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Thermodynamic clarification of interaction between antiseptic compounds and lipids consisting of stratum corneum

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Abstract

The interactions of antiseptic compounds with quaternary ammonium, such as benzalkonium chloride (BC), benzethonium chloride (BZC), dodecyldiaminoethyl-glycine hydrochloride (AEG), and chlorhexidine gluconate (CHG), with components of the stratum corneum were investigated by isothermal titration calorimetry at pH 7.5 and 25 °C. The different mechanisms for their permeation to stratum corneum were clarified. Cationic surfactants of BC and BZC bound to cholesterol and cholesterol sulfate with high affinity (10^5–$10^6\ M^{-1}$) to extract endogenous cholesterol and its derivatives from the stratum corneum and penetrated via an intercellular route. CHG also bound to cholesterol and accumulated in the stratum corneum without removing endogenous cholesterol. On the other hand, an amphoteric surfactant of AEG seemed to be incorporated into the lipid bilayer and bound to ceramide with its polar end close to the lipid polar heads by hydrophobic interaction.

Keywords: Benzalkonium chloride; Cationic surfactant; Chlorhexidine gluconate; Dodecyldiaminoethylglycine hydrochloride; Amphoteric surfactant; Microcalorimetry; Stratum corneum

1. Introduction

The skin is a multifunctional biological membrane. The most important of these functions is the barrier property to diffuse external substances, as well as prevent the evaporation of water. Skin is composed of three distinct layers, the outer stratum corneum layer, the epidermis and the inner dermis layer. The stratum corneum is a multilayered wall-like structure in which keratin-rich corneocytes are embedded in an intercellular lipid-rich matrix, and presents the greatest barrier to percutaneous absorption [1]. The intercellular lipids are arranged in multiple bilayers, and both polar and non-polar components create this organized structure. It is widely accepted that the intercellular lipid domain is the major rate-determining pathway by which most substances penetrate the stratum corneum. A variety of techniques have been used to study the structure of the stratum corneum and these lipids [2–4]. Interest is currently focusing on the molecular structure of the stratum corneum lipids. However, their roles with regard to the function of the barrier as well as the interactions with external substance are not yet sufficiently clear.

Recently, some investigators reported that a cationic surfactant of benzalkonium chloride (BC) and anionic surfactant of sodium dodecyl sulfate (SDS) elicited erythema and induced skin irritation by causing a disturbance of the epidermal barrier [5,6]. We also found that ^{14}C-benzyldimethyldodecyl ammonium chloride (^{14}C-BC) accumulated in epidermis and dermis after a 24 h application of ^{14}C-BC to the dorsal skin of rats and hairless mice by whole body and micro-autoradiography [7]. BC is widely used for the preoperative disinfection of skin, the disinfection of hands or instruments, and the treatment of superficial injuries or infected wounds as a topical antiseptic agent. The use of BC has several advantages over that of other antiseptic compounds; the sterilizing effect is strong, the sterilizing rate is rapid, and the antibacterial spectrum is broad. Even below the usual concentration of 0.1%, however, skin irritation and associated inflammatory responses are often induced by its application. Chemically, BC is a mixture of alkylbenzyldimethylammonium chloride consisting of three major homologues with straight carbon

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chain lengths of C_{12}, C_{14} and C_{16}. In general, the C_{12} homologue is most effective against yeast and fungi, the C_{14} homologue against Gram-positive bacteria, and the C_{16} homologue against Gram-negative bacteria [8]. However, the interaction of each homologue of BC with skin or stratum corneum and the mechanism by which BC passes through the stratum corneum or any other lamellar lipid phase, have not been well-characterized.

Of the bactericidal antimicrobial agents currently available on the market, BC and benzethonium chloride (BZC) which are cationic surfactants, diaminoethylglycine hydrochloride (AEZ) which is an amphoteric surfactant, and chlorhexidine gluconate (CHG) are all quaternary ammonium compounds. These antiseptic compounds have a disinfecting and cleaning action by causing a decrease in the surface tension and protein denaturation of the bacterial cell membrane, and skin irritation is often induced as a side effect of the application. In this study, the binding of the antiseptic compounds to the components of intercellular lipids in stratum corneum was investigated in order to clarify the mechanisms of the interaction and the permeation through the stratum corneum.

