Journal of Thermal Analysis and Calorimetry, Vol. 64 (2001) 293–297

INTERACTION OF STRATUM CORNEUM COMPONENTS WITH BENZALKONIUM CHLORIDE
Isothermal titration calorimetric study

H. Aki, E. Koizumi, Y. Okamoto and M. Yamamoto

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan

Abstract

The interactions of benzalkonium chloride (BC) with components of stratum corneum, a model system of intercellular lipids in human stratum corneum and homogenized rat stratum corneum were characterized in terms of thermodynamics at pH 7.5 and 37°C. BC was strongly bound to cholesterol and cholesterol sulfate with higher affinities (10^5~10^6 M^{-1}) than to any other components of the stratum corneum by hydrophobic interaction and ionic interaction, respectively. BC binding to the model system of intercellular lipids significantly decreased only in the absence of cholesterol. It is indicated that cholesterol and its derivatives play an important role in the penetration and/or accumulation of BC in stratum corneum.

Keywords: benzalkonium chloride, cholesterol, isothermal titration calorimetry, microcalorimetry, stratum corneum, thermodynamics

Introduction

Benzalkonium chloride (BC) is a cationic surfactant that is widely used for the preoperative disinfection of unbroken skin and the treatment of superficial injures or infected wounds as a topical antiseptic. Even below the usual concentration of 0.1%, the adverse reactions of BC such as skin irritation and associated inflammatory responses are often induced by its application to the skin. BC is a mixture of benzyldimethylalkylammonium chlorides consisting of three major homologues with straight carbon chain-lengths of dodecyl (BC12), tetradecyl (BC14) and hexadecyl (BC16). In a previous study, ^{14}C-benzyldimethyldodecylammonium chloride (^{14}C-BC12) solution was applied to the dorsal skin of rats and hairless mice. By whole body and micro autoradiography, heavy deposition of ^{14}C-BC12 in the surface of the skin and the penetration into epidermis and dermis at 24 h after the application were seen [1].

The primary barrier to transdermal diffusion for most substances is the stratum corneum, the thin outermost layer of skin. The stratum corneum is a multilayered wall-like structure in which keratin-rich corneocytes are embedded in an intercellular lipid-rich matrix [2]. There are two possible pathways for substance permeation through the intact stratum corneum: intercellular and intracellular routes. In this

1418–2874/2001/ $ 5.00
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study, the interaction of BC with the components of human stratum corneum was investigated to clarify the penetration mechanism of BC.

Experimental

Materials

BC, BC14 and BC16 were purchased from Sigma Chemical (St. Louis, MO, USA) and BC12 was from Sigma-Aldrich Chemie GmbH (Steinheim, Switzerland). Cholesterol monohydrate (CH), cholesterol 3-sulfate (CH·sulf), cholesteryl palmitate (CH·pal), ceramide, palmitic acid and oleic acid were used as intercellular lipids and keratin as the intracellular protein of stratum corneum. The stratum corneum sheets were prepared from the dorsal skin of rats by trypsin treatment [3], and homogenized in pH 7.5 Tris buffer solution.

Isothermal titration microcalorimetry

Microcalorimetric study was performed with a Thermal Activity Monitor 2277 system (Järfälla, Sweden) at 310.15 K. A sample cell was initially filled with a 3.0 ml suspension or solution of one of the model components or homogenized stratum corneum in pH 7.5 Tris buffer. Heat flow was measured by injecting 0.03~0.1% of each BC solution in 15~20 portions of 15 μl into the cell to a final concentration of 0.002~0.007%. The heat flow produced by the dilution of BC was separately measured using Tris buffer as a titrand. The binding and the thermodynamic parameters were computed from the calorimetric titration curve of the corrected heat effect by an iterative non-linear least-squares method [4].

Dissolution study of cholesterol in BC solutions

The equilibrium solubilities of CH, CH·pal and total CH of stratum corneum in pH 7.5 Tris buffer solutions were determined by adding BC12 (0.001~1.0%) with continuous agitation at 37°C for 24 h. The samples were filtered through a 0.45 μ cellulose membrane filter and analyzed by the HPLC method described by Duncan *et al.* [5].

