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Kinetic and Structural Aspects of the Adsorption of Sodium Dodecyl Sulfate on Phosphatidylcholine Liposomes

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The ability of sodium dodecyl sulfate (SDS) to be adsorbed on the surface of phosphatidylcholine (PC) bilayers was examined by fluorescence spectroscopy using the fluorescent probe 2-(*p*-toluidinyl)naphthalene-6-sodium sulfonate. The surfactant adsorption was quantified by means of the variations in the surface potential (ψ_o) of liposomes versus incubation time. Very low free SDS concentrations were detected already 10 s after the surfactant addition, indicating that the adsorption process was very fast and almost complete. The correlation between the lipid and SDS concentrations for a given number of monomers adsorbed was always linear, indicating no changes in the adsorption mechanism. Hence, a monomeric adsorption always occurred even in systems with a SDS concentration above its critical micelle concentration. Thus, the breaking of the micellar SDS structure into monomers needed for a monomeric adsorption does not seem to be the rate-limiting step of the process. The fact that the lowest values for the surfactant to lipid molar ratio (inversely related to the SDS ability to be adsorbed on liposomes) were always reached after 10 s of incubation corroborates the rapid kinetic of the process. The fall in the SDS partitioning for a number of surfactant molecules exceeding 15 000 was possibly due to the electrostatic repulsion between the free and the adsorbed monomers, which hindered the incorporation of new monomers on the charged surface of liposomes.

Introduction

The sublytic action of surfactants on phophatidylcholine (PC) vesicles leads to the adsorption of the surfactant molecules into these structures because of the partition equilibrium between bilayers and water.[1−5] Lasic[6] described that in systems with a surfactant concentration below its critical micelle concentration (cmc) the adsorption is monomeric either through the hydrophilic holes created by these monomers on the PC polar heads or via formation of short-lived complexes of surfactant−PC polar heads. In systems above the cmc, a monomeric adsorption is also expected, breaking the micellar surfactant structure. This adsorption is described as a two steps process: first, the release from micelles to monomers, and second, the incorporation of these monomers on the bilayer surface. However, the mechanism of this adsorption and the means by which it is generated remain relatively obscure.

In earlier papers we reported the structural changes resulting in the interaction of alkyl sulfates with PC liposomes.[7−10] Here, we studied the kinetics of partitioning of sodium dodecyl sulfate (SDS) between PC bilayers and water (partition coefficients) using the probe 2-(*p*-toluidinyl)naphthalene-6-sodium sulfonate (TNS), which reports on the surface potential (ψ_o) of membranes.[11−13] The surfactant adsorption is associated to the ψ_o changes of vesicles as a function of the time of incubation of the liposome/probe with SDS. The use of this simple spectroscopy technique may shed light on the not very well understood mechanism of adsorption of this biologically active surfactant on the surface of PC vesicles.

Materials and Methods

Phosphatidylcholine (PC) was purified from egg lecithin (Merck, Darmstadt, Germany)[14] and was shown to be pure by thin-layer chromatography (TLC). Sodium dodecyl sulfate (SDS) was supplied by Lancaster Synthesis Ltd. (Strasbourg, France) and further purified by column chromatography.[15] Tris(hydroximethyl)aminomethane (TRIS) was obtained from Merck. 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 2-(*p*-toluidinyl)naphthalene-6-sodium sulfonate (TNS)

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purchased from Sigma was prepared as 100 μM TNS in TRIS buffer and stored at 4 °C. Polycarbonate membranes were purchased from Nucleopore (Pleasanton, CA).

Preparation of PC Liposomes and Interaction with SDS. Unilamellar vesicles (LUV) of a defined size (of about 200 nm) were prepared from large unilamellar vesicles obtained by reverse phase evaporation[7] followed by a 10-fold passage through 800−200 nm polycarbonate membranes.[16] To incorporate the probe on the vesicle surface, TNS was added to liposomes. A period of about 30 min was needed to obtain a complete and stable incorporation of TNS on the vesicle surface (measured as a constant fluorescence intensity value of the liposome/probe). Afterward, equal volumes of appropriate surfactant solutions were added to liposome/probe suspensions (PC concentration ranging from 0.5 to 2.5 mM). After that, fluorescence measurements were performed at 25 °C.

