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Influence of the level of ceramides on the permeability of stratum corneum lipid liposomes caused by a C_{12}-betaine/sodium dodecyl sulfate mixture

M. Cócera, O. Lopez, L. Coderch, J.L. Parra, A. de la Maza *

Centro de Investigación y Desarrollo (C.I.D.), Consejo Superior de Investigaciones Científicas (C.S.I.C.), Departamento de Tensioactivos, Calle Jorge Girona 18-26, 08034 Barcelona, Spain

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Abstract

The sublytic interactions of a mixture of *N*-dodecyl-*N*,*N*-dimethylbetaine dodecyl betaine (C_{12}-Bet)/sodium dodecyl sulfate (SDS) (mole fraction of the zwitterionic surfactant = 0.6) with stratum corneum (SC) lipid liposomes varying the proportion of ceramides type III (Cer) were investigated. The surfactant/lipid molar ratios (Re) and the bilayer/aqueous phase partition coefficients (K) were determined by monitoring the changes in the fluorescence intensity of liposomes due to the 5(6) carboxyfluorescein (CF) released from the interior of vesicles. The fact that the free surfactant mixture concentration was always lower than its critical micelle concentration indicates that permeability changes were ruled by the action of surfactant monomers in all cases. Higher and lower Cer proportions than that of the SC lipids led to a fall and to a rise in the activity of the surfactant mixture on these bilayer structures. However, the surfactant partitioning into liposomes (or affinity with these bilayer structures) increased as the proportion of Cer increased up to the highest value was achieved for a Cer proportion similar to that in the SC lipids (about 40–45%). Thus, at low Cer proportions the ability of the surfactant mixture to alter the permeability of these bilayer structures was higher than that for liposomes approximating the SC lipid composition despite their reduced partitioning into liposomes. These findings are in agreement with the recently reported dependencies of the level of

Abbreviations: Cer, ceramides type III; CF, 5(6)-carboxyfluorescein; Chol, cholesterol; Chol-sulf, cholesteryl sulfate; CMC, critical micellar concentration; C_{12}-Bet, *N*-Dodecyl-*N*,*N*-dimethylbetaine (dodecyl betaine); K, bilayer/aqueous phase surfactant partition coefficient; $K_{50\%CF}$, bilayer/aqueous phase surfactant partition coefficient for 50% CF release; $K_{100\%CF}$, bilayer/aqueous phase surfactant partition coefficient for 100% CF release; PA, palmitic acid; PI, polydispersity index; PIPES, piperazine-1,4 bis(2-ethane-sulphonic acid); r^2, regression coefficient; Re, effective surfactant/lipid molar ratio; $Re_{50\%CF}$, effective surfactant/lipid molar ratio for 50% CF release; $Re_{100\%CF}$, effective surfactant/lipid molar ratio for 100% CF release; S_B, surfactant concentration in the bilayers; SC, stratum corneum; SDS, sodium dodecyl sulfate; S_W, surfactant concentration in the aqueous medium; $S_{W,50\%CF}$, surfactant concentration in the aqueous medium for 50% CF release; $S_{W,100\%CF}$, surfactant concentration in the aqueous medium for 100% CF release; $X_{zwitter}$, mole fraction of the zwitterionic component (dodecyl betaine).

* Corresponding author. Tel.: + 34-93-400-6161; fax: + 34-93-204-5904.

ceramides in skin lipids and function barrier abnormalities and could explain in part these dependencies. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

One of the key functions of stratum corneum (SC) lipids is to maintain the permeability barrier of the skin (Elias, 1981; Grubauer, et al., 1989). It has been established that the perturbations in the organized structure of these lipids affect the skin barrier function (Harada et al., 1992; Lavrijsen et al., 1995). Changes in the lipid composition are associated with different skin symptoms. Thus, there is a marked decrease in ceramide level in patients with atopic dermatitis, suggesting that an insufficiency of this lipid is an etiologic factor in atopic dry and barrier-disrupted skin (Holleran et al., 1991; Murata et al., 1996; Ponec et al., 1997). However, a physico-chemical study of this barrier abnormality using a simplified membrane model as SC lipid liposomes is still lacking.

