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ORIGINAL CONTRIBUTION

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Dodecyl maltoside as a solubilizing agent of stratum corneum lipid liposomes

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Abstract The lytic interactions of the nonionic surfactant dodecyl maltoside (DM) with liposomes formed by a mixture of lipids modeling the stratum corneum (SC) lipid composition were investigated. To this end, 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 concentration was always similar to its critical micelle concentration indicates that the liposome solubilization was mainly ruled by formation of mixed micelles. In addition, the linear dependence established between the level of SLS and Re indicates a progressive incorporation of DM in the liposomes as well as the progressive formation of mixed micelles. DM showed in all cases lower bilayer activity (higher Re values) and greater affinity with vesicles (higher K values) than those reported for its interaction with phosphatidylcholine (PC) liposomes. Thus, whereas the SC lipid liposomes were more resistant to the action of this surfactant, its degree of partitioning into SC bilayers was higher throughout the solubilization process than that exhibited in PC vesicles. Comparison of the present Re values with those reported for the lytic interaction of dodecyl glucoside (DG) with SC liposomes reveals that in the case of DM the bilayer activity was more than three times higher than that for DG in spite of the identical alkyl chain length.

Keywords Stratum corneum lipid liposomes · Dodecyl maltoside · Surfactant/lipid molar ratios · Surfactant partition coefficients · Surfactant critical micelle concentrations · Static light-scattering

Abbreviations *SC* stratum corneum · *PC* phosphatidylcholine · *PIPES* piperazine-1,4 bis(2-ethanesulphonic acid) · *Re* effective surfactant/lipid molar ratio · *K* normalized bilayer/aqueous phase surfactant partition coefficient · S_W surfactant concentration in the aqueous medium · S_B surfactant concentration in the bilayers · *Cer* ceramides type III · *Chol* cholesterol · *PA* palmitic acid · *Chol-sulf* cholesteryl sulfate · *DM* Dodecyl maltoside (n-dodecyl β-D-maltoside) · *TLC-FID* thin-layer chromatography/automated flame ionization detection system · *CMC* critical micellar concentration · *PI* polydispersity index · r^2 regression coefficient

Introduction

The stratum corneum (SC) consists of corneocytes that are separated by an intercellular matrix composed mainly of lipids. These lipids are organized into bilayers that have been postulated both to account for the permeability properties and to ensure the cohesiveness between corneocytes [1–4]. In all intercellular membranes, such 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 formed bilayers, Wertz et al. [5] prepared and characterized liposomes from lipid mixtures modeling the SC composition.

The nonionic surfactant dodecylmaltoside (DM) has been used for solubilization of cytochrome oxidase in active form [6, 7] and has been found to have good properties for solubilization of diverse membrane proteins [8–11].

We first studied the formation of liposomes from lipid mixtures modeling the SC composition [12], as well as the interaction of the surfactant model sodium dodecyl sulfate with these vesicles enriched in cholersteryl sulfate [13]. We also investigated the phase transitions involved in the interaction of DM with phosphatidylcholine (PC) liposomes [14]. Here, the effective surfactant to lipid molar ratios (Re) and the normalized bilayer/aqueous phase partition coefficients (K) corresponding to the lytic interaction of DM with SC lipid liposomes have been investigated in order to establish a criterion for the evaluation of its activity on a complex membrane such as the stratum corneum.

Materials and methods

The nonionic surfactant dodecylmaltoside (n-dodecyl β-D-maltoside, DM) was 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 [12]. Reagent grade organic solvents, ceramides type III (Cer), and cholesterol (Chol) were supplied by Sigma Chemical Co. (St Louis, MO), and palmitic acid (PA) (reagent grade) was purchased from Merck. Cholesteryl sulfate (Chol-sulf) was prepared by reaction of cholesterol with excess chlorosulphonic acid in pyridine and purified chromatographically [12].