2. Experimental

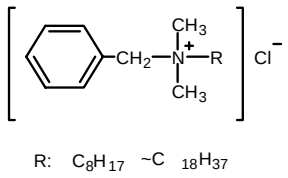
2.1. Materials

The antiseptic compounds used in this study were benzalkonium chloride (BC), benzyldimethylhexadecylammonium chloride (BC14), benzyldimethyltetradecylammonium chloride (BC16), benzethonium chloride (BZC) and chlorhexidine gluconate (CHG), which were purchased from Sigma Chemical (St. Louis, MO), benzyldimethyldodecyl-ammonium chloride (BC12) from Sigma-Aldrich Bhemie GmbH (Steinheim, Switzerland), and dodecyldiaminoethylglycine hydrochloride (AEZ) from Nissan Chemicals Co. (Tokyo, Japan). The chemical structures are shown in Fig. 1. Ceramide, cholesterol monohydrate (CH), cholesterol 2-sulfate (CHsulf), cholesterol palmitate (CHpal), and palmitic acid used as intercellular lipids and keratin from human epidermis as the intercellular protein of stratum corneum were purchased from Sigma Chemical and used without further purification. All other chemicals were of analytical grade.

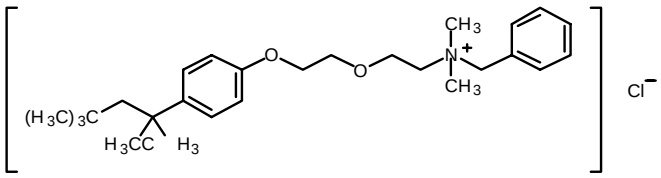
2.2. Preparation of rat stratum corneum samples

Dorsal full-thickness skin was excised from hairless rats (male Wister rats, at least 72-day-old, Kyudo Co., Ltd., Saga, Japan) and the subcutaneous fat and connective tissue were removed using forceps. The stratum corneum was separated from the epidermis by trypsin treatment [9]. The sheets of stratum corneum were rinsed repeatedly with distilled water, gently pressed between paper tissue, spread on filter paper and subsequently dried by storage in a desiccator over silica-gel at room temperature for a maximum of 1 week and powdered finely before use. Samples of lipid-extracted stratum corneum were obtained by soaking the dried stratum corneum sheets in chloroform–methanol (2:1, v/v) for 24 h at room temperature followed by soaking in acetone for 2 h and ethanol–diethyl ether (1:9, v/v) for 24 h [10]. The lipid-extracted samples were desiccated, as above, before use. Dried stratum corneum samples of known weight (16.0 mg/10 ml) were finely powdered and suspended in a pH 7.5 Tris buffer solution using an ultrasonic generator (UD-200, Tomy Co. Ltd., Tokyo, Japan) to prepare as a calorimetric titrand.

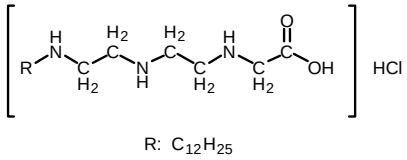
Benzalkonium chloride (BC)



Benzetonium chloride (BZC)



Dodecylaminoethylglycine hydrochloride (AEZ)



Chlorhexidine gluconate (CHG)

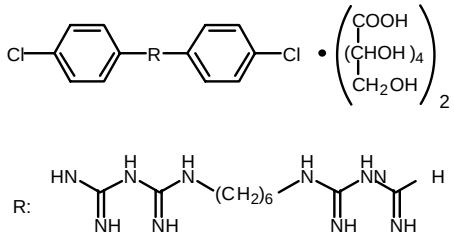


Fig. 1. Structures of antiseptic compounds used in this study.

2.3. Preparation of the suspension for lipid mixtures as a model system of intercellular lipids in human stratum corneum

For each series of calorimetric measurements as a model system of intercellular lipids in human stratum corneum, a suspension (2 mg/10 ml) of lipid mixtures was prepared. The mixtures studied were as follow: ceramide (46.2%, w/w), CH (30.2%), CHsulf (2.2%), CHpal (11.2%) and palmitic acid (10.2%) [11]. After weighting the mixture was co-solubilized in a chloroform/methanol 2:1 mixture solution. Chloroform/methanol was driven off by a nitrogen stream, and the mixture was stored under vacuum for at least 24 h. The resulting dry powders were finely ground up for 30 min with a ceramic vibrational ball mill (Fritsch, Germany), and were suspended in pH 7.5 Tris buffer by ultrasonication.

