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ORIGINAL CONTRIBUTION

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Influence of the hydrophobic tail of alkyl glucosides on their ability to solubilize stratum corneum lipid liposomes

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Abstract The lytic interactions of a series of alkyl glucosides (alkyl chain lengths ranging from C_8 to C_{12}) with liposomes formed by a mixture of lipids modeling the stratum corneum (SC) lipid composition were investigated. The surfactant-to-lipid molar ratios (Re) and the normalized bilayer/aqueous phase partition coefficients (K) were determined by monitoring the changes in the static light-scattering (SLS) of the system during solubilization. The fact that the free surfactant concentrations were always similar to their critical micelle concentrations indicates that the liposome solubilization was mainly ruled by the formation of mixed micelles. At the two interaction levels studied (100 and 0% SLS) the nonyl glucoside showed the highest ability to saturate and to solubilize liposomes (lowest Re values), whereas the dodecyl glucoside showed the highest degree of partitioning into liposomes or affinity with these structures (highest K values). Comparison of the data for octyl glucoside with that reported for the interaction of this surfactant with phosphatidylcholine (PC) liposomes shows that whereas the SC lipid liposomes were more resistant to the action of this surfactant (higher Re values), its degree of partitioning into SC bilayers was both in the saturation and solubilization of liposomes similar to that exhibited in PC vesicles (similar K values).

Key words Stratum corneum lipid liposomes · Alkyl glucoside surfactants · Surfactant/lipid molar ratios · Surfactant partition coefficients

Introduction

The stratum corneum (SC) of vertebrates is a major structural compartment that provides mechanical protection and prevents skin desiccation. This tissue is formed by corneocytes that are separated by an intercellular matrix mainly composed of lipids. The SC lipids, which have been described as approximately 40% ceramides (Cer), 25% cholesterol (Chol), 15% free fatty acids and small amounts of cholesterol sulfate (Cholsulf) and cholesterol esters [1], result in a very exceptional composition and organization not observed in other biological membranes. These lipids are organized into multilayers that have been postulated both to account for the permeability properties of SC and to ensure the appropriate cohesiveness between corneocytes [2, 3, 4, 5], although other structures, such as the corneodesmosomes, have to be considered in the cohesion–desquamation properties of this tissue [1]. In all cellular and intercellular membranes, the bilayer-forming lipids consist predominantly of phospholipids; 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. To find out whether SC lipids could form bilayers, Wertz et al. [6] prepared liposomes from lipid mixtures approximating the composition of the SC lipids and characterized these bilayer structures. These liposomes were useful to

study the interaction with sodium dodecyl sulfate and to determine the possible effect of this surfactant on human skin [6]. Another interest in these liposomes lies in their use as specific membrane models to study the adsorption of enhancer agents and to compare these results with those obtained in skin studies [7, 8]. One of the main differences between liposomes formed by SC lipids and by phospholipids is the fluidity of the resulting membranes. SC liposome membranes are more rigid than phospholipid liposomes; this characteristic seems to be related to membranes that separate aqueous and gaseous phases, compared to most other biological membranes which separate two aqueous phases [9].

One of the most commonly used surfactants in membrane solubilization and reconstitution experiments is octyl glucoside, which is believed to be a "mild" surfactant with respect to its denaturing effect on proteins [10, 11, 12, 13, 14, 15, 16, 17]. However, the use of alkyl glucosides with longer alkyl chains has been little investigated in spite of their excellent hydrolysis stability, improved ecological properties and lower toxicity as has been demonstrated in "in vivo" tests using *Daphnia magna* and *Photobacterium phosphoreum* [18, 19].

A number of studies have been devoted to the understanding of the principles governing the interaction of surfactants with phospholipid or SC lipid vesicles [20, 21, 22]. This interaction leads to the breakdown of lamellar structures and to the formation of lipid–surfactant mixed micelles. A significant contribution has been made by Lichtenberg [23], who postulated that the effective surfactant-to-lipid molar ratio (Re) producing vesicle solubilization depends on the surfactant critical micelle concentration (cmc) and its bilayer/aqueous medium distribution coefficients (K).