Results and discussion

BC binding to the components of stratum corneum

Figure 1 shows the calorimetric titration curves of BC binding to ceramide, CH and keratin. The initial concentrations of BC were used to be 0.1% BC12, 0.05% BC14 and 0.03% BC16, which were lower concentrations than critical micellar concentration. The concentrations of ceramide, cholesterol and keratin were 0.06, 0.005 and 0.16%, respectively. The heat effect was increased exothermally in proportion to BC concentration increases. The binding affinity (K) of BC to the components of intercellular lipids increased with increasing the alkyl chain-length of BC. BC was

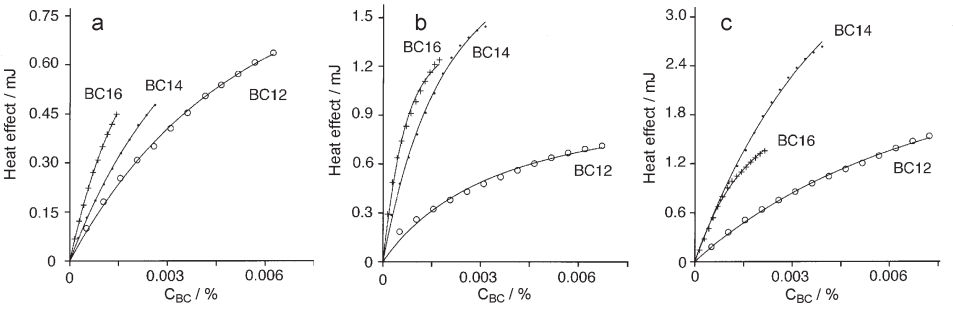

Fig. 1 Heat effect of BC binding to a – ceramide, b – cholesterol and c – keratin in pH 7.5 Tris buffer solution at 37°C. Points show the experimental data and solid lines represent the computer-generated best fit curves

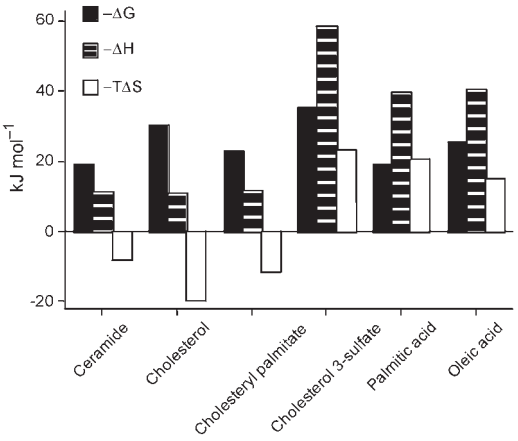

Fig. 2 Thermodynamic characterization of BC12 binding to the components of intercellular lipids in stratum corneum

most tightly bound to CH and CH·sulf with higher K values (10^5~10^6 M^{-1}) than to other components, whereas it was most weakly bound to keratin. The binding of BC to CH, CH·pal and ceramide was characterized by small negative ΔH and positive ΔS, reflecting hydrophobic interaction (Fig. 2). On the contrary, the binding of BC with CH·sulf and fatty acids was enthalpy driven, reflecting ionic interaction.

BC binding to the homogenized rat stratum corneum and to a model system of intercellular lipids in human stratum corneum

BC was bound to the homogenized stratum corneum of the dorsal skin of rats in pH 7.5 Tris buffer solution (Fig. 3a), where the titration curve was saturated with increasing BC concentration. Figure 3b shows the titration curves of BC12 binding to the lipid mixtures as a model system of intercellular lipids in human stratum corneum and to the lipid mixtures without every component. The lipid composition of the model system used was CH