Zwitterionic surfactants have strong interaction with anionic surfactants in water (Iwasaki et al., 1991). The effect of the micellar solution phase of these mixtures in avoiding or at least reducing the level of anionic/protein interaction has been suggested by several workers as a way of reducing the potential irritation of anionic surfactants (García Domínguez et al., 1981; Cooper and Berner, 1985; Rhein and Simion, 1991).

Liposomes have been used as membrane models to study the solubilizing effects caused by alkyl sulfates (Urbaneja et al., 1990; Inoue, 1996; Silvander et al., 1996). A significant contribution has been made by Lichtenberg (Lichtenberg et al., 1985), who postulated that the surfactant/lipid molar ratio (Re) producing liposome solubilization depends on the surfactant critical micelle concentration and on the bilayer/aqueous medium distribution coefficients (K). Wertz and Downing prepared liposomes from lipid mixtures approximating the SC composition and studied their interaction with sodium dodecyl sulfate (SDS) to determine its deleterious effect on human skin (Wertz et al., 1986; Wertz, 1992; Downing et al., 1993). SC lipid liposomes have been also used as membrane models to study the adsorption of enhancer agents and to compare these results with those obtained in skin studies (Miyajima et al., 1994; Yoneto et al., 1995, 1996; Suhonen et al., 1997).

The formation of liposomes by mixtures of four lipids approximating the SC lipid composition and the sublytic interactions of N-dodecyl-N,N-dimethylbetaine (dodecyl betaine) (C_{12}-Bet) and SDS (individually or in mixtures) with these liposomes was previously studied (de la Maza et al., 1995; de la Maza and Parra, 1996; de la Maza et al., 1997, 1998a,b). Here, it was seeked to extend these investigations by studying the influence of the content of ceramides in SC lipid liposomes on the ability of a C_{12}-Bet/SDS mixture to alter their permeability. To this end, the Re and K parameters were determined at two sublytic levels varying the proportion of ceramides type III (Cer). This information may shed light on the possible correlation between the proportion of ceramides in skin lipids and the abnormalities in the barrier function.

2. Materials and methods

C_{12}-Bet was specially prepared by Albright and Wilson (Warley, West Midlands, UK); the active matter was 30% in water and the amino free content was 0.20%. SDS was obtained from Merck and further purified by a column chromatographic method (Rosen, 1981). Piperazine-1,4-bis(2-ethanesulphonic acid) (PIPES) was obtained from Merck (Darmstadt, Germany).

PIPES buffer 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). Polycarbonate membranes and membrane holders were purchased from Nucleopore (Pleasanton, CA). Reagent grade organic solvents, Cer, cholesterol (Chol) and palmitic acid (PA) were supplied by Sigma (St Louis, MO). Cholesteryl sulfate (Chol-sulf) was prepared by reaction of Chol with excess chlorosulphonic acid in pyridine and purified chromatographically.

The molecular weight of Cer used in the lipid mixtures was determined by low resolution fast atom bombardment mass spectrometry using a Fisons VG Auto Spec Q (Manchester, UK) with a caesium gun operating at 20 Kv. From this analysis a molecular weight of 671 g was obtained, being the value used to calculate the molarity of the lipid mixtures investigated (de la Maza et al., 1995). The lipids of the highest purity grade available were stored in chloroform/methanol 2:1 under nitrogen at $-20°C$ until use.

2.1. Liposome preparation and characterization

Liposomes formed by a mixture of lipids approximating the SC lipid composition (40% Cer, 25% Chol, 25% PA and 10% Chol-sulf) and those prepared varying the percentage of Cer from 30 to 50%, the relative proportion of the other lipids remaining constant, were prepared at pH 7.20 following the method described by Wertz (Wertz et al., 1986; de la Maza et al., 1995). The lipid composition of these liposomes is given in Table 1. Liposomes (lipid concentration 0.5–5.0 mM) were prepared in PIPES buffer containing 100 mM CF. Vesicles were freed of unencapsulated dye by passage through Sephadex G-50 medium resin (Pharmacia, Uppsala, Sweden) by column chromatography (de la Maza et al., 1998b).