The molecular weight of ceramide used was determined by low resolution fast atom bombardment mass spectrometry [12]. A molecular weight of 671 g was obtained for the majority 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 P.W. Wertz [15], despite the fact that the ceramide type III used was a mixture of ceramides of different chain length (purity approx 99%). Hence, we used the molecular weight obtained to calculate the molarity of the lipid mixture investigated.

Preparation and characterization of SC lipid liposomes

We reported the formation of liposomes using a mixture of lipids modeling the SC composition [12], which was prepared following the method described by Wertz et al. [5]. The size of vesicles was determined using a photon correlator spectrometer (Malvern Autosizer 4700c PS/MV; Malvern, England) [16, 17]. The lipid composition and concentration of liposomes were determined using thin-layer chromatography coupled to an automated flame ionization detection system (TLC-FID, Iatroscan MK-5, Iatron Lab. Inc. Tokyo, Japan) [18]. To find out whether all the components of the lipid mixture formed liposomes, vesicular dispersions were analyzed for these lipids [18]. The dispersions were then spun at 140000 g at 25 °C for 4 h to remove the vesicles [19]. 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 ^{1}H NMR showing a value of 55–56 °C [12].

Parameters involved in the interaction of DM with SC lipid liposomes

In the analysis of the equilibrium partition model proposed by Schurtenberger et al. [20] for bile salt/lecithin systems, Lichtenberg [21] and Almog et al. [19] have shown that for a mixing of lipids [lipid conc L (mM)] and surfactant in dilute aqueous media, the distribution of surfactant between lipid bilayers and water obeys a partition coefficient K, by:

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

where S_B and S_W are the surfactant concentrations in the bilayers (mM) and in the aqueous medium (mM). For $L \gg S_B$, the definition of K, as given by Schurtenberger, applies:

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

where Re is the effective molar ratio of surfactant to lipid in the bilayers ($Re = S_B/L$). Under any other conditions Eq. 1 has to be employed to define K; this yields:

$$K = Re/(S_W[1 + Re]) \quad (3)$$

This approach is consistent with the experimental data offered by these authors for various surfactant/lipid systems over wide ranges of Re. Given that the lipid concentration range used is similar to that used by Almog et al. to test his equilibrium partition model, the K parameter has been determined using this equation.

It has been previously demonstrated that static light scattering technique (SLS) constituted a very convenient technique for the quantitative study of the liposome solubilization by surfactants [22–24]. Accordingly, the solubilizing perturbations produced by DM in SC lipid liposomes was monitored using this technique. The solubilization of liposomes was characterized by determining the different levels of SLS of the system throughout the solubilization process. To this end, liposomes were adjusted to the appropriate lipid concentration (from 0.5 to 5.0 mM). Equal volumes of the appropriate 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 (mM) was calculated for each mixture. This time was chosen as the optimum period needed to achieve a complete equilibrium surfactant/liposome in the lipid concentration range used [24, 25]. The temperature of 25 °C was selected for the following reasons: (i) the reasonable stability of the SC liposomes under these conditions, (ii) similar experimental conditions to those used to study the interaction of dodecyl maltoside with PC liposomes (iii) these experimental conditions are generally used in "in vivo" tests to study the interaction of surfactants with skin [26–28]. SLS measurements were made at 25 °C using a spectrofluorophoto-

meter Shimadzu RF-540 (Kioto, Japan) with both monochromators adjusted to 500 nm [14]. The assays were carried out in triplicate and the results given are the average of those obtained.

The determination of Re and K parameters was carried out on the basis of the linear dependence existing between the surfactant concentrations required to achieve different levels of SLS and the lipid concentration (L), which can be described by the equations:

$$S_T = S_W + Re \cdot [L] \quad (4)$$

where S_T is the total surfactant concentrations. The surfactant to lipid molar ratio Re and the aqueous concentration of surfactant S_W is in each curve respectively the slope and the ordinate at the origin (zero lipid concentration). The K parameters (normalized bilayer/aqueous phase surfactant partition coefficients) were calculated using Eq. 3.