We first studied the phase transitions involved in the interaction of octyl glucoside with phosphatidylcholine (PC) liposomes [24, 25] and the sublytic interactions of a series of alkyl glucosides (alkyl chain lengths ranging from C_8 to C_{12}) with these vesicles [26]. We also studied the formation of liposomes from lipid mixtures approximating the SC composition [27]. Here, we seek to extend these studies by characterizing the Re and K parameters for lytic interactions of these surfactants with SC lipid liposomes. This study will provide new information about the influence of the alkyl chain length of these surfactants on their ability to saturate and solubilize specific biological membranes such as the SC.

Materials and methods

The nonionic surfactants *n*-octyl β-D-glucopyranoside (C_8-Glu), *n*-nonyl β-D-glucopyranoside (C_9-Glu), *n*-decyl β-D-glucopyranoside (C_{10}-Glu), *n*-undecyl β-D-glucopyranoside (C_{11}-Glu) and *n*-dodecyl β-D-glucopyranoside (C_{12}-Glu) were purchased from Sigma Chemicals Co. (St. Louis, Mo.). Piperazine-1,4 bis(2-ethanesulphonic acid) (PIPES) was obtained from Merck. PIPES buffer was prepared as 10 mM PIPES containing 110 mM Na_2SO_4, and adjusted to pH 7.2 with NaOH. Polycarbonate membranes and membrane holders were purchased from Nucleopore (Pleasanton, Calif.).

Reagent grade organic solvents, ceramide type III (Cer) and Chol were supplied by Sigma Chemical Co. (St Louis, Mo.), and palmitic acid (PA) (reagent grade) was purchased from Merck. Chol-sulf was prepared by reaction of cholesterol with excess chlorosulphonic acid in pyridine and was purified chromatographically.

The molecular weight of the ceramide type III used in the lipid mixture 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/mol was obtained for the major compound of the ceramides type III used (Sigma). This value was similar to the molecular weight of ceramide 3 (667 g) calculated from the structure of this compound reported by Wertz [28], despite the fact that the ceramide type III used was a mixture of ceramides of different chain lengths (purity approximately 99%). As a consequence, we used the molecular weight obtained to calculate the molarity of the lipid mixture investigated. The lipids of the highest purity grade available were stored in 2:1 chloroform/methanol under nitrogen at −20 °C until use.

Preparation and characterization of SC lipid liposomes

We reported the formation and characterization of liposomes using a mixture of lipids modeling the composition of the SC [27], which were prepared according to the composition (40% Cer, 25% Chol, 25% PA and 10% Chol-sulf, percentages in weight) and the method described by Wertz et al. [6]. After preparation, the vesicles were annealed at 60 °C for 30 min and incubated at 25 °C under a nitrogen atmosphere. The range of lipid concentrations in the liposomes was 0.1–10.0 mM. The vesicle size distribution of the liposomes after preparation in this lipid concentration range was determined by dynamic light scattering using a photon correlator spectrometer (Malvern Autosizer 4700c PS/MV; Malvern, UK) [24, 27]. The sample was adjusted to the appropriate lipid concentration with PIPES buffer. Measurements were taken at 25 °C at a scattering angle of 90°.

The lipid composition and concentration of the liposomes after preparation were determined using thin-layer chromatography coupled to an automated flame ionization detection system (Iatroscan MK-5, Iatron Lab., Tokyo, Japan) [29]. In order to find out whether all the components of the lipid mixture formed liposomes, vesicular dispersions were analyzed for these lipids [29]. The dispersions were then spun at 140,000 *g* at 25 °C for 4 h to remove the vesicles [11]. The supernatants were tested again for these components. No lipids were detected in any of the supernatants.

The phase-transition temperature of the lipid mixture forming liposomes was determined by proton magnetic resonance (^{1}H NMR) and had a value of 55–56 °C [27].

