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Adsorption of Sodium Lauryl Ether Sulfate on Liposomes by Means of a Fluorescent Probe: Effect of the Ethylene Oxide Groups

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Introduction

The anionic surfactant sodium lauryl ether sulfate (SLES) is a widely used surfactant as an ingredient in many household and personal care products because of its good foaming properties. The molecular structure of SLES is similar to that of sodium dodecyl sulfate (SDS). The unique structural difference is the presence in the SLES molecule of ethylene oxide (EO) units spacing the hydrophilic and the hydrophobic parts. This surfactant has been demonstrated to be less irritating than SDS.[1] In particular, the addition of EO units promotes lower in vivo irritation and decreases the values in corneosurfametry.[2,3] In fact, some authors reported that SDS had a higher capacity of irritation in vivo than SLES (at 1% concentration) using the soap chamber test;[4] others observed a decreased erythema when mixtures of 20% SDS and 10% SLES were applied in human volunteers in relation to the use of 20% SDS alone.[5] Thus, the presence of EO moles appears to be the cause of this milder behavior on the skin. In this sense, the use of liposomes as a membrane model could help in understanding the EO effect on the irritant activity of surfactants. Although we are aware that the PC liposomes are a simple membrane model, they can provide valuable information that would be difficult to attain in vivo.

In earlier works, we studied several aspects involving the interaction of SDS with phosphatidylcholine (PC) and stratum corneum lipid liposomes.[6−8] These works reported information about the solubilization process and the mechanism governing the initial incorporation of surfactant on the liposomes. In the present work, we seek to extend these investigations by studying some kinetic and structural aspects of the adsorption of SLES with two EO units on PC bilayers. To this end, we used the probe 2-(*p*-toluidinyl)-naphthalene-6-sodium sulfonate (TNS), which reports on the surface potential (ψ_0) of membranes.[9−12] The surfactant adsorption is associated with the ψ_0 changes of the liposome/probe systems as a function of the SLES concentration and of the incubation time. The use of this simple spectroscopy technique may shed light on how the EO units affect the SLES adsorption mechanism on the PC vesicles in relation to the data reported for SDS. Our investigations could be useful to clarify why the EO moles reduce the irritant activity of SLES on the skin.

Materials and Methods

Lipoid S100 as PC from soybean lecithin was purchased from Lipoid GmbH (Ludwigshafen, Germany). SLES containing 2 EO moles (27%) was supplied by Albright and Wilson Iberica (Barcelona, Spain). Tris(hydroximethyl)-aminomethane (TRIS) was obtained from Merck (Darmstadt, Germany). TRIS buffer was prepared as 5.0 mM TRIS buffer adjusted to pH 7.4 with HCl and containing 100 mM NaCl. The fluorescent agent TNS purchased from Sigma Chemical Co. (St. Louis, MO) was prepared as 100 μM TNS in TRIS buffer and stored at 4 °C. Polycarbonate membranes of 800, 400, and 200 nm pore size were purchased from Nucleopore (Pleasanton, CA).

Determination of Surfactant Critical Micelle Concentration. The surface tension of the surfactant solutions in 5 mM TRIS buffer containing 100 mM NaCl adjusted to pH 7.4 was measured at room temperature using a Wilhelmy plate method (Krüss K-12 Processor Tensiometer), which determines directly the real surface tension values at equilibrium. The surfactant critical micelle concentration (cmc) was determined from the abrupt change in the slope of the surface tension values versus surfactant concentration.

Preparation of PC Liposomes with the TNS Probe and Interaction with SLES. A PC film was obtained by rotary evaporation of the solvent from a chloroform-containing PC solution. Unilamellar liposomes of a defined size (about 200 nm) were prepared by a 10-fold passage through 800−400−200 nm polycarbonate membranes[13] of vesicles previously obtained by hydration of the PC film with TRIS buffer. To incorporate the probe on the vesicle surface, the TNS buffered system was added to liposomes. A period of about 30 min was needed to obtain a complete and stable incorporation of TNS on the liposomes (measured as a constant fluorescence intensity value of the liposome/probe). Afterward, equal volumes of appropriate surfactant solutions were added to the liposome/probe suspensions (PC concentration ranging from 0.25 to 2.0 mM), and the fluorescence measurements were performed at 25 °C.