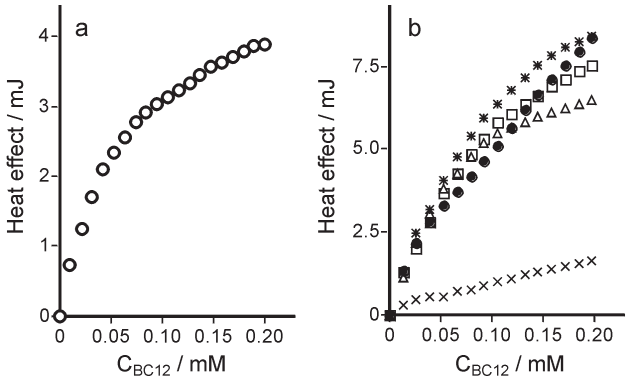

Fig. 3 Heat effect of BC12 binding to homogenized stratum corneum of dorsal skin of rats (a) and to model systems of intercellular lipids in human stratum corneum in pH 7.5 Tris buffer at 37°C. The homogenized stratum corneum used in this experiment was about 1 mg ml^{-1} dry mass. o – homogenized suspension of stratum corneum, ● – a model system of intercellular lipids, × – a model system without CH, ✳ – a model system without ceramide, □ – a model system without CH·pal, and Δ – a model system without CH·sulf

(30.2% in mass/mass), CH·pal (11.2%), CH·sulf (2.2%), ceramide (46.2%), and palmitic acid (10.2%) [6]. The BC12 binding to the model system significantly decreased only in the absence of CH. Incorporated palmitic acid was essential in forming suspensions in the lipid mixtures. It was suggested that CH played an important role in the binding interaction between BC and stratum corneum lipids.

Dissolution behavior of stratum corneum CH in BC solution at pH 7.5

The solubilities of CH increased, depending on the concentration of BC12, with marked rapidity from 0.08 to 0.1% BC12. In suspensions of homogenized stratum corneum, the concentration of total CH was also increased with increasing BC12 concentration

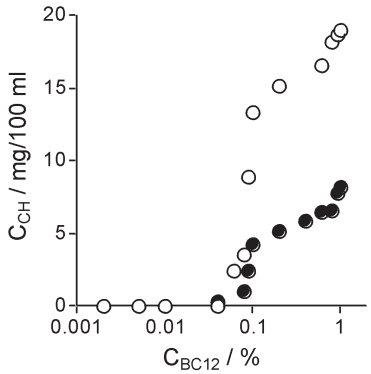

Fig. 4 Solubilities of cholesterol monohydrate and total cholesterol in stratum corneum of the dorsal skin of rats in BC12 solutions at 37°C. o – CH, and ● – total cholesterol in stratum corneum

(Fig. 4). This seems to be owing to the extraction of endogenous CH from the stratum corneum by BC12. The results support previous reports that in bile acid solution, cholesterol dissolution rates could be greatly increased by the addition of BC which probably reduced the charge repulsion and lowers interfacial resistance [7, 8].

Penetration mechanism of BC through stratum corneum

Stratum corneum lipid bilayers consist mainly of three fractions: ceramide, fatty acids and CH and its derivatives. Ceramide has been suggested to have a central role in the barrier function of stratum corneum. CH, another major component of stratum corneum, is required for the permeability barrier homeostasis and ordering the bilayer array.

Results showed that the intercellular lipids, especially CH and its derivatives were responsible for the penetration of BC in the stratum corneum. Treatment with BC had some effects on the lipid matrix in the stratum corneum. It was suggested that BC interacted with stratum corneum lipids, especially with CH, to remove CH and its derivatives or to coexist as patches in the ordered stratum corneum lipid structure. Thus, BC probably penetrated and/or accumulated in the stratum corneum through disordering of the multiple lipid bilayer structure by extracting CH.

Conclusions

The isothermal titration microcalorimetry has been employed to investigate the interaction between BC and the components of stratum corneum. BC was strongly bound to CH and its derivatives in the intercellular lipids, which were most likely extracted from the stratum corneum with increasing BC concentration. Thus, CH and its derivatives would be essential to the percutaneous absorption of BC in the stratum corneum.

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This work was financially supported in part by Hayashi Memorial Foundation for Female Natural Scientists in Japan to H. Aki.

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