Influence of the level of cholesteryl sulfate in the solubilization of stratum corneum lipid liposomes by sodium dodecyl sulfate

Abstract The role played by cholesteryl sulfate (Chol-sulf) in the solubilization of liposomes modeling the stratum corneum (SC) lipids by sodium dodecyl sulfate (SDS) was studied. We determined the surfactant-to-lipid molar ratios and the bilayer/aqueous phase surfactant partition coefficients of this interaction by varying the proportion of Chol-sulf, the relative proportions of the other lipids remaining constant. These parameters were determined by monitoring the changes in the static light scattering of the system during solubilization. The fact that the free surfactant concentration was always similar to its critical micelle concentration indicates that the liposome solubilization was mainly ruled by the formation of mixed micelles. The SDS ability to saturate and solubilize SC liposomes decreased as the proportion of Chol-sulf in the bilayers increased until a minimum was reached for a Chol-sulf proportion of about 15%. Inversely, the SDS partitioning into liposomes (or affinity with these bilayers) increased as the proportion of Chol-sulf increased until a maximum was reached at similar Chol-sulf proportions (10–15%). Hence, in these Chol-sulf proportions (similar to that existing in the intercellular lipids, which was 10%) the ability of SDS molecules to interact with liposomes exhibits a minimum despite their enhanced partitioning into liposomes. These effects may be related to the reported dependencies of the level of Chol-sulf on the abnormalities in the skin barrier function and on the SC intercellular cohesion.

Key words Stratum corneum lipid liposomes · Sodium dodecyl sulfate · Stratum corneum liposome solubilization · Influence of cholesteryl sulfate in stratum corneum lipid liposome solubilization · Dynamic light scattering changes

Introduction

The stratum corneum (SC) forms a continuous sheath of alternating squamae (protein-enriched corneocytes) embedded in an intercellular matrix enriched in nonpolar lipids displayed as lamellar sheets. The proportion of cholesterol and cholesteryl sulfate (Chol-sulf) in these lipids is claimed to play an important role in the stability properties of the SC (cohesion and desquamation) and in the regulation of the skin barrier function [1–5]. Thus, patients with recessive X-linked ichthyosis show elevated proportions of Chol-sulf due to steroid sulfatase deficiency [6], whereas tissues with extremely tenacious intercellular cohesion also present higher Chol-sulf proportions than that existing in skin lipids [7]. SC lipid liposomes have been used to study the role played by each lipid in the SC lipid phase behavior [8–11]; however, the
molecular mechanism by which Chol-sulf affects SC shedding is not clear.

Sodium dodecyl sulfate (SDS) has frequently been used as a model substance to induce structural changes in the epidermal surface and in the SC transcutaneous permeability barrier [12–15]. The interaction of SDS with lipid bilayers leads to the breakdown of lamellar structures and to the formation of lipid–surfactant mixed micelles [16–18]. A significant contribution in this area has been made by Lichtenberg et al. [19], who postulated that the surfactant/lipid molar ratio (Re) producing liposome solubilization depends on the surfactant critical micelle concentration (cmc) and on the bilayer/aqueous medium partition coefficients (K).

We studied the formation of liposomes using a mixture of four lipids modeling the SC composition and the interactions of alkyl sulfates and mixtures of SDS/alkyl betaines with these liposomes [20–23]. We also investigated the role played by the ceramides in the interaction of SDS with SC lipid liposomes [24]. Here, we seek to extend these studies by characterizing the influence of the Chol-sulf on the resistance of SC lipid liposomes to be solubilized by SDS. To this end, we determined the Re and K parameters of this interaction at lytic level by varying the proportion of Chol-sulf in the bilayers. This information may shed light on the possible correlation between the level of Chol-sulf in the bilayers and the abnormalities in the skin barrier function and in the SC cohesion.