Parameters involved in the interaction of surfactants with SC lipid liposomes

In the analysis of the equilibrium partition model proposed by Schurtenberger et al. [30] for bile salt/lecithin systems, Lichtenberg [23] and Almog et al. [11] have shown that for a mixing of lipids (at a lipid concentration L, millimol · liter^{-1}) and surfactant (at a concentration, S_T, millimol · liter^{-1}), in dilute aqueous media, the distribution of surfactant between lipid bilayers and water obeys a partition coefficient, K:

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

where S_B and S_W are the surfactant concentration in the bilayers (millimol · liter^{-1}) and in the aqueous medium (millimol · liter^{-1}). For $L \gg S_B$, the definition of K, as given by Schurtenberger, applies:

$$K = S_B/(LS_W) = \mathrm{Re}/S_W, \quad (2)$$

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:

$$K = \mathrm{Re}/S_W(1 + \mathrm{Re}). \quad (3)$$

This approach is consistent with the experimental data offered by Lichtenberg [23] and Almog et al. [11] for different surfactant lipid mixtures over a wide ranges of Re values. Given that the range of lipid concentrations used was similar to that used by Almog et al. to test their equilibrium partition model, the K parameter was determined using this equation.

The solubilization of liposomes was characterized by two parameters termed $\mathrm{Re_{SAT}}$ and $\mathrm{Re_{SOL}}$ (according to the nomenclature adopted by Lichtenberg [23]), corresponding to the Re ratios at which static light scattering (SLS) starts to decrease with respect to the original value and shows no further decrease. These parameters corresponded to the surfactant/lipid molar ratios at which the surfactant saturated liposomes and led to a complete liposome solubilization.

Equal volumes of surfactant solutions were added to the liposome suspensions and the resulting mixtures were left to equilibrate for 24 h at 25 °C. The final surfactant concentration was calculated for each mixture. This time was chosen as the optimum period needed to achieve complete surfactant/liposome equilibrium in the lipid concentration range used [21, 31]. The temperature of 25 °C was selected for the following reasons:

1. The reasonable stability of the SC liposomes under these conditions.
2. Similar experimental conditions to those used to study the interaction of octyl glucoside with PC liposomes.
3. These experimental conditions are generally used in "in vivo" tests to study the interaction of surfactants with skin [1, 32, 33].

SLS measurements were made at 25 °C using a Shimadzu RF-540 spectrofluorophotometer (Kyoto, Japan) with both monochromators adjusted to 500 nm [34]. The assays were carried out in triplicate and the results given are the average of those obtained.

The determination of these parameters was carried out on the basis of the linear dependence existing between the surfactant concentrations required to saturate or solubilize the liposomes and the lipid concentration (L), which can be described by the equations

$$S_{SAT} = S_{W,SAT} + \mathrm{Re_{SAT}}L, \quad (4)$$

$$S_{SOL} = S_{W,SOL} + \mathrm{Re_{SOL}}L, \quad (5)$$

where S_{SAT} and S_{SOL} are the total surfactant concentrations. The surfactant-to-lipid molar ratios $\mathrm{Re_{SAT}}$ and $\mathrm{Re_{SOL}}$ and the aqueous concentration of surfactant $S_{W,SAT}$ and $S_{W,SOL}$ are in each curve, respectively, the slope and the ordinate at the origin (zero lipid concentration). The K_{SAT} and K_{SOL} parameters (normalized bilayer/aqueous phase surfactant partition coefficients for saturation and complete solubilization of liposomes) were calculated using Eq. (3).

Results and discussion

The characterization of the geometric properties of the liposomes used in the present study was reported previously [27]. This study demonstrated that these liposomes were formed by unilamellar vesicles in all cases. Furthermore, the vesicle size distribution of the liposomes after preparation varied little, showing in all cases a similar value of about 200 nm. The vesicle size after addition of equal volumes of PIPES buffer and equilibration for 24 h at 25 °C always had values similar to those obtained after preparation. Hence, the liposomes investigated were reasonably stable in the absence of surfactants under the experimental conditions used.