Fluorescence Measurements. TNS is a probe of membrane surface potential introduced by Eisenberg et al.[12] The main pathway in the excited state of TNS is a twisted intramolecular charge transfer, which is quite fast in the highly polar aqueous media. Hence, the quantum yield of fluorescence of this probe is extremely low in water.[17] The addition of TNS to liposomes led to the adsorption of these molecules on the bilayer surface. This adsorption produces a marked increase in their fluorescence, which is quenched by the presence of negative charges as those of SDS. This variation allows calculation of the surface potential (ψ_o) of the charged vesicles. This probe has been used as an alternative to other markers to characterize the incorporation of anionic surfactants as alkyl sulfates to bilayers as well as the self-association of bile salts in water.[10,13] Fluorescence measurements were performed on a spectrofluorometer (Shimadzu RF-540, Kyoto Japan) with excitation wavelength (λ_{exc}) at 321 nm and emission (λ_{em}) at 446 nm.

The surface potential of vesicles (ψ_o) 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

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

where $f(0)$ and $f(-)$ are the fluorescence intensity 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 is 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 surface potential values (ψ_o) (expressed in volts), the surface charge density (σ_o) expressed in μC·cm^{-2} for symmetrical electrolytes may be calculated by means of[18]

$$\sigma_o = 11.74\ c^{1/2} \sin h(ze\psi_o/2kT) \quad (2)$$

where c is the electrolyte concentration in mol·L^{-1}, z is the valence of 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

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

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

Parameters Involved in the Interaction of SDS with PC Liposomes. In the analysis of the equilibrium partition model proposed by Schurtenberger et al.[19] for bile salt/lecithin systems, Lichtenberg et al.[20] and Almog et al.[21] 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

We first checked the fluorescence lifetime of the probe and the optimal ratio lipid/probe. Fluorescence intensity was almost constant during 6 h after mixing liposomes with the probe, the molar ratio PC/TNS 100 being the most appropriate. Around this ratio, the net TNS fluorescence was proportional to the number of probe molecules adsorbed to the membrane and its fluorescence in aqueous solution was negligible with respect to that of the TNS bound to lipid vesicles. Given that no changes in the fluorescence intensity of the liposome/probe occurred during 6 h, we may assume that under our experimental conditions (buffered system adjusted to pH 7.4) no migration of the probe to the interior of vesicles occurred. These findings are in line with the assays reported by Eastman et al., in relation to the partitioning of TNS into the interior lipid monolayer as a function of the pH gradient (transmembrane pH gradient).[11] This partitioning would be expected to result in variations in the fluorescence intensity due to the different aqueous to lipid volume ratio in the LUV interior.

To determine the Re, S_W, and K parameters for the adsorption of a given number of surfactant molecules on the liposome surface, we determined the variations in the surface potential (ψ_o) versus the incubation time liposome/probe−surfactant. To this end, the anionic probe TNS was used. Thus, fluorescence intensity changes of liposomes (PC/TNS molar ratio 100) containing increasing amounts of SDS after different times of incubation were determined. The curves for 10 s of incubation (PC concentration ranging from 0.5 to 2.5 mM) are plotted in Figure 1. The assays

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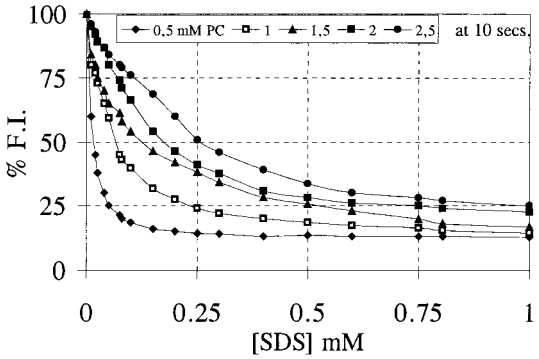

Figure 1. Variation in the fluorescence intensity of PC bilayers (PC concentration ranging from 0.5 to 2.5 mM and PC/TNS molar ratio 100) containing increasing amounts of sodium dodecyl sulfate after 10 s of incubation. In each curve fluorescence intensity 100% corresponded to that of liposomes/probe lacking surfactant after the same period of time. PC concentrations were 0.5 mM (♦), 1.0 mM (□), 1.5 mM (▲), 2.0 mM (■), 2.5 mM (●).

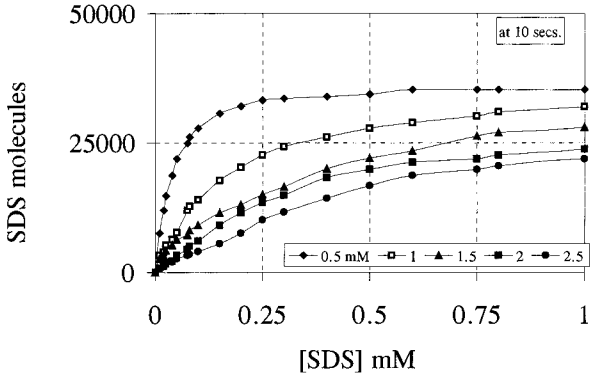

Figure 2. Variation in the number of surfactant molecules adsorbed on the bilayer surface per vesicle (PC concentration ranging from 0.5 to 2.5 mM and PC/TNS molar ratio 100) versus the sodium dodecyl sulfate concentration in the system after 10 s of incubation. PC concentrations were 0.5 mM (♦), 1.0 mM (□), 1.5 mM (▲), 2.0 mM (■), 2.5 mM (●).