2.4. Isothermal titration microcalorimetry

Microcalorimetric measurements were performed with a Thermal Activity Monitor 2277 system (ThermoMetric AB, Järfälla, Sweden). A titration cell with a titration volume of 4.0 ml was used. The reference cell was filled completely with pH 7.5 Tris buffer and the sample cell was initially filled with a 3.0 ml suspension or solution of stratum corneum and the components in pH 7.5 Tris buffer. The 0.03–0.1% solution of the antiseptic compounds was injected as 15–20 portions of 15 μl in to the sample cell at $25.000 \pm 0.001\,^{\circ}C$. The concentrations of compounds used were below the critical micellization concentration (c.m.c.). The dilution heat of each compound (q_{dil}, μW) was measured separately using Tris buffer as a titrand and subtracted from the reaction heat. The reaction heat flow (q, μW) was integrated for every peak to give the heat effect, which was corrected by the corresponding heat of dilution of the compound. A calorimetric titration curve was obtained by plotting the heat effect (Q, mJ) versus the total concentration of the compounds added.

The value of Q is proportional to the amount of the compounds bound with the component of the stratum corneum (A_p), that is

$$Q = \Delta H A_p = \Delta H L_b V \tag{1}$$

where ΔH is the enthalpy change associated with the reaction, L_b the bound concentration of titrant ligand and V the volume of solution in the reaction cell. For a one-step reaction, the equilibrium aspect of the interaction was correlated through the mass law, yielding the following equation:

$$\frac{L_b}{S_t} = \frac{KL_f}{1 + KL_f} \tag{2}$$

where K is the binding constant, S_t the total concentration of the titrand, and L_f the concentration of free ligand. Thus, Eq. (2) can be expressed as a function of the total concentration of the ligand, as follows:

$$Q = \frac{\Delta H V}{2}\left(\alpha - \sqrt{\alpha^2 - 4 S_t L_t}\right)$$

where

$$L_t = L_f + L_b \tag{3}$$

$$\alpha = \frac{1}{K} + L_t + S_t \tag{4}$$

The best-fitted values of K and ΔH can be computed directly using the non-linear least squares regression method [12] and the other thermodynamic parameters can then be calculated from the following equation:

$$\Delta G = -RT \ln K = \Delta H - T\Delta S \tag{5}$$

2.5. Solubility of CH in aqueous solution of antiseptic compounds

The equilibrium solubility of CH was determined by adding excess of CH to pH 7.5 Tris buffer solutions of 0.001–1.9% (w/v) BC, BZC, AEZ or CHG and stirring continuously for 24 h at 37 °C. The samples were passed through a 0.45 μm filter (Toyo Co. Ltd., Japan) to analyze CH by the HPLC method described by Duncan et al. [13]. The HPLC system (Shimadzu Co., Kyoto, Japan) consisted of an ultraviolet (UV) spectrometric detector (Shimadzu SPD-6AV), and chromatopac (Shimadzu C-R6A). The column used was TSKgel ODS-80Ts (ID 4.6 mm × L 150 mm, Toso Co., Kyoto, Japan) and the mobile phase was acetonitrile/water (2:1, v/v). The UV detector was set at 295 nm and the flow rate was 1.0 ml/min at 30 °C.

2.6. Extraction of CH from stratum corneum by antiseptic compounds

After separating stratum corneum from epidermis by trypsin treatment [9], a sheet of stratum corneum (30 mg wet weight) was homogenized in 20 ml of an aqueous solution containing each antiseptic compound. The suspension was shaken violently for 30 min and centrifuged at 1000 × g for 10 min, the supernatant was treated as described above, and the total amount of CH and its derivatives extracted from the stratum corneum was measured using a kit (Cholesterol E-test WAKO, Wako Co. Ltd., Osaka, Japan). The amount of CH extracted in a methanol solution was used as a control. For each experiment, the samples and the control were taken from the same skin. Each set of experiments was performed in seven rats.