Determination of the Fluorescence Variations Using the TNS Probe. TNS is a probe of membrane surface potential (ψ_0) introduced by Eisenberg et al.[9] The fluorescence of this probe is extremely low in water. The addition of TNS to liposomes led to the adsorption of these molecules on the bilayer surface. Kachel et al. located TNS within the polar headgroup region of the phospholipids (at 16−18 Å from the bilayer center), that is, with its charged sulfonic acid group anchored at a shallow location.[10] This adsorption produces a marked increase in its fluorescence, which is quenched by negative charges such as those of SLES. This probe has been used to determine the ψ_0 of liposomes containing either anionic lipids[11,12] or surfactants[14,15] and to study

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the self-association of bile salts in water.[16] Fluorescence measurements were performed on a spectrofluorometer (Shimadzu RF-540, Kyoto, Japan) with the excitation wavelength (λ_{exc}) at 321 nm and emission (λ_{em}) at 446 nm.

The ψ_0 of vesicles was calculated on the basis of the ratio of fluorescence of pure liposomes and those containing surfactant molecules at the same lipid concentration using the relation

$$f(-)/f(0) = \exp\{F\psi_0/RT\} \quad (1)$$

where $f(0)$ and $f(-)$ are the fluorescence intensities in the absence and in the presence of quencher, F is the Faraday constant, R is the gas constant, and T is the absolute temperature. This equation is useful when a small fraction of binding sites are occupied by TNS at the liposome surface and when the aggregates provide very similar surface environments for TNS to give equal lifetimes, as occurred in our experimental conditions. From the Ψ_0 values (expressed in volts), the surface charge density (σ_0) expressed in μC cm^{-2} for symmetrical electrolytes may be calculated by means of[17]

$$\sigma_0 = 11.74\sqrt{C}\,\sinh\{ze\Psi_0/2kT\} \quad (2)$$

where C is the electrolyte concentration in mol L^{-1}, z is the valence of the ions, e is the elementary charge, k is the Boltzmann constant, and T is the absolute temperature. The number of charged molecules (n) can be obtained from

$$n = (\sigma_0/1.60219 \times 10^{-19})S_{ext} \times 10^{-22} \quad (3)$$

where S_{ext} is the external surface of a vesicle expressed in Å^2.

Parameters Involved in the Interaction of SLES with PC Liposomes. In the analysis of the equilibrium partition model proposed by Schurtenberger et al.[18] for bile salt/lecithin systems, Lichtenberg et al.[19] and Almog et al.[20] 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

$$K = \mathrm{Re}/S_W\,[1 + \mathrm{Re}] \quad (4)$$

where Re is the effective surfactant to lipid molar ratio in the bilayers and S_W is the surfactant concentration in the aqueous medium (mM). The Re, S_W, and K parameters were determined on the basis of the linear dependence between the surfactant concentrations needed for the adsorption of a given number of surfactant molecules on the bilayer surface and the PC concentration, which is described by the equation

$$S_T = S_W + \mathrm{Re}[PC] \quad (5)$$

where S_T is the total surfactant concentration. The surfactant to lipid molar ratios Re and the surfactant aqueous concentration S_W are in each curve the slope and the ordinate at the origin (zero PC concentration), respectively. The K parameters (bilayer/aqueous phase partition coefficient) were determined from eq 4.

Results and Discussion

The cmc of SLES was 0.12 mM in the experimental conditions described above. In the same conditions, the higher cmc reported for SDS (0.75 mM[14]) shows that the EO units in the SLES structure drastically decrease its cmc, in line with the results reported by Vollhardt et al.[21] These authors associated the introduction of EO units with an increase in the surface activity. However, this work did not consider the value of the surface tension at the cmc (γ_{cmc}), which has been used as one criterion of surface activity of the system by other authors (the lower the γ_{cmc}, the higher the surface activity[22]). In our experiments, γ_{cmc} for SLES and SDS were 29.9 and 26.4 mN/m, respectively. Hence, we may assume that the EO units in SLES decrease its surface activity.