Results

The characterization of the geometric properties of the SC liposomes demonstrated that these liposomes were formed by unilamellar vesicles in all cases [12]. The size distribution curve of the vesicles obtained by photon correlator spectrometry just after their preparation exhibited a monomodal distribution (one peak) with a hydrodynamic diameter (HD) of about 200 nm. The polydispersity index (PI) was in all cases lower than 0.10, indicating a homogeneous size distribution in all cases. The vesicle size just after the addition of equal volumes of PIPES buffer and equilibration for 60 min at 25 °C always showed values similar to those obtained after preparation, with a slight rise in PI (between 0.10 and 0.14). Hence, the liposomes appeared to be reasonably stable in the absence of DM under the experimental conditions used.

Parameters involved in the interaction of DM with SC lipid liposomes

We first studied the validity of the equilibrium partition model proposed by Almog et al. and Lichtenberg [19, 21] based on the Eq. 1 for DM. 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. These authors demonstrated the validity of this model for octyl glucoside/PC liposome systems in the range of lipid and surfactant concentration used in the present work.

To test the validity of the model for DM using SC liposomes, vesicles were mixed with varying sublytic DM concentrations (S_T). The resultant surfactant-containing vesicles were then spun at 140 000 g at 25 °C for 4 h to remove the vesicles [19]. No lipids were detected in the supernatants [18]. The DM concentration in the supernatants (S_W) was determined by HPLC [29], and its concentration in bilayers was calculated ($S_B = S_T – S_W$). The S_B and S_W values thus obtained (in the same range of lipid and DM concentrations used to determine K) were plotted in terms of the dependence of L/S_B on $1/S_W$. A straight line was obtained ($r^2 = 0.991$), which was dependent on L and intersected with the L/S_B axis at $–0.96 \pm 0.11$. Both the linearity of this dependence and the proximity of the intercept to –1 support the validity of this model to determine K for this system.

To determine the Re and S_W parameters, a systematic investigation of SLS variations in SC liposomes due to the addition of DM was carried out for various lipid concentrations. The curves obtained (lipid concentration ranging from 0.5 to 5.0 mM) are given in Fig. 1. Addition of surfactant to 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 liposomes.

The DM concentrations resulting in different percentages of SLS were graphically obtained and plotted versus lipid concentration (results not shown). An acceptable linear relationship was established in each case. The r^2 statistic values (regression coefficients r^2, Table 1) of these straight lines demonstrate the goodness of the fit. These findings confirm that the straight lines for the Eq. 4 were appropriate to determine Re and S_W. This method has also been demonstrated to be valid for the study of the interaction of various surfactants with SC lipid liposomes [13, 17]. The Re, K and S_W values obtained are given in Table 1, in which it is observed that the Re, S_W and K values progressively increase as the SLS of the system decrease.

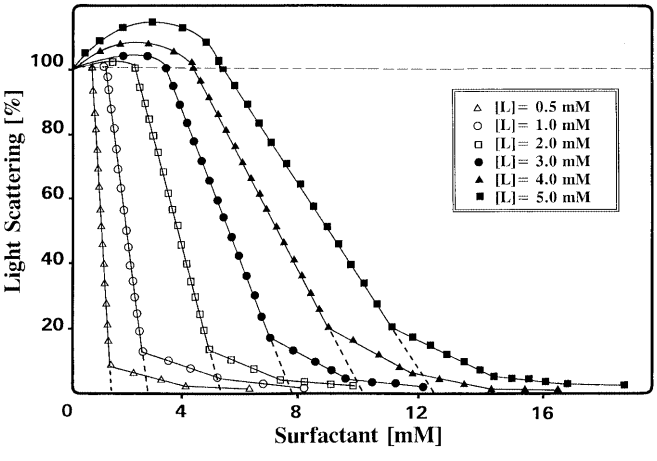

Fig. 1 Intensity percentage changes in static light-scattering of SC lipid liposomes, (lipid concentration ranging from 0.5 to 5.0 mM), induced by the presence of increasing concentrations of DM. SC lipid concentration symbols : (△) 0.5 mM, (○) 1.0 mM, (□) 2.0 mM, (●) 3.0 mM, (▲) 4.0 mM and (■) 5.0 mM