3. Results

3.1. The binding of antiseptic compounds with stratum corneum of rats and a model system of intercellular lipids in human stratum corneum

The calorimetric titrations of rat stratum corneum with antiseptic compounds at pH 7.5 and 37 °C are shown in Fig. 2a. The initial concentrations of the antiseptic compounds were

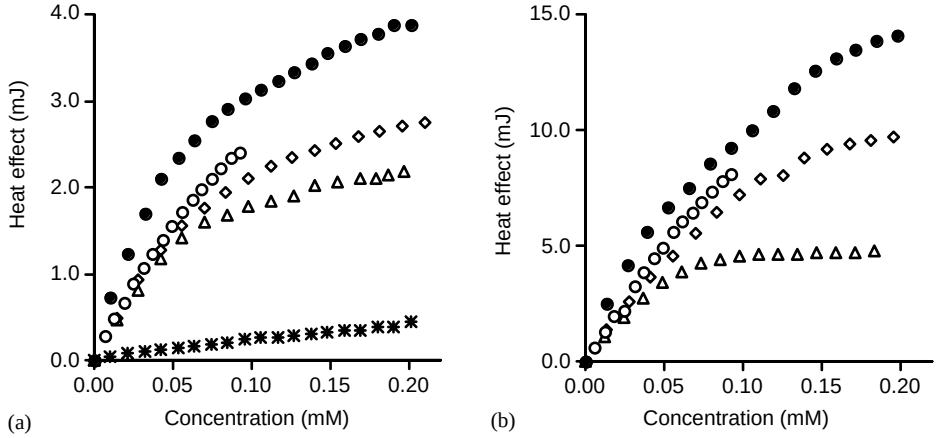


Fig. 2. Heat effects of antiseptic compounds binding to stratum corneum of dorsal skin of rats (a) and to a model system of intercellular lipids in human stratum corneum (b) in pH 7.5 Tris buffer at 25 °C. The amount of stratum corneum used in this study was 4.8 mg dry weight in the cell. (●) BC, (○) BZC, (◇) AEZ, (△) CHG and (∗) BC binding to delipidized stratum corneum.

0.1% BC12, 0.055% BZC, 0.1% AEG and 0.2% CHG, and the reacting concentrations were lower than each c.m.c. value. For the normal and lipid-extracted stratum corneum of rats, 4.8 mg dry weight was finely powdered and suspended in 3.0 ml of pH 7.5 Tris buffer solution using the ultrasonic method. All of the antiseptic compounds were bound to the normal stratum corneum at pH 7.5, where the titration curves were saturated with increasing concentrations. While, the heat effect of the binding to lipid-extracted stratum corneum was much lower than that to the normal stratum corneum. Fig. 2b shows the heat effects of the binding to the lipid mixtures as a model system of intercellular lipids in human stratum corneum (2 mg/10 ml of suspension in pH 7.5 Tris buffer). The lipid composition of the model system used was ceramide (33.1 mol%), CH (37.4 mol%), CHpal (8.3 mol%), CHsulf (2.2 mol%) and palmitic acid (19.0 mol%). The calorimetric titration curves were similar to those of the normal stratum corneum as shown in Fig. 2a. These results indicate that these antiseptic compounds are mainly bound to the intercellular lipids in stratum corneum.

The heat effects of the antiseptics binding to the lipid mixtures without one of the components in the model system are shown in Fig. 3. The heat of binding for all compounds significantly decreased only in the absence of CH and palmitic acid. But incorporated palmitic acid was essential in forming suspensions in the lipid mixtures. In the case of CHG, the heat of binding increased in the absence of ceramide or CHpal and no heat effect was observed in the absence of CH. Thus, CH played an important role in the interaction between the antiseptic compounds and stratum corneum lipids.