The bilayer lipid composition was determined by thin-layer chromatography coupled to an automated flame ionization detection system (Ackman et al., 1990). In order to find out whether all the components of the lipid mixtures formed liposomes, vesicular dispersions were analyzed for these lipids (Ackman et al., 1990). The dispersions were then spun at $140\,000 \times g$ at 25°C for 4 h to remove the vesicles (Almog et al., 1990). The supernatants were tested again for these components. No lipids were detected in any of the supernatants.

Analyses of proton magnetic resonance (^1H NMR) were carried out at temperatures ranging from 25 to 90°C to determine the phase transition temperature of the lipid mixtures forming liposomes showing values ranging from 55 to 59°C (de la Maza et al., 1995).

The vesicle size distribution and polydispersity index (PI) of liposomes after preparation was determined by dynamic light-scattering using a photon correlator spectrometer (Malvern Autosizer 4700c PS/MV) (de la Maza et al., 1998b).

2.2. Parameters involved in the interaction of the surfactant mixture with SC lipid liposomes

In the analysis of the equilibrium partition model proposed by Schurtenberger (Schurtenberger et al., 1985) for bile salt/lecithin systems, Lichtenberg (Lichtenberg et al., 1985) and Almog (Almog et al., 1990) have shown that for a mixing of lipids (at a lipid concentration L (mM)) and surfactant (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:

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

Table 1
Liposome lipid composition corresponding to the five experiments, in which the percentage of ceramides varied from 30 to 50% and the relative proportion of the other lipids remained constant

Exp no.	Liposome lipid composition %			
	Cer	Chol	PA	Chol-sulf
1	30	29.16	29.16	11.66
2	35	27.08	27.08	10.83
3	40	25	25	10
4	45	22.91	22.91	9.16
5	50	20.83	20.83	8.33

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, applies:

$$K = S_B/(L \cdot S_W) = \mathrm{Re}/S_W \quad (2)$$

where Re is the effective molar ratio of surfactant to lipid in the bilayers ($\mathrm{Re} = S_B/L$). Under any other conditions, Eq. (2) has to be employed to define K; this yields:

$$K = \mathrm{Re}/S_W[1 + \mathrm{Re}]$$

This approach is consistent with the experimental data offered by Lichtenberg (Lichtenberg et al., 1985) and Almog (Almog et al., 1990) for different surfactant lipid mixtures over wide ranges of Re values. The validity of this model for the surfactant/liposome systems investigated has been studied in the Section 3.

The Re, S_W and K parameters were determined on the basis of the linear dependence existing between the surfactant concentrations required to achieve 50% and 100% CF release and the SC lipid concentration (SCL). These linear dependences are described by the equations:

$$S_{T,50\%CF} = S_{W,50\%CF} + \mathrm{Re}_{50\%CF} \cdot [\mathrm{SCL}] \quad (4)$$

$$S_{T,100\%CF} = S_{W,100\%CF} + \mathrm{Re}_{100\%CF} \cdot [\mathrm{SCL}] \quad (5)$$

where $S_{T,50\%CF}$ and $S_{T,100\%CF}$ are the total surfactant concentrations. The effective surfactant/lipid molar ratios for 50% CF release ($\mathrm{Re}_{50\%CF}$) and 100% CF release ($\mathrm{Re}_{100\%CF}$) and the aqueous surfactant concentrations surfactant concentration in the aqueous medium for 50% CF release ($S_{W,50\%CF}$) and 100% CF release ($S_{W,100\%CF}$) are in each curve respectively the slope and the ordinate at the origin (zero lipid concentration) (de la Maza et al., 1998b).