Figure 2 shows the variation of the SLS versus Re in the interaction DM with SC lipid liposomes. It is interesting to note that a linear relationship was established between both parameters. Thus, the progressive incorporation of surfactant in the lipid bilayers results in a progressive formation of mixed micelles surfactant-lipid, which is reflected by a progressive decrease of the SLS of the system. Figure 3 shows the variation in K versus Re throughout the solubilization process. The increase in Re results in a increase in K which was more pronounced in the Re interval ranging from 1.1 to 1.9.

Discussion

In general the SLS behavior during the solubilization process of SC liposomes by DM is similar to that reported for the interaction of DM with PC liposomes [14] although showing in all cases a more pronounced initial SLS increase.

Concerning the results reported on Table 1; the fact that S_W always showed similar values than the DM critical micelle concentration (0.125 mM [14]) confirms the generally admitted assumption that the liposome solubilization process was mainly ruled by the action of surfactant-lipid mixed micelles [21].

Comparison of the Re and K values obtained in this study with those reported for the interaction DM with PC liposomes [14] shows that at the same interaction step (same SLS percentage) both parameters always exhibited higher values. Given that the surfactant capacity to interact with liposomes is inversely related to the Re value, we assume that the DM activity was always lesser than that exhibited with PC liposomes. Hence, the SC lipid vesicles were more resistant to the surfactant perturbations than PC vesicles [14]. However, the increased K values indicated that the affinity of DM molecules with SC liposomes was always higher than that for PC vesicles in spite of its decreased bilayer activity. Comparison of the variation in K versus Re (Fig. 3) for the interaction of DM with SC liposomes with that reported for the interaction of DM with PC liposomes [14] also confirm this fact. Both curves exhibit a similar tendency at the same interaction step. However, the Re and K values for SC liposomes were higher than those for PC vesicles. Hence, in the case of SC lipid liposomes more DM molecules were needed to achieve the same level of solubilization. These differences may be explained by taking into account the more hydrophilic nature of PC, which could facilitate the permeation of water and DM in PC vesicles either through the hydrophilic holes created on the polar heads or via formation of short-lived complexes DM-PC polar heads, the subsequent transfer through the bilayers via flip-flop

Table 1 Surfactant to lipid molar ratios, normalized partition coefficients and surfactant concentrations in the aqueous medium resulting in the solubilizing interaction of DM with SC lipid liposomes. The regression coefficients of the straight lines obtained are included

Light-scattering (%)	S_W (mM)	Re (mole/mole)	r^2	K (Mm^{-1})
100	0.124	1.03	0.993	4.09
90	0.124	1.19	0.994	4.38
80	0.125	1.36	0.997	4.61
70	0.125	1.49	0.990	4.78
60	0.126	1.65	0.992	4.94
50	0.126	1.78	0.994	5.08
40	0.127	1.93	0.992	5.18
30	0.127	2.08	0.998	5.31
20	0.128	2.23	0.995	5.39
10	0.128	2.41	0.991	5.52
0	0.129	2.54	0.996	5.56

S_W, surfactant concentration; Re, surfactant to lipid molar ratio; r^2, regression coefficient; K, normalized partition coefficient

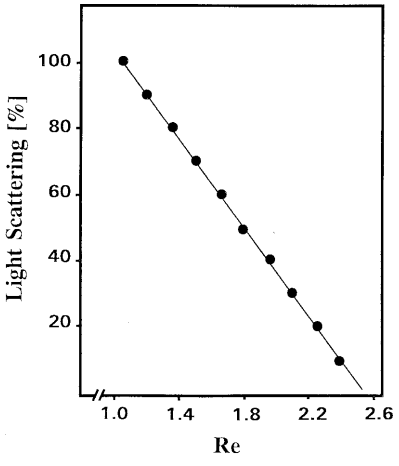

Fig. 2 Variation in the static light-scattering of the system versus the surfactant to SC lipid molar ratio (Re)