3.2. Binding characteristics of the antiseptic compounds for each component of stratum corneum

The heat of binding for antiseptic compounds with the components of intercellular lipids and keratin was examined

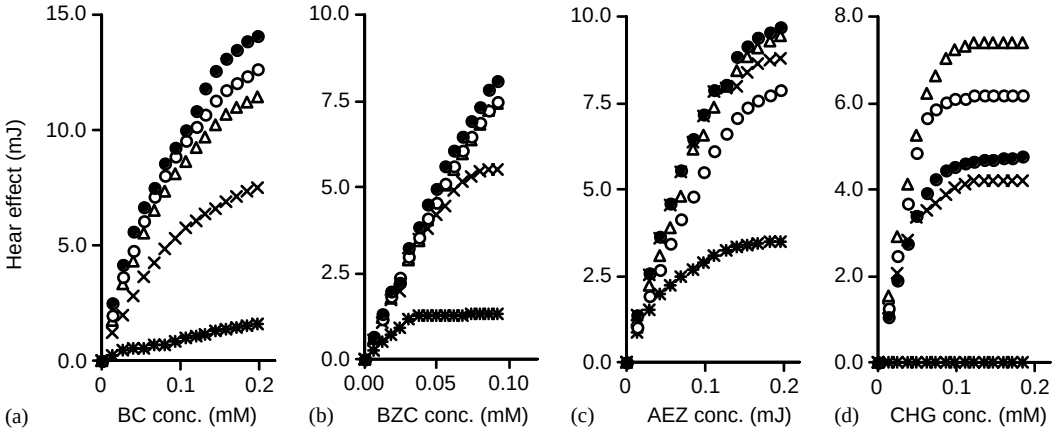


Fig. 3. Heats effects for the interaction of BC (a), BZC (b), AEZ (c) and CHG (d) with a model system of intercellular lipids in human stratum corneum in pH 7.5 Tris buffer solution at 25 °C. (●) a model system of intercellular lipids; (○) the model system without ceramide; (△) the model system without CHpal; (×) the model system without CHsulf and (✱) the model system without CH.

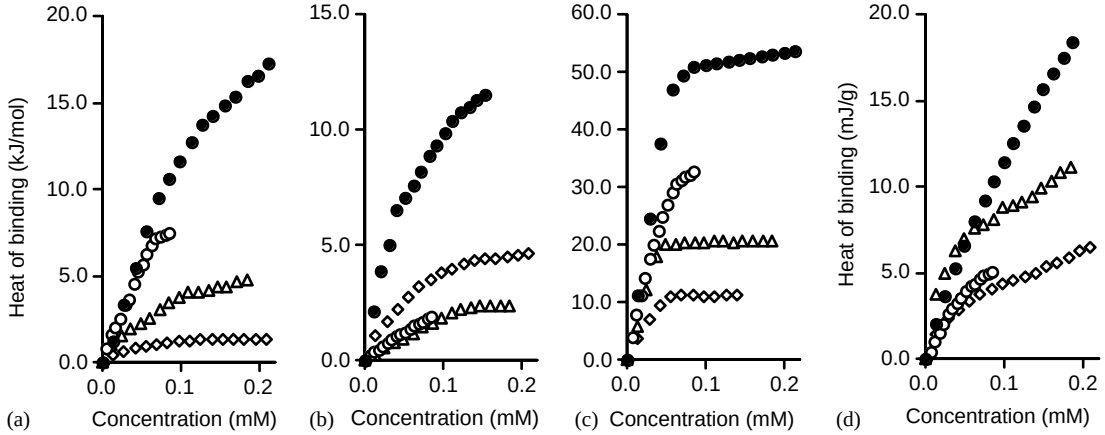


Fig. 4. Heat of binding of antiseptic compounds with ceramide (a), CH (b), CHsulf (c), and keratin (d) at pH 7.5 and 25 °C. (●) BC12, (○) BZC, (◇) AEZ and (△) CHG.

at 37 °C. Each component was finely powdered and suspended in a pH 7.5 Tris buffer by ultrasonication. Fig. 4a–c show the calorimetric titration curves of the compounds binding to ceramide, CH and CHsulf, respectively. The initial concentrations of the compounds were the same as in the binding experiment on the stratum corneum of rats. The concentrations of ceramide, CH and CHsulf in the titration cell were 0.05–0.2 mM, 0.05–0.2 mM and 0.01–0.05 mM, respectively. The calorimetric data are shown best as a plot of the heat produced per mole of each component (Q, kJ/mol) versus the final concentration of antiseptic compounds in the titration cell. The binding constant (K) and thermodynamic parameters (ΔG, ΔH, ΔS) for the binding to the components of intercellular lipids are listed in Table 1. The compounds were mainly bound to ceramide, CH and CHsulf in the intercellular lipids. In the binding experiment on intracellular protein, keratin was ground to a fine powder and suspended in pH 7.5 Tris buffer to 0.02–0.03% (w/v) by the ultrasonic method. The calorimetric titration curves are shown as the heat effect per gram of keratin (Q, mJ/g) in Fig. 4d. Keratin bound most weakly to all antiseptic compounds in the components of stratum corneum.