We reported the variation of the surface tensions of the surfactant solutions in PIPES buffer for the alkyl glucosides investigated as a function of total surfactant concentration as well as their cmcs)[26]. The increase in the alkyl chain length drastically reduced the cmcs of the surfactant (Table 1) and slightly reduced the surface tensions in all cases (from 31 m Nm^{-1} for C_8-Glu to 28.5 m Nm^{-1} for C_{12}-Glu). Given that the surface tension at the cmc (γ_{cmc}) is used as one of the criteria of surface activity of the system (the lower the γ_{cmc}, the higher the surface activity [35]), we may assume that the longer the surfactant hydrophobic tail, the higher its surface activity in the system.

To determine the partition coefficients of the surfactants tested between bilayers and the aqueous phase, we first studied the validity of the equilibrium partition model proposed by Almog et al. [11] and Lichtenberg [23] based on Eq. (1) for the surfactant investigated. 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

Table 1 Surfactant to lipid molar ratios (Re), normalized partition coefficients (K) and surfactant concentrations in the aqueous medium (S_W) resulting in the interaction of alkyl glucosides with stratum corneum lipid liposomes. The critical micelle concentrations (*cmcs*)of each surfactant tested are also included together with the regression coefficients of the straight lines obtained

	cmc (mM)	$S_{W,SAT}$ (mM)	$S_{W,SOL}$ (mM)	$\mathrm{Re_{SAT}}$ mol/mol	$\mathrm{Re_{SOL}}$ mol/mol	K_{SAT} (mM^{-1})	K_{SOL} (mM^{-1})	r^2 SAT	r^2 SOL
C_8-Glu	18.0	18.0	18.50	1.56	4.14	0.03	0.04	0.998	0.993
C_9-Glu	5.6	5.60	5.75	1.45	4.01	0.11	0.14	0.994	0.992
C_{10}-Glu	1.80	1.82	1.90	1.80	4.80	0.35	0.43	0.996	0.995
C_{11}-Glu	0.58	0.58	0.62	2.70	6.35	1.26	1.39	0.995	0.998
C_{12}-Glu	0.18	0.18	0.19	3.90	8.40	4.42	4.70	0.990	0.991

$1/S_W$; this line should have a slope of $1/K$, intersect with the L/S_B axis at -1 and intersect with the $1/S_W$ axis at K. These authors demonstrated the validity of this model for C_8-Glu/PC liposome systems in the range of lipid and surfactant concentrations used in the present work [11].

To test the validity of the model for the alkyl glucosides investigated using SC lipid liposomes, vesicles were mixed with varying sublytic surfactant concentrations (S_T). The resulting surfactant-containing vesicles were then spun at 140,000*g* at 25 °C for 4 h to remove the vesicles [11]. No lipid was detected in the supernatants [29]. The concentration of each surfactant in the supernatants (S_W) was determined by high-pressure liquid chromatography [36], and their concentration in the lipid bilayers was calculated ($S_B = S_T - S_W$). The results of the experiments in which S_B and S_W were measured (for each surfactant 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 surfactant ($r^2 = 0.991$, 0.992, 0.989, 0.994 and 0.990 for C_8-Glu, C_9-Glu, C_{10}-Glu, C_{11}-Glu and C_{12}-Glu, respectively), which were dependent on L and always intersected the L/S_B axis at -0.97 ± 0.12. Both the linearity of these dependences and the proximity of the intercept to -1 support the validity of this model to determine K for these surfactant/liposome systems.

To determine the Re and S_W parameters, a systematic investigation of SLS variations in SC liposomes due to the addition of the alkyl glucosides investigated was carried out for various lipid concentrations. The curves obtained for C_8-Glu (lipid concentration ranging from 1.0 to 10.0 mM) are given in Fig. 1. Addition of surfactant to the liposomes led to an initial increase and a subsequent fall in the scattered intensity of the system until a low constant value was achieved, corresponding to the complete solubilization of the liposomes. The curves obtained for the other alkyl glucosides investigated showed similar trends to those exhibited by C_8-Glu (results not shown). This SLS behaviour is similar to that reported for the interaction of C_8-Glu with PC liposomes [24, 25] although showing in all cases a more pronounced initial SLS increase.