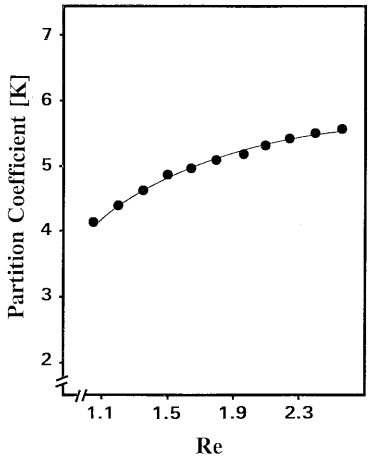

Fig. 3 Variation in the normalized bilayer/aqueous phase partition coefficients versus the surfactant to SC lipid molar ratio (Re)

[30], and the subsequent formation of mixed micelles surfactant-lipid. In addition, other factors such as the different phase transition temperatures of the PC (below 0 °C) and SC lipids (about 65 °C) and the different polarity of the PC and the SC lipids should be considered. From a physiological viewpoint, the different behavior of PC and SC liposomes could be related to the different functionality of the membranes depending on their lipid composition. Whereas the phospholipids constitute the main lipid of the cellular membranes, these lipids are absent from the SC lamellar structure, where the predominant lipids are cholesterol, free fatty acids and ceramides. The chemical nature of the SC lipids has been described as an important factor responsible for the barrier function of the skin in avoiding the transepidermal water loss. This function is specific to the SC membranes in contrast with the more usual membranes formed by phospholipids, which are highly permeable. Probably, the resistance of the SC lipid liposomes against the surfactants could be related to this fact.

Comparison of the present Re and K values with those reported for the lytic interaction of dodecyl glucoside (DG) with SC lipid liposomes [31] reveals that in the case of DM, the Re values for liposome saturation (100% SLS, corresponding to the Re_{SAT} according to the nomenclature adopted by Lichtenberg [21]) and complete liposome solubilization (0% SLS, corresponding to the Re_{SOL} [21]) were more than three times lower than those for DG. However, the K values for DM for bilayer saturation were slightly lower, and for complete solubilization slightly higher, than those for DG. The increased DM activity could be explained by bearing in mind that its molecular structure appears to be more equilibrated in terms of hydrophilic-lipophilic balance (HLB) than that of the DG in spite of the identical alkyl chain length of both compounds. In fact, the adsorption of the surfactant molecules and subsequent incorporation into bilayers are correlated with the HLB of each surfactant as well as with the composition and physicochemical characteristics of the bilayer structure.

Conclusions

In the interaction with the SC lipid liposomes, the DM showed lower bilayer activity (higher Re values) and greater affinity with vesicles (higher K values) than those reported for its interaction with PC liposomes. Hence, a higher concentration of DM was needed to induce the same effects in SC lipid liposomes, despite the higher degree of partitioning of this surfactant throughout the solubilization process. Thus, we can conclude that SC lipid liposomes were more resistant to the action of this surfactant than the PC liposomes.

Comparison of the solubilizing parameters of the DM with those reported for the lytic interaction of dodecyl glucoside (DG) with SC liposomes shows that the DM was more active than the DG in spite of the identical alkyl chain length of the surfactants. This fact could be probably due to the different hydrophilic nature of the surfactants.