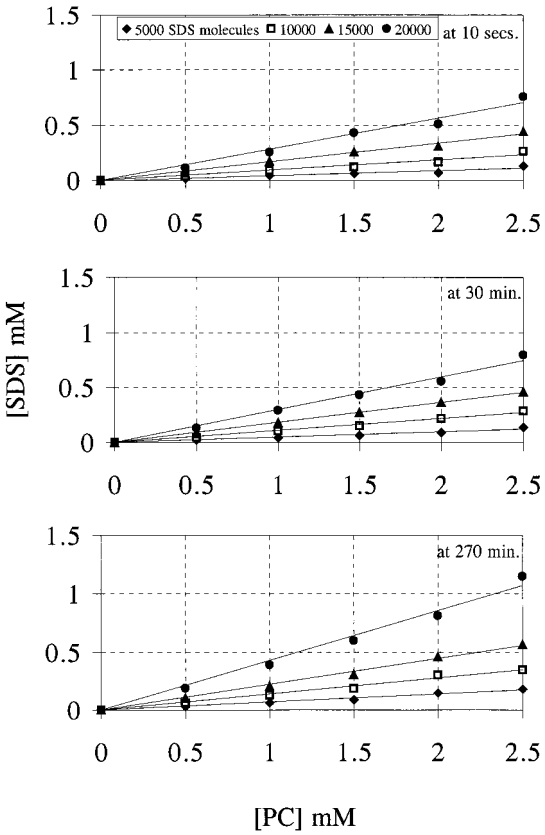

Figure 3. Surfactant concentration resulting in different numbers of surfactant molecules incorporated in the outer surface of liposomes (PC concentrations ranging from 0.5 to 2.5 mM and PC/TNS molar ratio 100) versus the liposome PC concentration, after 10 s, 30 min, and 270 min of incubation. The number of SDS molecules incorporated were 5000 (♦), 10 000 (□), 15 000 (▲), and 20 000 (●).

were carried out in triplicate, and the results given 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. The addition of a very low concentration of surfactant monomers (SDS concentration markedly lower than its cmc, which was 0.75 mM) always resulted in an abrupt decrease in the fluorescence intensity. This finding is in line with our previous electrokinetic studies of the interaction of different alkyl sulfates with PC liposomes using TNS.[10]

The theoretical surface potentials (ψ_o) calculated from the fluorescence intensity values applying eq 1 allow determination of the variations in charge surface density (σ_o) of liposomes (eq 2) and, consequently, the variation in the number of SDS molecules adsorbed on the outer membrane leaflet per vesicle (eq 3). This variation quantifies the adsorption of surfactant molecules per vesicle as a function of the time of incubation. Given that the liposomes used were unilamellar and formed by spherical vesicles with a diameter of 190 nm,[10,22] and assuming that the surface area on the lipid molecules was 70 Å^2 and the thickness of the bilayers was 4 nm,[23] an outer vesicle surface of 11.3×10^6 Å^2 (S_{ext}, eq 3) was obtained with 160 000 molecules of lipid in this surface. This value is consistent with the data reported by Lasic.[23]

The variations in the number of SDS molecules adsorbed on the liposome surface (per vesicle) versus the concentration of the added SDS after 10 s of incubation (PC concentration ranging from 0.5 to 2.5 mM) are plotted in Figure 2. The surfactant concentrations needed to produce the adsorption of increasing amount of surfactant molecules (from 2500 to 20 000) are plotted versus the PC concentration in Figure 3. A linear relationship was established in each case for incubation periods of 10 s, 30 min, and 270 min. The straight lines obtained corresponded to the eq 5 from which Re and S_W were determined. The ordinate in the origin (zero lipid concentration) showed always very low values indicating the very low concentration of the free surfactant in all cases. The Re, S_W, and K values, including the regression coefficients (r^2) of the straight lines, are given in Table 1. This linear correlation indicated that the adsorption process exhibited the same behavior in the SDS concentrations range studied. Given that in most systems the surfactant concentration was smaller than its cmc, we can assume that this adsorption was monomeric even when a SDS concentration above the cmc was used. In this case, the breaking of micellar surfactant structure into monomers is expected to occur previous to a monomeric incorporation on the bilayer surface. This breaking