Changes in the release of the CF trapped into SC lipid vesicles were determined quantitatively by monitoring the increase in the fluorescence intensity of liposomes due to the CF liberated (Weinstein et al., 1986; de la Maza et al., 1995). Liposomes of different composition (Table 1) were adjusted to the appropriate lipid concentration (from 1.0 to 10.0 mM). Equal volumes of the appropriate C_{12}-Bet/SDS solution (2.0 ml) were added to these liposomes and the resulting systems were left to equilibrate for periods of time ranging from 45 to 80 min, depending on the Cer concentration in bilayers. These intervals were chosen as the minimum periods of time needed to achieve in each case a constant level of CF release for the lipid concentration range investigated. The fluorescence intensity measurements were taken at 25°C using a spectrofluorophotometer Shimadzu RF-540 (Kyoto Japan) (de la Maza et al., 1998b). The experimental determination of these intervals is given in the Section 3.

3. Results and discussion

The surfactant mixture was selected for the mole fraction of the zwitterionic component 0.6, given that exhibited the highest sublytic activity on SC lipid liposomes. Its critical micelle concentration (CMC) in PIPES buffer was 0.210 mM (de la Maza et al., 1998b).

The characterization of the liposomes used in this study, based on the determination of the mean size and the internal volume of vesicles, demonstrated that these liposomes were formed by unilamellar vesicles regardless of the Cer proportion in bilayers (de la Maza et al., 1995). The size distribution of vesicles after preparation varied very little showing always a value of about 200 nm (PI lower than 0.1), thereby indicating that the liposomes used were very homogeneous. The size of vesicles after the addition of equal volumes of PIPES buffer and equilibration for two hours showed values similar to those obtained after preparation with a slight PI increase (between 0.10 and 0.12). Hence, the liposomes studied were reasonably stable in the absence of surfactant under the experimental conditions used.

3.1. Influence of Cer in the interaction of C_{12}-Bet/SDS with SC lipid liposomes

The suitable sonication conditions of the lipid mixtures used by preparing liposomes at temperatures approximating their phase transition tem-

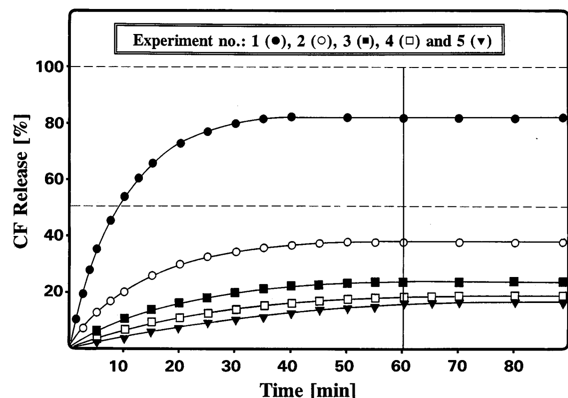

Fig. 1. Time curves of the release of 5(6)-carboxyfluorescein (CF) trapped into stratum corneum (SC) lipid liposomes at the five lipid compositions given in Table 1 and caused by addition of *N*-dodecyl-*N*,*N*-dimethylbetaine (C_{12}-Bet)/sodium dodecyl sulfate (SDS) mixture (mole fraction of the zwitterionic surfactant $X_{\text{zwitter}} = 0.6$). Lipid concentration in liposomes 1.0 mM and surfactant mixture concentration 0.1 mM. Experiment no., 1 (●), 2 (○), 3 (■), 4 (□), and 5 (▼).

peratures (55–59°C) were first determined. It was found that temperatures exceeding this range by more than 10°C caused alterations in Cer and Chol-sulf. Hence, the lipid mixtures studied were sonicated at 60°C.

A systematic study of CF release changes in liposomes (prepared varying the proportion of Cer) caused by the presence of a specific C_{12}-Bet/SDS mixture (mole fraction of the zwitterionic component 0.6) was carried out. This study was performed to shed light on the possible dependencies between the level of Cer in skin lipids and function barrier abnormalities. The percent of Cer varied from 30 to 50% (lower and higher values than that in SC lipids), 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 of different compositions were treated with the surfactant mixture and the subsequent CF release was studied as a function of time. The CF release curves for 1.0 mM lipid liposomes treated with 0.1 mM surfactant mixture are given in Fig. 1. The CF release was in all cases a biphasic process, in which increasing amounts of Cer resulted in an increased period of time to achieve a CF release plateaux. Hence, although the liposomes mimicking the SC lipid composition (experiment no. 3) 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).