References

1. Wertz PW, Downing DT (1989) Transdermal drug delivery. Developemental issues and research iniciatives. Marcel Dekker, New York
2. Friberg SE, Goldsmith LB, Kayali I, Suhaimi H. (1991) Lipid structure of stratum corneum. In: Bender M (ed) Interfacial phenomena in biological systems, Surfactant science series, vol. 39. Marcel Dekker, New York, chap 1
3. Imokawa G, Abe A, Jin K, Higaki Y, Kamashima M, Hidano A (1991) J Invest Dermatol 96:523–526
4. Bouwstra JA, Gooris GS, Bras W, Downing DT (1995) J Lipid Res 36:685–695
5. Wertz PW, Abraham W, Landman L, Downing DT (1986) J Invest Dermatol 87:582–584
6. Suarez MD, Revzin A, Narlock R, Kempner ES, Thompson DA, Ferguson-Miller S (1984) J Biol Chem 259:13791–13799
7. Bolli R, Nalecz KA, Azzi A (1985) Arch Biochem Biophys 240:102–116
8. Foresta B, Henao F, Champeil P (1992) Eur J Biochem 209:1023–1034
9. le Marie M, Garrigos M, Møller, JV (1992) Technologies on protein studies and purification. vol 5. G.R.B.P., Villebon sur Yvette, France, pp 75–85
10. Kragh-Hansen U, le Marie, M, Nöel J.P, Gulik-Krzywicki T, Møller JV (1993) Biochemistry 32:1648–1656
11. Lambert O, Levy D, Ranck JL, Leblanc G, Rigaud JL (1998) Biophys J 74:918–930
12. de la Maza A, Manich AM, Coderch L, Bosch P, Parra JL (1995) Colloids surfaces A 101:9–19
13. Cócera M, López O, Coderch L. Parra JL, de la Maza A (2000) Colloid Polym Sci 278: 794–799
14. de la Maza A, Parra JL (1997) Biophys J 72:1668–1675
15. Wertz PW (1992) Liposome dermatics. Chemical aspects of the skin approach. In: Braun-Falco O, Korting HC, Maibach H (eds) Liposome dermatics (Griesbach conference). Springer, Berlin Heidelberg New York, pp 38–43
16. de la Maza A, Parra JL (1997) Colloid Polym Sci 275:821–829
17. de la Maza A, López O, Cócera M, Coderch L. Parra JL, Guinea J (1999) Colloid Polym Sci 277:1200–1204
18. Ackman RG, Mc Leod CA, Banerjee AK (1990) J Planar Chrom 3:450–490
19. Almog S, Litman BJ, Wimley W, Cohen J, Wachtel EJ, Barenholz Y, Ben-Shaul A, Lichtenberg D (1990) Biochemistry 29:4582–4592
20. Schurtenberger P, Mazer N, Känzig W (1985) J Phys Chem 89:1042–1049
21. Lichtenberg D (1985) Biochim Biophys Acta 821:470–478

22. Urbaneja MA, Alonso A, Gonzalez Mañas JM, Goñi FM, Partearroyo MA, Tribout M, Paredes S (1990) Biochem J 270:305–308
23. Ruiz J, Prado A, Goñi FM, Alonso A (1994) Biochim Biophys Acta 1193:301–306
24. Partearroyo MA, Alonso A, Goñi FM, Tribout M, Paredes S (1996) J Colloid Interface Sci 178:156–159
25. Ruiz J, Goñi FM, Alonso A (1988) Biochim Biophys Acta 937:127–134
26. Schäfer-Korting M (1992) Liposome dermatics. Chemical aspects of the skin lipid approach. In: Braun-Falco O, Korting HC, Maibach H. (eds) Liposome dermatics (Griesbach conference). Springer, Berlin Heidelberg New York, pp 299–307
27. Wilheim KP, Surber C, Maibach HI (1991) J Invest Dermatol 97:927–932
28. Wilheim KP, Surber C, Maibach HI (1991) J Invest Dermatol 96:963–976
29. Seino H, Uchibori T, Nishitani T, Inamasu S (1984) J Am Oil Chem Soc 61:1761–1765
30. Lasic DD (1993) Liposomes from Lipids to Applications. Elsevier, Amsterdam, pp 56–62
31. López O, Cócera M, Parra JL, de la Maza A, (2001) Colloid Polym Sci 279:909–915