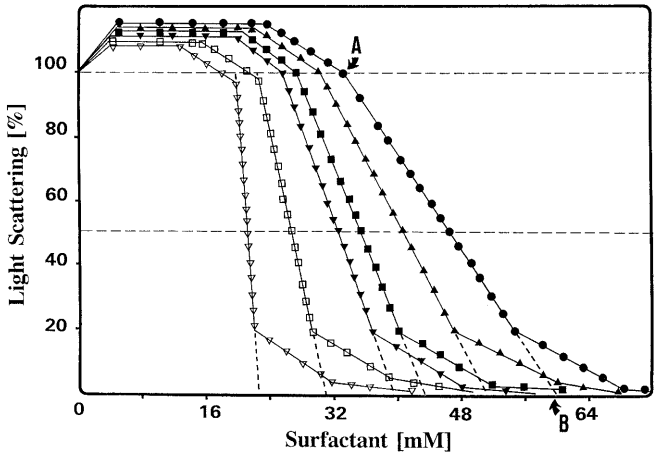

Fig. 1 Intensity percentage changes in static light scattering of stratum corneum (*SC*) lipid liposomes, (lipid concentration ranging from 1.0 to 10.0 mM), induced by the presence of increasing concentrations of C_8-Glu. SC lipid concentrations: 1.0 mM (▽), 3.0 mM (□), 5.0 mM (▼), 6.0 mM (■), 8.0 mM (▲) and 10.0 mM (●)

Surfactant concentrations producing 100% (S_{SAT}) and 0% (S_{SOL}) of SLS were obtained for each lipid concentration by graphical methods. Arrows A and B (curve for 10.0 mM lipid concentration, Fig. 1) correspond to these two values.

When plotting the surfactant concentrations thus obtained versus the lipid concentration, curves were obtained in each case. The straight lines corresponded to Eqs. (4) and (5), from which the Re and S_W parameters were determined. The r^2 statistic (r^2 regression coefficients given in Table 1) indicated that the straight lines obtained explained more that 98.3% of the surfactant concentration variability versus lipid concentration. Therefore, a very good linear fit was established in each case. These findings confirm that these straight lines were appropriate to determine Re and S_W for the surfactants tested. This method has also been demonstrated to be valid for the study of the interactions of different surfactants with SC lipid liposomes [37, 38]. The Re, K and S_W values obtained and the surfactant cmcs are given in Table 1.

The free surfactant concentrations ($S_{W,SAT}$, $S_{W,SOL}$) always decreased as the alkyl chain length of the surfactant increased, although they had similar values to those for the cmcs of the surfactant in all cases. These findings are in line with that reported for the interaction of C_8-Glu with PC liposomes [24, 25]. Given that S_W is the surfactant concentration in the aqueous phase this finding extends to the SC lipid liposomes the observation made with PC liposomes that the free surfactant concentration must reach the cmc for solubilization to commence and indicates that liposome solubilization was mainly ruled by the formation of mixed micelles [23, 24].

As for the Re parameters, these values always increased from liposome saturation (Re_{SAT}) to complete solubilization of these bilayer structures (Re_{SOL}); C_9-Glu had the lowest values in both cases. Given that the surfactant capacity to saturate or solubilize liposomes is inversely related to the Re value, the maximum activity at these two interaction levels corresponded to C_9-Glu, whereas the minimum corresponded to C_{12}-Glu (highest Re values). Thus, from C_{12}-Glu to C_9-Glu the lower the surfactant alkyl chain length (or the hydrophobic moiety), the higher its ability to saturate and solubilize

the liposomes. This finding is surprising taking into account that the C_{12}-Glu exhibits the lowest γ_{cmc} and, consequently, the highest surface activity [35].

Comparison of the Re values for C_8-Glu with those reported for the interaction of this surfactant with PC liposomes [24, 25] shows that the ability of this surfactant to saturate and solubilize SC liposomes was less (higher Re values) than that exhibited with PC liposomes. Hence, the SC lipid vesicles exhibited more resistance to the surfactant perturbations than the PC vesicles. This different behaviour may be explained bearing in mind the more hydrophilic nature of PC, which could facilitate the surfactant permeation in PC vesicles, their saturation and subsequent formation of mixed micelles [39].