This biphasic behaviour may be due to the release of the CF trapped into the vesicles through holes, or channels, created in the membranes. The incorporation of surfactant monomers in membranes may induce the formation of hydrophilic pores in these structures or merely stabilize transient holes. This hypothesis is in line with the concept of transient channels proposed in the surfactant-mediated increase in membrane permeability for nonionic and anionic surfactants (Edwards and Almgren, 1990, 1992; Silvander et al., 1996). The possibility that CF would be associated with the headgroups of the lipids leading to a faster release during the first 30 min of the experiment may be also considered.

To determine the *K* parameters, the validity of the equilibrium partition model proposed by Lichtenberg and Almog (Lichtenberg et al., 1985; Almog et al., 1990) based on the Eq. (1) for the systems investigated was studied first. According to these authors this equation may be expressed by: $L/S_B = (1/K)(1/S_W) - 1$. Hence, this validity requires a linear dependence between L/S_B and $1/S_W$; this line should have a slope of $1/K$, intersect with the L/S_B axis at -1 and intersect with the $1/S_W$ at *K*.

To test the validity of this model, liposomes at different lipid composition (Table 1) were mixed with varying sublytic concentrations of the surfactant mixture studied (S_T). The resultant surfactant-containing vesicles were spun at $140\,000 \times g$ at 25°C for 4 h to remove the vesicles (Almog et al., 1990). No lipids were detected in the supernatants (Ackman et al., 1990). The concentration of each surfactant component in the supernatants (S_W) was determined by high-performance liquid chromatography (HPLC) (Nakamura and Morikawa, 1984; Kanesato et al., 1987) and their concentration in the lipid bilayers was calculated ($S_B = S_T - S_W$). The S_B and S_W values, thus measured, (in the same range of lipid and surfactant concentrations used to determine *K*) were plotted

in terms of the dependence of L/S_B on $1/S_W$. Straight lines were obtained for each liposome tested ($r^2 = 0.990$, 0.992, 0.988, 0.993 and 0.991 for the experiments 1, 2, 3, 4 and 5, respectively), which were dependent on L and intersected with the L/S_B axis always at -0.95 ± 0.11. Both the linearity of these dependences and the proximity of the intercept to -1 support the validity of this model to determine K for the systems studied.

To determine the Re and S_W parameters the CF released from liposomes was studied for a lipid concentration ranging from 0.5 to 5.0 mM. Permeability changes were measured 45, 50, 60, 70 and 80 min after the addition of the surfactant mixture to the liposomes at 25°C for the experiments 1, 2, 3, 4 and 5, respectively (Table 1). These periods of time were obtained from the CF release curves given in Fig. 1. The spontaneous release of the fluorescent agent encapsulated into liposomes in the absence of surfactant in these periods of time was negligible in all cases. The CF release variations of liposomes (lipid composition for the experiment 3 and concentration ranging from 0.5 to 5.0 mM) versus the concentration of the added surfactant mixture (60 min after mixing) are given in Fig. 2A. The surfactant concentrations resulting in 50 and 100% of CF release for each system tested were plotted versus the liposome lipid concentration. A linear relationship was established in each case. The straight lines for the experiment no. 3 are plotted as an example in Fig. 2B. The error bars are S.D. and represent the error of three replicates. The straight lines obtained corresponded to the Eqs. (4) and (5) from which Re and S_W were determined. The Re and S_W values for the five experiments investigated, including the regression coefficients (r^2) of the straight lines, are given in Table 2.

Incresing amounts of Cer in liposomes led to a rise in the Re parameters. Given that the surfactant capacity to release the encapsulated dye is inversely related to the Re values, the higher the Cer proportion in liposomes the lower the ability of the surfactant mixture to alter the permeability of these bilayers (increased resistance of liposomes to the surfactant perturbations). Thus, higher and lower Cer proportion than that existing in the SC lipids ($\sim 40\%$) led to a fall and to a rise in the surfactant activity at the two interaction levels investigated. The partitioning of the surfactant mixture between bilayers and the aqueous medium also increased as the percent of Cer increased (increase in K parameters) up the highest value was achieved for a Cer proportion of about 40–45%.