To optimize the experimental conditions, the fluorescence lifetime of the probe and the optimal ratio lipid/probe were checked. The fluorescence intensity remained almost constant for a minimum of 6 h after mixing liposomes with TNS. This indicates that under our experimental conditions no migration of the probe to the interior of vesicles occurred. This fact is in line with the work of Eastman et al.,[23] who reported that the movement of TNS into a lipid monolayer required a transmembrane pH gradient. The most appropriate molar ratio PC/TNS was 100. Around this ratio, the net fluorescence was proportional to the number of TNS molecules adsorbed on the bilayer and the probe fluorescence in aqueous solution was negligible with respect to the TNS bound to vesicles. The fluorescence intensity of the probe when it is added to SLES-containing PC liposomes at equilibrium (20 h after mixing) is constant with time (data not shown). This control experiment allows us to conclude that TNS does not translocate to the inner monolayer and, hence, the changes in the fluorescence emission described in this work are promoted by the surfactant adsorption on liposomes. The fluorescence intensity of liposome/probe solutions with increasing surfactant amounts after different incubation times was determined. The assays were carried out in triplicate, and the final results are the averages. The standard deviations of the data for each point were lower than 1.1%. In each curve, 100% fluorescence intensity corresponded to that of liposomes/probe lacking surfactant after the same period of time.

To determine the Re, S_W, and K parameters for the adsorption of a given number of surfactant molecules on the liposome surface, we first measured the changes of the fluorescence intensity of the systems versus SLES concentration. The ψ_0 calculated from these values by applying eq 1 allows to determine the changes in the surface charge density (σ_0) of the liposomes (eq 2) and, hence, the variation in the number of SLES molecules adsorbed per vesicle on the outer membrane leaflet (eq 3). Unilamellar and spherical liposomes were used with a diameter of 190 nm.[14] In previous works, only slight changes in the liposome size (mixed vesicles) were determined by transmission electron microscopy and dynamic light scattering (DLS) at subsolubilizing concentrations of anionic surfactants.[6,24] Thus, we can assume that the liposome size remained almost constant throughout the experiment. Taking into account a surface area of 70 Å^2 for each lipid molecule, we obtained an outer vesicle surface of 11.3×10^6 Å^2 (S_{ext}, eq 3).

The fluorescence intensity curves 10 s after mixing liposomes (PC concentration ranging from 0.25 to 2.0 mM) with surfactant are plotted in Figure 1. The addition of

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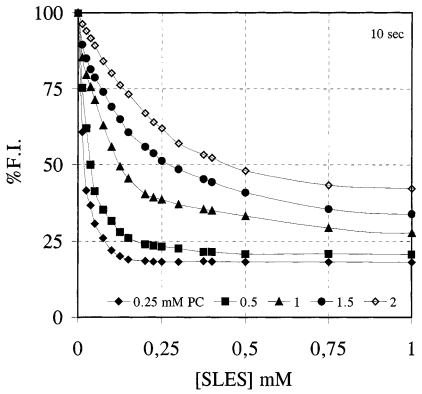

Figure 1. Variation in the fluorescence intensity of PC bilayers (PC concentration ranging from 0.25 to 2.0 mM and PC/TNS molar ratio of 100) containing increasing amounts of SLES after 10 s of incubation. In each curve, a fluorescence intensity of 100% corresponded to that of liposomes/probe lacking surfactant after the same period of time. PC concentration: 0.25 mM (◆), 0.5 mM (■), 1.0 mM (▲), 1.5 mM (●), and 2.0 mM (◇).

