26.4	5.0	0.060	0.225	0.28	0.82	3.64	2.0	0.997	0.997
3	40.0	25.0	25.0	10.0	0.083	0.289	0.35	1.0	3.12	1.73	0.994	0.996
4	37.8	23.6	23.6	15.0	0.100	0.320	0.36	1.04	2.64	1.59	0.996	0.992
5	35.6	22.2	22.2	20.0	0.100	0.330	0.33	1.03	2.48	1.54	0.998	0.995
6	33.4	20.8	20.8	25.0	0.095	0.330	0.29	1.0	2.36	1.51	0.994	0.996

The liposome lipid composition, varying the percentage of Chol-sulf from 1 to 25% and the relative proportions of the other lipids remaining constant, is also included.

Table 3
DLS data corresponding to the interaction of SDS with SCL liposomes (1.0 mM) for the experiments 1 and 4 of Table 2

SDS (mM)	Experiment no. 1				Experiment no. 4			
	Large particles		Mixed micelles		Large particles		Mixed micelles	
	nm	%	nm	%	nm	%	nm	%
0	105	100	–	–	105	100	–	–
0.5	104	100	–	–	105	100	–	–
0.9	100	97	8.2	3	105	100	–	–
1.0	78	76	8.2	24	104	100	–	–
1.1	57	54	8.3	46	104	100	–	–
1.2	37	32	8.3	68	104	100	–	–
1.5	–	0	8.3	100	104	100	–	–
2.0	–	–	8.3	100	90	87	8.5	13
2.5	–	–	8.3	100	72	67	8.5	33
3.0	–	–	8.4	100	48	44	8.5	56
3.5	–	–	8.4	100	27	22	8.6	78
4.0	–	–	8.4	100	7	2	8.6	98
4.5	–	–	8.4	100	–	–	8.6	100

group(s) area [70]. This modification might affect the supramolecular organization of bilayers, which plays an important role in the fluidization of these structures. In fact, in recessive X-linked ichthyosis, the absence of the enzyme steroid sulfatase produces the accumulation of Chol-sulf [72,73]. This abnormality results in an harmful increase in cell-to-cell cohesion within the SC affecting the supramolecular assembly of the intercellular lipids. Thus, it is interesting to note that the accumulation of Chol-sulf in liposomes and in the living SC caused a same effect: an increase in the cohesion of the lipid bilayers; however, the consequences of this effect are different on these two substrates. An increased cohesion results in a higher stability of the liposomes but in an anomalous desquamation of the SC [63,72,73]. Consequently, the extrapolation of data in these systems is limited. However, the stability detected for liposomes containing high Chol-sulf proportions against SDS might be considered to explain in some way the mechanism involved in the anomalous desquamation of the skin.

Dynamic light scattering (DLS) experiments. A series of DLS experiments were performed to shed light on the correlation between the Chol-sulf level in skin lipids and their resistance to be solubilized by SDS. To this end, we studied the size distribution of the aggregates 24 h after mixing increasing amounts of SDS with liposomes varying the level of Chol-sulf (Table 2) [63,74].

The DLS curves (at a scattering angle of 90°) of micellar SDS solutions (SDS concentration ranging