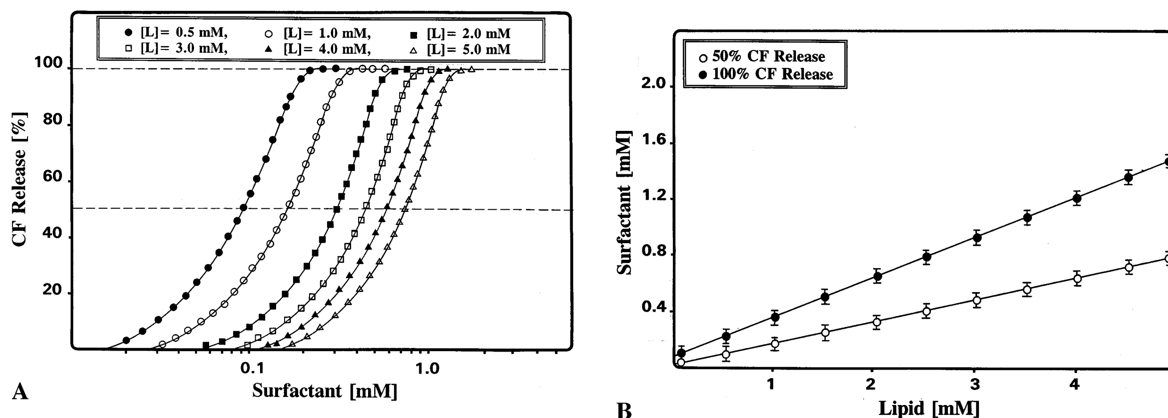

Fig. 2. (A) Percentage changes in 5(6)-carboxyfluorescein (CF) release of stratum corneum (SC) lipid liposomes (lipid composition corresponding to experiment no. 3), the lipid concentration ranging from 0.5 to 5.0 mM, induced by the presence of increasing concentrations of the N-dodecyl-N,N-dimethylbetaine (C_{12}-Bet)/sodium dodecyl sulfate (SDS) mixture (mole fraction of the zwitterionic surfactant $X_{zwitter} = 0.6$). 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 vs lipid concentration of SC lipid liposomes for the experiment no. 3. Fifty percent CF release (○), and 100% CF release (●).

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 the N-dodecyl-N,N-dimethylbetaine (C_{12}-Bet)/sodium dodecyl sulfate (SDS) mixture (mole fraction of the zwitterionic surfactant $X_{zwitter} = 0.6$) with stratum corneum (SC) lipid liposomes at the two interaction levels investigated (50 and 100% CF release), varying the liposome lipid composition (Table 1)

Exp. No.	$S_{W,50\%CF}$ (mM)	$S_{W,100\%FC}$ (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)
1	0.019	0.088	0.045	0.135	2.27	1.35	0.996	0.992
2	0.020	0.090	0.110	0.238	4.95	2.14	0.994	0.998
3	0.021	0.092	0.154	0.281	6.36	2.38	0.999	0.995
4	0.023	0.095	0.180	0.300	6.64	2.43	0.997	0.991
5	0.025	0.098	0.190	0.310	6.39	2.42	0.993	0.995

The fact that the surfactant mixture showed, at 100% CF release, lower K values than those for 50% may be explained assuming that at low Re only the outer vesicle leaflet was available for interaction with surfactant molecules, the binding of additional molecules being hampered at slightly higher Re values. These findings are in agreement with those reported by Schubert (Schubert et al., 1986) for the interaction sodium cholate/phosphatidylcholine liposomes. The increasing difference between bilayer/aqueous phase surfactant partition coefficient for 50% CF release ($K_{50\%CF}$) and for 100% CF release ($K_{100\%CF}$) with the concentration of Cer (up to 45% Cer) suggests that the presence of this lipid progressively hampers the incorporation of surfactant monomers into the outer vesicle leaflet, in agreement with the results given in the Fig. 1.