The normalized surfactant partition coefficients between bilayers and the aqueous phase both for saturation and solubilization (K_{SAT} and K_{SOL}, Table 1) show that the C_{12}-Glu molecules had the highest values, whereas the C_8-Glu showed the lowest. In addition, the K values increased from saturation to complete bilayer solubilization regardless of surfactant alkyl chain length. This means that the affinity of surfactant molecules for the lipids building the liposome structure appears to be greater in the complete bilayer solubilization (micellization process) than during the previous step of bilayer saturation (formation of mixed vesicles). This result contrasts with that reported by Ueno [40], who postulated that in the interaction of C_8-Glu with PC liposomes, K was independent of the surfactant concentration at low free surfactant concentrations.

The relationship between K_{SAT} and K_{SOL} for each surfactant may be correlated with a dynamic surfactant/lipid equilibrium in transition steps from mixed vesicles to mixed micelles. Comparison of these two parameters reveals that the higher the surfactant alkyl chain length, the higher the quotient K_{SAT}/K_{SOL}. Hence, at the saturation level the surfactant partitioning into liposomes increased with respect to that for solubilization as the length of the surfactant hydrophobic tail rose (K_{SAT}/K_{SOL} values from 0.75 for C_8-Glu to 0.94 for C_{12}-Glu). Thus, although the length of the hydrophobic tail improved, the partitioning of surfactant molecules also resulted in a relative decrease in their ability to be associated with lipid molecules to form mixed micelles. Possibly, the first-order phase transition from mixed vesicles into mixed micelles is hampered by the increasing size of the hydrophobic tail.

Comparison of the K values for C_8-Glu with those reported for PC liposomes [24, 25] shows that the degree of partitioning of this surfactant into SC bilayers both at saturation and solubilization levels was similar to that for PC vesicles in spite of the different degree of saturation and nature of polar heads of the lipid building these two bilayer structures.

If the Re and K values are plotted versus the surfactant cmc the graphs shown in Figs 2 and 3 are obtained. A decrease in Re_{SAT} and Re_{SOL} took place as

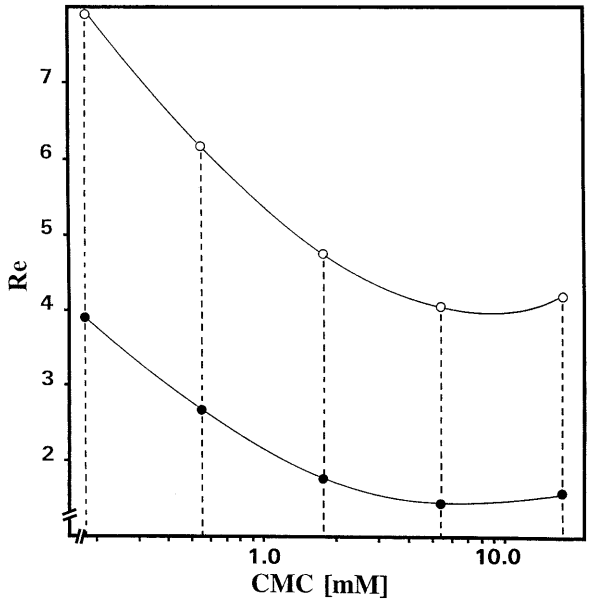

Fig. 2 Variation in the effective surfactant-to-lipid molar ratios (Re_{SAT} and Re_{SOL}) in the SC lipid liposomes for the alkyl glucosides tested versus the critical micelle concentrations (*cmc*) of the surfactant. Re_{SAT} (●) and 100% Re_{SOL} (○)