The binding affinities (K) of the antiseptic compounds for ceramide increased in the order BC12 < CHG < BZC < BC14 < BC16 < AEZ and these bindings were characterized by small negative ΔH and positive ΔS values, reflecting hydrophobic interaction. Although the binding to CH was also induced by hydrophobic interaction, K values conversely increased in the order AEZ < BC12 < BZC < CHG < BC14 < BC16. BC was most tightly bound to CH with higher K values than to other components and the binding affinity of BC increased as the alkyl chain-length increased. On the other hand, the binding of BC and BZC to CHsulf was characterized by large negative ΔH and ΔS, and the K values of BC decreased with the alkyl chain-length, indicating that the binding was induced by ionic interaction. The bindings of AEZ and CHG to the intercellular lipids were characterized by smaller ΔH and larger positive ΔS values than those of BC and BZC.

3.3. Dissolution behavior of stratum corneum CH in antiseptic solutions

The solubility of CH was determined in antiseptic solutions at various concentrations at 37 °C (Fig. 5). CH was little dissolved at concentrations lower than 0.1% (w/v) of the

Table 1
Binding affinities and thermodynamic parameters for antiseptic compounds binding to components of intercellular lipids in stratum corneum at pH 7.5 and 37 °C

	K[a] (10^3 M^{-1})	$-\Delta H$[a] (kJ mol^{-1})	$-\Delta G$[b] (kJ mol^{-1})	ΔS[b] (mol^{-1} K^{-1})
Ceramide				
BC12	5.20 ± 0.39	16.3 ± 3.0	22.1	18.7
BC14	54.1 ± 9.0	15.1 ± 4.3	28.1	41.8
BC16	75.4 ± 4.4	15.5 ± 2.0	28.9	43.5
BZC	34.7 ± 1.4	12.4 ± 3.2	27.0	46.8
AEZ	92.5 ± 6.5	1.54 ± 0.5	29.5	90.1
CHG	14.6 ± 1.4	4.43 ± 1.4	24.7	65.4
Cholesterol				
BC12	61.7 ± 7.8	11.5 ± 0.4	28.4	54.7
BC14	192.8 ± 19.1	16.7 ± 2.5	31.4	47.2
BC16	479.7 ± 14.3	24.0 ± 2.5	33.7	31.4
BZC	69.5 ± 2.3	2.80 ± 0.8	28.7	83.7
AEZ	22.3 ± 1.9	4.98 ± 1.4	25.8	67.2
GCH	135.4 ± 12.0	2.74 ± 0.2	30.5	89.4
Cholesterol sulfate				
BC12	93.0 ± 11.4	52.2 ± 1.7	29.5	−73.3
BC14	49.4 ± 7.9	55.3 ± 0.6	27.9	−88.5
BC16	8.42 ± 1.76	46.6 ± 2.7	23.3	−75.2
BZC	38.3 ± 5.7	45.9 ± 1.5	27.2	−60.3
AEZ	37.7 ± 4.4	12.4 ± 1.4	27.2	47.6
GCH	33.9 ± 2.2	22.5 ± 3.9	26.9	14.1
Cholesterol palmitate				
BC12	0.25 ± 0.03	1.50 ± 0.50	14.2	41.1
BC14	1.23 ± 0.05	1.41 ± 0.25	18.3	54.6
BC16	1.66 ± 0.03	1.76 ± 0.33	19.1	56.0
Palmitic acid				
BC12	1.86 ± 0.14	8.32 ± 0.61	19.4	35.7
BC14	3.54 ± 0.77	8.69 ± 0.98	21.1	39.9
BC16	7.79 ± 0.36	8.90 ± 0.51	23.1	45.8

[a] Mean ± S.D. ($n = 3$).
[b] Mean values.