**Materials and methods**

SDS was obtained from Merck and was further purified by column chromatography [25]. Piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) was obtained from Merck (Darmstadt, Germany). PIPES buffer was prepared as 20 mM PIPES containing 110 mM Na₂SO₄ and adjusted to pH 7.20 with NaOH. Polycarbonate membranes and membrane holders were purchased from Nucleopore (Pleasanton, Calif.). Reagent grade organic solvents, ceramides type III (Cer), cholesterol and palmitic acid were supplied by Sigma Chemical Co. (St Louis, Mo.). Chol-sulf was prepared by reaction of cholesterol with excess chlorosulfonic acid in pyridine and was 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. A molecular weight of 671 g was obtained for the major component of the ceramides used (Sigma). This value was used to calculate the molarity of the lipid mixture investigated. The lipids of the highest purity grade available were stored in chloroform/methanol 2:1 under nitrogen at –20 °C until use.

**Liposome preparation and characterization**

Liposomes formed by mixtures of SC lipids by varying the percentage of Chol-sulf from 1 to 25%, the relative proportions of the other lipids remaining constant, were prepared following the method described by Wertz et al. [8]. The lipid compositions investigated are given in Table 1. After preparation the liposomes were annealed at 60 °C for 30 min and incubated at 25 °C under a N₂ atmosphere. The final volumes of the liposomes were adjusted with PIPES buffer to provide a final lipid concentration ranging from 0.5 to 5.0 mM. Experiment 3 corresponded to the composition of the intercellular lipids, in accordance with the data reported by Wertz et al. [8]. The lipid composition of the liposomes after preparation was determined by thin-layer chromatography coupled to an automated flame ionization detection system (Iatroscan MK-5, Iatron Lab., Tokyo, Japan) [20, 26].

In order to find out whether all the lipid mixture components formed liposomes, vesicular dispersions were analyzed for these lipids [26]. The dispersions were then spun at 140 000 g at 25 °C for 4 h to remove the vesicles [27]. The supernatants were tested again for these components. No lipids were detected in any of the supernatants. The phase transition temperatures of the lipid mixtures forming liposomes were determined by proton magnetic resonance, showing values ranging from 55 to 59 °C [20]. The size distribution and polydispersity index (PI) of the liposomes after preparation were determined by dynamic light scattering measurements using a photon correlator spectrometer (Malvern Autosizer 4700c PS/MV). The samples were adjusted to the appropriate concentration range with PIPES buffer. Measurements were taken at 25 °C at a scattering angle of 90°.

**Table 1** Liposome lipid composition corresponding to the six experiments, in which the percentage of cholesterol sulfite (Chol-sulf) varied from 1 to 25% and the relative proportions of the other lipids remained constant

<table>
<thead>
<tr>
<th>Exp no.</th>
<th>Liposome lipid composition (%)</th>
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<tbody>
<tr>
<td></td>
<td>Ceramides type III</td>
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<tr>
<td>1</td>
<td>44.0</td>
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<tr>
<td>2</td>
<td>42.2</td>
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<tr>
<td>3</td>
<td>40.0</td>
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<td>4</td>
<td>37.8</td>
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<td>5</td>
<td>35.6</td>
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<td>6</td>
<td>33.4</td>
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Parameters involved in the interaction of SDS with SC lipid liposomes

In the analysis of the equilibrium partition model proposed by Schurtenberger et al. [28] for bile salt/lecithin systems, Lichtenberg et al. [19] and Almog et al. [27] have shown that for mixing lipids, at a lipid concentration L (millimoles) and surfactant, at a concentration S₇ (millimoles), in dilute aqueous media, the distribution of surfactant between lipid bilayers and aqueous media obeys a partition coefficient K, given (in reciprocal millimoles) by

\[ K = S_B / [L + S_B]S_W \] ,

where \( S_W \) is the concentration of surfactant in the bilayers (millimoles) and \( S_B \) is the surfactant concentration in the aqueous medium (millimoles). For \( L \gg S_B \), the definition of \( K \), as given by Schurtenberger et al., applies:

\[ K = S_B / L S_W = \text{Re} / S_W \] ,

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

\[ K = \text{Re} / S_W [1 + \text{Re}] \] .

This approach is consistent with the experimental data offered by Lichtenberg et al. [19] and Almog et al. [27] for different