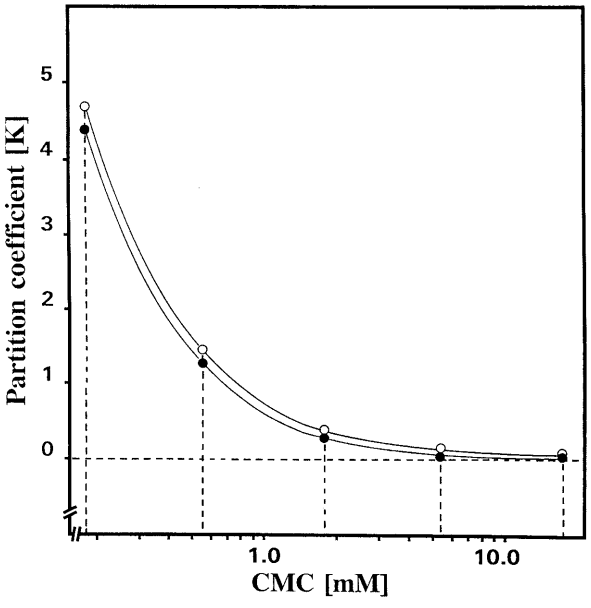

Fig. 3 Variation in the normalized bilayer/aqueous phase partition coefficients (K_{SAT} and K_{SOL}) in SC lipid liposomes for the alkyl glucosides tested versus the cmcs of the surfactant. K_{SAT} (●) and K_{SOL} (○)

the surfactant cmc rose (or the alkyl chain length decreased), although showing a minimum for C_9-Glu. The fall in Re was more pronounced for the surfactants with lower cmcs.

The structural changes in the molecular surfactant structure due to the decrease in its alkyl chain length (increase in its cmc) also resulted in an abrupt fall in K_{SAT} and K_{SOL}, which was also especially noticeable for the surfactants with lower cmcs. Thus, the degree of surfactant partitioning into bilayers (or affinity with these structures) drastically decreased as the surfactant alkyl chain length decreased or its cmc increased.

Hence, two opposite trends in the interaction of alkyl glucosides with SC lipid liposomes may be observed when comparing the variation of Re and K versus the surfactant cmc. The structural changes corresponding to the increase in its cmc led to a rise in the surfactant ability to saturate or solubilize the liposomes and inversely to an abrupt decrease in its affinity with these vesicles. The overall balance of these two tendencies indicates that the molecular structure of C_9-Glu was the more appropriate in terms of the hydrophilic–lipophilic balance to obtain an improved activity against SC lipid vesicles.

From a practical viewpoint, comparison of the surfactant amounts needed to saturate and solubilize liposomes for C_8-Glu and C_9-Glu (1.0 mM lipid concentration) shows that when using C_9-Glu only a concentration approximately 2.5 times lower than that used for the C_8-Glu was needed to produce the same effects in these vesicles. Hence, the use of C_9-Glu may be considered as an alternative with respect to the use of the conventional C_8-Glu, given its higher lytic activity, lower toxicity, appropriate solubility in water and improved ecological properties [17, 18]. In addition, the results presented in this work could be useful in the study of transfersomes and other deformable vesicles, whose bilayers are formed by a combination of lipids and surfactants [41, 42]. Firstly, knowledge of the interaction of liposomes with surfactant is essential in the development of these types of delivery systems. Secondly, the study of alkyl glucosides and the effect of the hydrophobic tail in the liposome–surfactant interaction could be very useful in the design of more specific deformable vesicles for transdermal application that would induce permeation enhancement without damaging the skin.