The rise in the Cer percent also resulted in a slight increase in both $S_{W,50\%CF}$ and $S_{W,100\%CF}$, although these parameters showing smaller values than the surfactant mixture CMC (0.210 mM) (de la Maza et al., 1998b). Hence, permeability changes were mainly ruled by the action of surfactant monomers, in agreement with the reported sublytic interactions of this surfactant mixture with SC liposomes (de la Maza et al., 1998b).

The variations of Re and K versus the percent of Cer in liposomes at the two interaction levels investigated are plotted in Fig. 3A,B, respectively. A progressive increase in Re occurred as the Cer percent rose, this increase being more pronounced at low Cer proportions, specially for $Re_{100\%CF}$. Both the $K_{50\%CF}$ and $K_{100\%CF}$ parameters increased with the Cer concentration up to the highest value was achieved for a Cer proportion of about 40–45%. However, the increase of $K_{50\%CF}$ was more pronounced than that of $K_{100\%CF}$, specially at low Cer proportions (from 30 to 40%). Hence, despite the reduced partitioning of the surfactant mixture molecules in liposomes containing low proportion of Cer (low affinity with these bilayer structures) their ability to alter the permeability of these bilayer structures was higher than that for bilayers approximating the SC lipid composition (percentage of Cer of about 40%). This finding emphasizes the low resistance of these bilayers against the surfactant mixture action. Inversely, the increased surfactant partitioning and Re parameters at the Cer level for the SC lipids reveals that although an increased number of surfactant molecules were incorporated into bilayers these molecules were less able to alter the permeability of these bilayers. These findings are in agreement with the recent studies on the dependencies of the level of Cer in skin on function barrier abnormalities (Murata et al., 1996; Ponec et al., 1997) and could help to explain in part these dependencies.

It is known 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 present membrane model has been shown to be useful in establishing a correlation between the level of Cer in liposomes and the resistance of these bilayer

structures against the effect of a very biologically active surfactant mixture.

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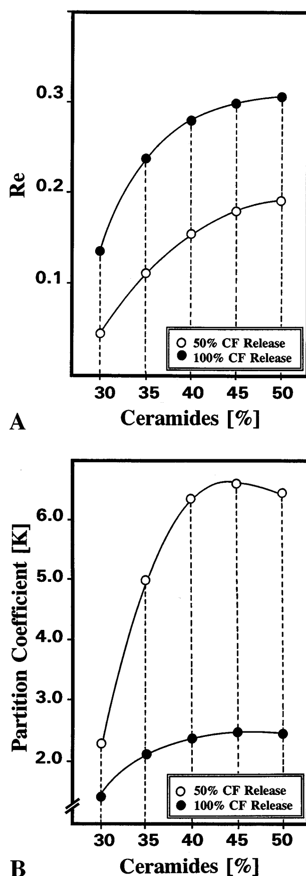

Fig. 3. (A) Effective surfactant to lipid molar ratios for 50 and 100% CF release ($Re_{50\%CF}$ and $Re_{100\%CF}$) in SC lipid liposomes for the *N*-dodecyl-*N*,*N*-dimethylbetaine (C_{12}-Bet)/sodium dodecyl sulfate (SDS) mixture (mole fraction of the zwitterionic surfactant $X_{zwitter} = 0.6$) vs the percentage of ceramides in liposomes. $Re_{50\%CF}$ (○) and $Re_{100\%CF}$ (●). (B) Bilayer/aqueous phase surfactant partition coefficients for 50 and 100% CF release ($K_{50\%CF}$ and $K_{100\%CF}$) in stratum corneum (SC) lipid liposomes for the C_{12}-Bet/SDS mixture (mole fraction of the zwitterionic surfactant $X_{zwitter} = 0.6$) vs the percentage of ceramides in liposomes. $K_{50\%CF}$ (○) and $K_{100\%CF}$ (●).

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