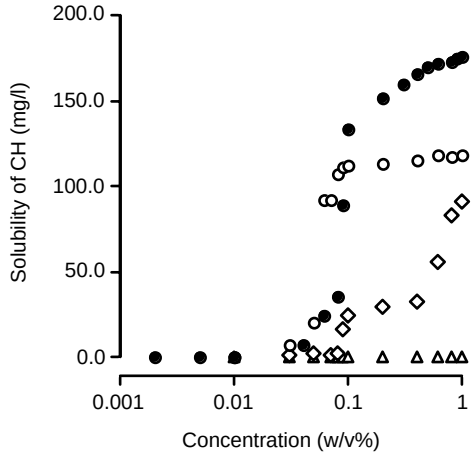


Fig. 5. Solubility of CH in antiseptic solutions. (●) BC12, (○) BZC, (◇) AEZ and (△) CHG.

compound and the solubility increased sharply from 0.08 to 0.1% of BC, BZC and AEZ. Thus, cationic surfactants such as BC and BZC and an amphoteric surfactant such as AEZ were effective as solubility enhancers for CH at concentrations above the c.m.c. values. The interaction between the surfactants and endogenous CH resulted in CH being incorporated into the micelle of the surfactants. CH, however, was quite insoluble in the CHG solution.

In the suspension of homogenized stratum corneum, the concentration of total CH also increased with the concentrations of BC, BZC and AEZ. The relative amount of endogenous CH extracted from the stratum corneum of hairless rats by the antiseptic compounds is shown in Fig. 6. The concentrations of antiseptic compounds employed in this experiment were typical of those used for the disinfection of hands. Results were expressed as a percentage of the total amount of CH extracted from stratum corneum by methanol. About 80, 60 and 20% of total CH were extracted in stratum corneum treated with 0.1% BC, 0.1% BZC and 0.2% AEZ, respectively. These results indicated that only cationic surfactants were effective as solubility enhancers for CH. It

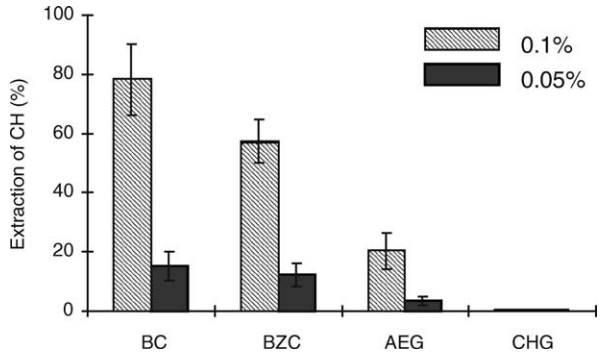


Fig. 6. Endogenous CH extracted from stratum corneum of hairless rats in aqueous solution containing antiseptic compounds. Results were expressed as a percentage in comparison with the total CH extracted from stratum corneum by methanol. Each column represents the mean ± S.D. of seven experiments.

seemed that at concentrations above the c.m.c. of the compounds, the interaction between the cationic surfactant and endogenous CH resulted in CH being incorporated into the micelle of the cationic surfactant.

4. Discussion

The stratum corneum is a complex composite of proteins and lipids, and essentially consists of flattened keratinocytes embedded in a matrix of multi-lamellar lipid bilayers [1]. The intercellular lipids play a predominant role in the barrier function of stratum corneum and consist mainly of three fractions, namely, ceramides, fatty acids, and CH and its derivatives [11]. Ceramides are *N*-acyl-linked sphingolipids and have been suggested to have a central role in the barrier function [14]. The free hydroxyl groups and the amide group can form an extensive hydrogen bonded network. CH and free acids, other major components of stratum corneum, are required for the permeability barrier homeostasis and ordering the bilayer array.