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References

1. Schäfer-Korting M (1992) In: Braun-Falco O, Korting HC, Maibach H (eds) Liposome dermatics (Griesbach conference). Springer, Berlin Heidelberg New York, pp 299–307
2. Wertz PW, Downing DT (1989) Transdermal drug delivery. Developmental issues and research initiatives. Dekker, New York
3. Friberg SE, Goldsmith LB, Kayali I, Suhaimi H (1991) In: Bender M (ed) Interfacial phenomena in biological systems. Surfactant science series 39. Dekker, New York, pp 3–32
4. Imokawa G, Abe A, Jin K, Higaki Y, Kamashima M, Hidano A (1991) J Invest Dermatol 96:523
5. Bouwstra JA, Gooris GS, Bras W, Downing DT (1995) J Lipid Res 36:685
6. Wertz PW, Abraham W, Landman L, Downing DT (1986) J Invest Dermatol 87:582
7. Yoneto K, Li SK, Higuchi WI, Jiskoot W, Herron JN (1996) J Pharm Sci 85:511
8. Suhonen TM, Pirskanen L, Ra M, Kosonen K, Rytting JH, Paronen P, Urtti A (1997) J Controlled Release 43:251
9. Ghyczy M, Vacata V (2001) In: Rowlings AW, Leyden J (eds) Skin moisturisers. Dekker, New York, pp (in press)
10. Vinson PK, Talmon Y, Walter A (1989) Biophys J 56:669
11. Almog S, Litman BJ, Wimley W, Cohen J, Wachtel EJ, Barenholz Y, Ben-Shaul A, Lichtenberg D (1990) Biochemistry 29:4582
12. Cully DF, Paress PS (1991) Mol Pharmacol 40:326
13. Lummis SCR, Martin IL (1992) Mol Pharmacol 41:18
14. Oku N, Tsudera J, Kurohani K, Okada S (1996) Chem Pharm Bull 44:1928
15. Tetlo IJ, Bowsher CG, Emes MJ (1996) Biochem J 319:717
16. Seras M, Edwards K, Almgren M, Carlson G, Ollivon M, Lesieur S (1996) Langmuir 12:330
17. García MT, Ribosa I, Campos E, Sanchez Leal J (1997) Chemosphere 35:545
18. Balzer D, (1997) Specialist surfactants. Blackie, London
19. Downing DT, Abraham W, Wegner BK, Willman KW, Marshall JM (1993) Arch Dermatol Res 285:151
20. Silvander M, Karlsson G, Edwards K (1996) J Colloid Interface Sci 179:104
21. Partearroyo MA, Alonso A, Goñi FM, Tribout M, Paredes S (1996) J Colloid Interface Sci 178:156
22. Cladera J, Rigaud JL, Villaverde J, Duñach M (1997) Eur J Biochem 243:798
23. Lichtenberg D (1985) Biochim Biophys Acta 821:470
24. de la Maza A, Parra JL (1994) Eur J Biochem 226:1029
25. de la Maza A, Parra JL (1996) J Am Oil Chem Soc 73:877
26. de la Maza A, Coderch L, Gonzalez P, Parra JL (1998) J Controlled Release 52:159
27. de la Maza A, Manich AM, Coderch L, Bosch P, Parra JL (1995) Colloids Surf A 101:9
28. Wertz PW (1992) In: Braun-Falco O, Korting HC, Maibach H (eds) Liposome dermatics (Griesbach conference). Springer, Berlin Heidelberg New York, pp 38–43
29. Ackman RG, Mc Leod CA, Banerjee AK (1990) J Planar Chromatogr-Mod TLC 3:450
30. Schurtenberger P, Mazer N, Känzig W (1985) J Phys Chem 89:1042
31. Ruiz J, Goñi FM, Alonso A (1988) Biochim Biophys Acta 937:127

32. Wilheim KP, Surber C, Maibach HI (1991) J Invest Dermatol 97:927
33. Wilheim KP, Surber C, Maibach HI (1991) J Invest Dermatol 96:963
34. de la Maza A, Parra JL (1997) Biophys J 72:1668
35. Rosen MJ (1974) J Am Oil Chem Soc 51:461
36. Seino H, Uchibori T, Nishitani T, Inamasu S (1984) J Am Oil Chem Soc 61:1761
37. de la Maza A, Parra JL (1997) Colloid Polym Sci 275:821
38. Cócera M, López O, Coderch L, Parra JL, de la Maza A (2000) Colloid Polym Sci 278:794
39. Lasic DD (1993) In: Lasic DD (ed) Liposomes from lipids to applications. Elsevier, Amsterdam, pp 56–62
40. Ueno M (1989) Biochemistry 28:5631
41. Cevc G (1996) Crit Rev Ther Drug Carrier Syst 13:257
42. Schatzlein A, Cevc G (1998) Br J Dermatol 138:583