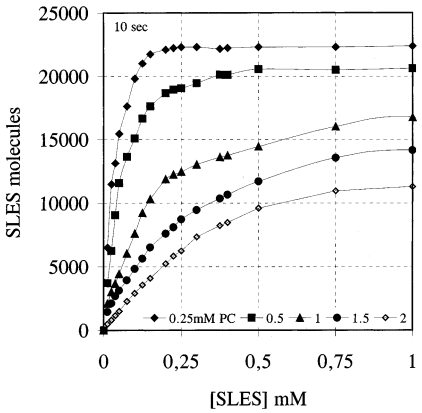

Figure 2. Variation in the number of surfactant molecules adsorbed on the bilayer surface per vesicle (PC concentration ranging from 0.25 to 2.0 mM and PC/TNS molar ratio of 100) versus the SLES concentration in the system after 10 s of incubation. PC concentration: 0.25 mM (◆), 0.5 mM (■), 1.0 mM (▲), 1.5 mM (●), and 2.0 mM (◇).

a very low SLES concentration (lower than its cmc) always resulted in an abrupt decrease in the fluorescence intensity. This initial decrease and the slight fluorescence changes with time (data not shown) indicate that the adsorption process was fast and almost complete, in line with our previous studies of interaction of anionic surfactants with PC liposomes using TNS.[7,15] This rapid adsorption (initial step of the solubilization process) was also detected by the DLS technique for SDS.[24] The slight variations (increase) in the fluorescence intensity with time after SLES addition could be attributed to the inner leaflet migration of the surfactant. This phenomenon was described for the analogue SDS in a previous work.[25] Kragh-Hansen et al. demonstrated a slow flip-flop for SDS because of the marked hydrophilic properties of its headgroup,[26] which is very similar to that of SLES.

Figure 2 plots the number of SLES molecules adsorbed per vesicle versus the added SLES concentration after 10 s of incubation (PC concentration ranging from 0.25 to 2.0 mM). Comparison of the present data with those previously reported by our group for SDS[7] shows that for the same

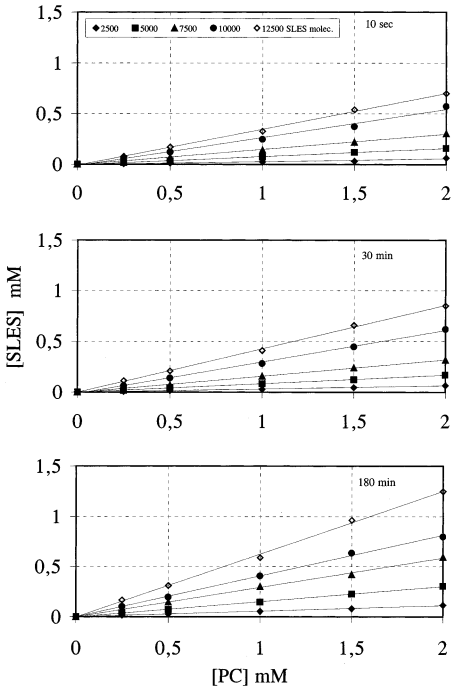

Figure 3. Surfactant concentration resulting in different numbers of surfactant molecules incorporated in the outer surface of liposomes (PC concentration ranging from 0.25 to 2.0 mM and PC/TNS molar ratio of 100) versus the liposome PC concentration, after 10 s, 30 min, and 180 min of incubation. Number of SLES molecules incorporated: 2500 (◆), 5000 (■), 7500 (▲), 10 000 (●), and 12 500 (◇).

PC and surfactant concentrations only half of the SLES molecules were incorporated onto the liposome surface. A similar effect occurred after 30, 180, and 270 min of incubation (results not shown). Hence, the EO units in the molecular structure of SLES hinder its adsorption capacity despite the similar solubilizing ability reported for both surfactants.[27,28] As a consequence, the milder effect on the skin reported for SLES[1−5] seems to be more related to this decreased adsorption than to its capacity to saturate or to solubilize liposomes. In fact, it is commonly accepted that a potential irritant needs to penetrate through the stratum corneum lipids before it can exert its toxic effect on the skin.