Under the experimental conditions, the overall binding of the antiseptic compounds with stratum corneum was exothermic as indicated by the negative enthalpy changes. Since the heat of binding to keratin and lipid-extracted stratum corneum of rats was very low, it was found that these compounds mainly bound and interacted with intercellular lipids in the stratum corneum. Especially, ΔH values of the binding of BC to each component in the intercellular lipids were more negative than those of other compounds, indicating that BC strongly interacted with stratum corneum. BC consists of a variable mixture of C_8–C_{18} alkylbenzyldimethylammonium chlorides with BC12, BC14 and BC16 homologues predominating. The homologues possess different physical, chemical and microbiological properties. In the intercellular lipids, the strength of the binding to ceramide and CH increased with the alkyl chain length of BC-based on the hydrophobic interaction, but the binding to CHsulf decreased as the alkyl chain lengthened (Table 1). Cationic surfactants, BC and BZC, dissolved CH at the concentration higher than c.m.c. (Fig. 5). In stratum corneum treated with 0.1% BC and BZC, more than 50% of the endogenous CH was extracted (Fig. 6), suggesting that the cationic surfactants increase the permeability of the skin. In fact, BC showed enhanced activity for percutaneous absorption of enoxacin compared to an anionic surfactant of sodium laurylsulfate and a non-ionic surfactant of Polysorbate 80 [15]. Higuchi et al. reported that the dissolution of cholesterol solid phases in bile acid solution could be greatly increased by the addition of BC [16,17]. Thus, cationic surfactants, BC and BZC, mainly interacted with CH and its derivatives in patches of the ordered intercellular lipid structure, and probably penetrated the stratum corneum by extracting CH and disrupting the lipid bilayer at concentrations above 0.1%. As the results, the accumulation of BC in epidermis and dermis was found after the

24 h application to the dorsal skin of rats and hairless mice [7].

The amphoteric surfactant of AEZ most strongly bound to ceramide with the smallest ΔH among the antiseptic compounds used in this study. AEZ possesses an aliphatic carbon chain (C12) plus a diaminoethylglycine group and is completely different in chemical structure from BC and BZC. From the results, AEZ was probably incorporated into the lipid bilayer and bound to ceramide with its polar end close to the lipid polar heads. Sznitowska et al. reported that polar lipids such as ceramide, free sterols and fatty acids were important to the polar pathway of penetration of the model zwitterion baclofen across stratum corneum [18]. However, further study is needed to elucidate whether AEZ disrupts and increases the fluidity of the lipid region.

The ΔH for the binding of CHG to each component of the intercellular lipids was smaller than that of any other compounds. Although CHG bound to CH with high affinity ($10^6\,M^{-1}$), the mechanism of the interaction between CHG and the intercellular lipids was significantly different from that of BC and BZC. CH was practically insoluble in the CHG solution (0.2 mg in 100 ml of CHG). Even when the concentration of CHG increased, the solubility of CH hardly changed (Fig. 6). Thus, it seemed that the CHG bound to the intercellular lipids through CH was adsorbed into the stratum corneum without the removal of CH. Also, CHG could not penetrate the stratum corneum because of its size (mw = 898).

For anionic surfactants such as sodium dodecyl sulfate, several authors indicated that SDS interacted with the intracellular protein matrix to cause conformational changes in keratin at the c.m.c. (0.24%) [19]. On the other hand, no removal of lipid was found when SDS was applied to the skin at concentrations below the c.m.c. [20]. The previous studies did not focus on the binding of antiseptic compounds to the stratum corneum. We did not observe an additional promotion in the calorimetric titration of the binding of SDS to each component of the stratum corneum. However, a different phenomenon from the cationic and amphoteric surfactants for the penetration of stratum corneum may be expected.

In conclusion, isothermal titration microcalorimetry was employed to investigate the interactions between antiseptic compounds with quaternary ammonium and the components of the stratum corneum. Cationic surfactants such as BC and BZC were mainly bound to CH and CHsulf by hydrophobic and ionic interaction, respectively. BC was more strongly bound to CH and its derivatives than any other compound, and extracted CH from the intracellular lipids of the stratum corneum of concentrations above the c.m.c. value. It was suggested that BC penetrated the stratum corneum by disrupting the structure of the lipid bilayer through extraction of CH. AEZ, an amphoteric surfactant, was incorporated into the lipid bilayer and bound to ceramide with high affinity by hydrophobic interaction. GCH was bound to only CH and accumulated in the stratum corneum.

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