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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 Number of Surfactant Molecules Adsorbed on PC Liposomes and for Increasing Periods of Incubation Surfactant/Liposome

adsorbed SDS molecules per vesicle	10 s incubation				30 min incubation				270 min incubation			
	S_W (mM)	Re mole/mole	K (mM^{-1})	r^2	S_W (mM)	Re mole/mole	K (mM^{-1})	r^2	S_W (mM)	Re mole/mole	K (mM^{-1})	r^2
2 500	0.006	0.021	3.43	0.921	0.007	0.029	4.03	0.918	0.009	0.044	4.68	0.963
5 000	0.006	0.047	7.48	0.936	0.007	0.053	7.19	0.967	0.009	0.070	7.26	0.983
7 500	0.007	0.078	10.34	0.942	0.008	0.087	10.00	0.976	0.010	0.119	10.63	0.982
10 000	0.007	0.098	12.75	0.962	0.008	0.113	12.69	0.994	0.010	0.144	12.59	0.989
12 500	0.007	0.128	16.21	0.987	0.008	0.146	15.92	0.981	0.010	0.191	16.04	0.985
15 000	0.007	0.172	20.97	0.989	0.008	0.186	19.62	0.999	0.010	0.229	18.63	0.995
17 500	0.014	0.211	12.44	0.987	0.016	0.234	11.85	0.978	0.020	0.378	13.72	0.942
20 000	0.024	0.297	9.54	0.982	0.025	0.308	9.41	0.989	0.036	0.447	8.59	0.989

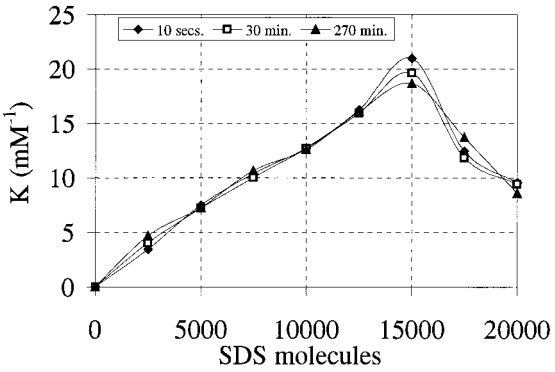

Figure 4. Variation of K versus the number of surfactant molecules incorporated in the outer surface of liposomes (PC concentrations ranging from 0.5 to 2.5 mM and PC/TNS molar ratio 100) after 10 s, 30 min, and 270 min of incubation. Incubation times were 10 s (♦), 30 min (□), and 270 min (▲).

is associated with a micellar relaxation time that although it has been described by other authors as a slow process[24] does not seem to be the rate-limiting step of the adsorption process.

The fact that the free surfactant concentration (S_W) was very low even after 10 s of incubation (values in all cases lower than 5% of the surfactant cmc) reveals the fast kinetics of the adsorption process, in which most of the surfactant molecules were incorporated on the vesicle surface. Hence, the structural and the effective charges would be equivalent, and the fractional charge of the surfactant molecules in the PC liposomes would be almost the unity.[25,26] This fact allow us to obtain conclusions at a structural level based on a fluorescence technique that is sensitive to effective charge. This finding extends that reported for the kinetics of liposome solubilization by SDS using a dynamic light-scattering technique,[9] in which no free surfactant micelles were detected in periods higher than 30 s of incubation.

The increase in the time of incubation liposome/probe−surfactant resulted in a progressive increase in the Re values (see Table 1). The ability of the SDS molecules to be incorporated on the liposome surface is inversely related to the Re parameter. Thus, we may assume that in the range of PC concentrations investigated the maximum surfactant ability of incorporation took place 10 s after incubation, regardless of the number of SDS molecules incorporated (from 2500 to 20 000). This finding confirms the rapid kinetic of adsorption of surfactant monomers on the outer bilayer leaflet of liposomes.

The variation in the partition coefficients versus the number of surfactant molecules adsorbed per vesicle at three incubation times (10 s, 30 min, and 270 min) is plotted in Figure 4. Although up to 15 000 molecules adsorbed the SDS partitioning increase, the adsorption of higher surfactant amounts resulted in a clear decrease in K. This indicated a loss in the affinity of the surfactant by the vesicles, possibly due to the electrostatic repulsion between the free and the incorporated surfactant monomers. This repulsion would hinder the incorporation of new surfactant monomers on the charged surface of liposomes.

Abbreviations

PC, phosphatidylcholine
SDS, sodium dodecyl sulfate
TRIS, tris(hydroxymethyl)aminomethane
TNS, 2-(*p*-toluidinyl)naphthalene-6-sodium sulfonate
ψ_o, surface potential
Re, effective surfactant to PC molar ratio
K, bilayer/aqueous phase surfactant partition coefficient
cmc, critical micellar concentration

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