The SLES concentrations needed to produce the adsorption of increasing numbers of surfactant molecules per vesicle (from 2500 to 12 500) were plotted versus the PC concentration and are shown in Figure 3. A linear relationship was established in each case (incubation periods of 10 s, 30 min, and 180 min). This linear correlation indicated a similar adsorption behavior in the range of SLES and PC concentrations used. Given that the surfactant concentrations used were lower and higher than its cmc, we can assume that this adsorption was always monomeric even using SLES concentrations above its cmc. This adsorption could follow the mechanism proposed by Lasic,[29] who reported adsorption either through hydrophilic holes created by monomers on the PC polar heads or via formation of short-lived complexes of surfactant−PC polar heads. The monomeric adsorption at SLES concentrations above its cmc implicates a breaking of the micellar structure into monomers previous

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Table 1. Surfactant Concentrations in the Aqueous Medium (S_W), Surfactant to Lipid Molar Ratios (Re), and Partition Coefficients (K) for Different Numbers of Surfactant Molecules Adsorbed on PC Liposomes and for Increasing Periods of Incubation of Surfactant/Liposomes

absorbed SLES molecules/vesicle	10 s incubation				30 min incubation				180 min incubation			
	S_W (mM)	Re mol/mol	K (mM^{-1})	r^2	S_W (mM)	Re mol/mol	K (mM^{-1})	r^2	S_W (mM)	Re mol/mol	K (mM^{-1})	r^2
2500	0.003	0.031	10.02	0.942	0.003	0.033	10.65	0.997	0.004	0.056	13.26	0.997
5000	0.003	0.082	25.26	0.997	0.003	0.085	26.11	0.999	0.005	0.152	26.39	0.999
7500	0.004	0.152	32.99	0.999	0.004	0.161	34.67	0.999	0.006	0.294	37.87	0.998
10000	0.015	0.279	14.54	0.990	0.016	0.310	14.79	0.998	0.018	0.409	16.13	0.998
12000	0.03	0.355	8.73	0.998	0.04	0.428	7.49	0.999	0.04	0.628	9.64	0.998

to its incorporation on the bilayer surface. The kinetics of this process is governed by the micellar relaxation time (τ_2), which was determined by Patist et al.[30] for SDS. These authors determined for 2.5 mM SDS τ_2 values of 10^{-4} s and reported that the presence of an increasing number of EO units in nonionic surfactants reduced this period. Thus, a lower τ_2 is expected for SLES than for SDS indicating that the micellar breaking would not be a rate-limiting step in SLES adsorption on liposomes.

Taking into account eq 5 and the data from Figure 3, Re, K, and S_W were determined. These parameters for different numbers of surfactant molecules adsorbed and various incubation times, together with the regression coefficients (r^2) for each straight line, are given in Table 1.

A very low free surfactant concentration (S_W) was detected even 10 s after incubation. This fact confirms the fast kinetics of the adsorption process, in which most of the surfactant molecules were incorporated on the vesicle surface after this short period of time. This finding is related to that reported for the kinetics of liposome solubilization by SDS using a DLS technique,[24] in which no free surfactant micelles were detected in periods higher than 30 s of incubation.

As for the Re values, the increase in the incubation time of liposome/probe−surfactant led to a progressive rise in these values. Given that the ability of the SLES molecules to be incorporated on the liposome surface is inversely related to the Re value, we may assume that the maximum ability always occurred 10 s after incubation, corroborating a very fast adsorption kinetics. Comparison of these Re values with those reported for SDS[7] for the same number of molecules incorporated shows that SLES always exhibits higher values. This fact indicates the relatively lower incorporation ability of SLES, in line with the results discussed in Figure 2 and with its aforementioned lower surface activity (γ_{cmc}).

To compare the relative incorporation ability of both surfactants, the variation of the effective molar ratio quotients (Re_{SDS}/Re_{SLES}) for different numbers of adsorbed molecules at different periods of time was studied and is plotted in Figure 4. This quotient decreased as the bound surfactant molecules increased regardless of the incubation time. This indicates a decrease in the relative adsorption capacity of SLES, possibly due to the steric impediment caused by the EOs present in its structure.