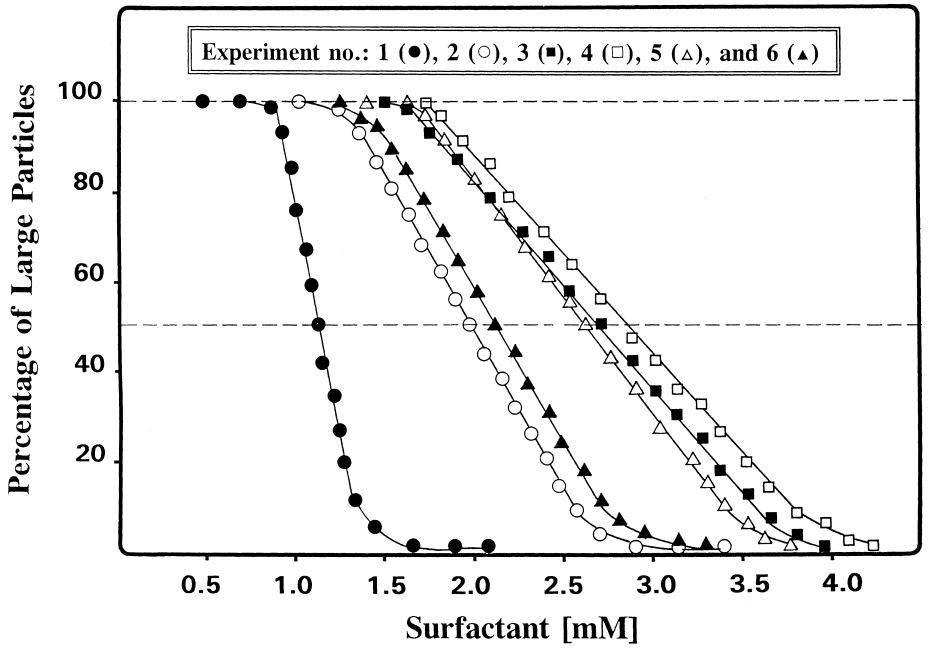

Fig. 5. Variation in the percentage of large particles during the solubilization of SCL liposomes (1.0 mM) by the SDS. The proportion of Chol-sulf in bilayers varied from 1 to 25% (Table 2). Experiment symbols: 1 (●), 2 (○), 3 (■), 4 (□), 5 (△) and 6 (▲).

from 1 to 5 mM) showed always a monomodal distribution with a peak at 2 nm. The DLS data for SCL vesicles and for some surfactant/lipid systems (experiments 1 and 4, Table 2) are given in Table 3. SC liposomes showed a monomodal distribution with a hydrodynamic diameter (HD) of 105 nm in both cases. The addition of low SDS amounts to liposomes did not produce noticeable changes in the size of the surfactant–lipid mixed vesicles formed. When the SDS concentration exceeded 0.9 and 2.0 mM (experiments 1 and 4, respectively), a new peak in the size distribution curve appeared (about 8.0 nm) corresponding to the formation of surfactant–lipid mixed micelles. Increasing surfactant amounts led to a progressive rise in the proportion of mixed micelles and to a fall in that for mixed vesicles (large particles). It is noteworthy that in this interval the HD of mixed micelles remained almost unaltered, whereas that for large particles decreased up to the complete solubilization of liposomes. Hence, in this interval, large particles and mixed micelles coexisted in different proportions (bimodal size distribution curves). The SDS concentrations of 1.5 and 4.5 mM (experiments 1 and 4, respectively) showed again a monomodal size distribution curve for mixed micelles (about 8.4–8.6 nm).

Fig. 5 shows the percent variation of large particles vs. the SDS concentration during the liposome solubilization process for all the lipid compositions studied. It may be seen that experiment 4 (15% Chol-sulf) exhibited the highest resistance to be solubilized by SDS, whereas experiment 1 (1% Chol-sulf) showed the lowest. Comparison of data of Table 2 and Fig. 5 shows that the Chol-sulf concentration range for the highest stability against SDS at sublytic level (10–15% Chol-sulf) also included the percentage for the highest resistance of liposomes to be solubilized by this surfactant via mixed micelles formation (15%).

Comparison of the present findings with those reported for the interaction of this surfactant with SCL liposomes varying the proportion of Cer [62] shows noticeable differences. Thus, whereas the increasing presence of Cer resulted in increasing surfactant partitioning between bilayers and the aqueous phase, increasing Chol-sulf proportions led to fall in this partitioning. This effect may be attributed to the variations in both the electrostatic interactions lipid–surfactant and the hydrophilic lipophilic balance of the lipid mixtures, in which Cer is replaced by the major polar lipid of the mixture (Chol-sulf). As aforementioned, the more hydrophilic nature of Chol-sulf could affect the Ninham packing ratio of these structures affecting the fluidization of these structures and, consequently, the surfactant partitioning in bilayers.

We are aware of the fact that the lipids used in this work are not exactly the same as those existing in the SC and that this tissue shows a complex structure, in which proteins building corneocytes and corneocyte envelopes play an important role in skin barrier function. Nevertheless, the simplified membrane model used has shown to be useful in establishing a relation between the level of Cer and Chol-sulf and the resistance of liposomes against the action of the anionic surfactant SDS. Thus, from this review, some specific correlations might be considered between the role played by Cer and Chol-sulf in SCL liposomes against SDS and some skin barrier function abnormalities.

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