The K variation versus the number of SLES molecules adsorbed at three incubation times is plotted in Figure 5. Although for up to 7500 molecules adsorbed the SLES partition coefficient increased, the adsorption of more molecules led to a decrease in K. This indicates a loss in the surfactant affinity with the vesicles, possibly due to the electrostatic repulsion between the free and the bound surfactant monomers, which would hinder the incorporation of new monomers on the charged surface of liposomes. Comparison of these K values with those reported for SDS[7]

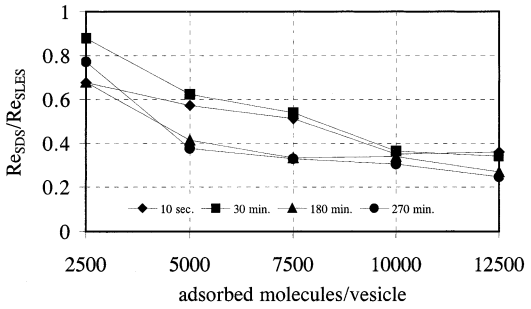

Figure 4. Variation of the quotient of the effective ratios of SDS to SLES (Re_{SDS}/Re_{SLES}) versus the number of adsorbed molecules of surfactant per vesicle at different incubation times: 10 s (◆), 30 min (■), 180 min (▲), and 270 min (●).

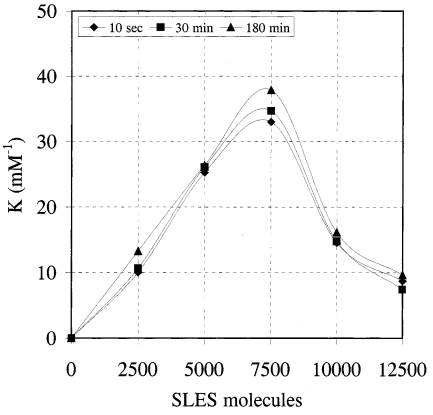

Figure 5. Variation of K versus the number of surfactant molecules incorporated on the outer surface of liposomes (PC concentration ranging from 0.25 to 2.0 mM and PC/TNS molar ratio of 100) after 10 s, 30 min, and 180 min of incubation. Time of incubation: 10 s (◆), 30 min (■), and 180 min (▲).

for the same number of adsorbed molecules and for the same periods of incubation shows that SLES had always higher values. These increased K values for SLES could be attributed to the EO units in its molecular structure, which lend to the surfactant molecule a more hydrophilic character.

In the present study, we have demonstrated the suitability of this fluorescence spectroscopy technique to study the adsorption of SLES on PC liposomes. The analysis of the physicochemical parameters described here leads to very detailed results about the adsorption of this EO-containing surfactant. Despite the complexity of these data, a simple and general aspect should be underlined: the important role of the EO units on surfactant properties. For SLES, these properties seem to be more related to the adsorption than to the solubilizing ability of the surfactant. Hence, the milder effect on the skin reported for SLES would be associated with its decreased adsorption. These perspectives should be considered in future work related to the design and application of new topical compounds.

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Abbreviations

PC = phosphatidylcholine
SLES = sodium lauryl ether sulfate
SDS = sodium dodecyl sulfate
EO = ethylene oxide
TRIS = tris(hydroximethyl)-aminomethane
TNS = 2-(*p*-toluidinyl)-naphthalene-6-sodium sulfonate
ψ_0 = surface potential
σ_0 = surface charge density
S_{ext} = outer vesicle surface
S_W = the surfactant concentration in the aqueous medium
Re = effective surfactant to PC molar ratio
Re_{SDS}/Re_{SLES} = quotient of the effective ratios of SDS to SLES
K = bilayer/aqueous phase surfactant partition coefficient
r^2 = the regression coefficients
cmc = critical micelle concentration
DLS = dynamic light scattering
τ_2 = micellar relaxation time

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