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Short Communication

**EVIDENCE FOR THE HYDROLYSIS OF TOPICAL APPLIED  
LIPOSOMAL LIPIDS IN HUMAN STRATUM CORNEUM**

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**Abstract:** Liposomes were incubated with an extract of human plantar stratum corneum. The liposomal lipids were hydrolysed, if composed of soy-bean phosphatidylcholine or phosphatidylglycerol. Rigid lipids were not degraded. The temperature optimum of the hydrolysis was between 30-35°C. CaCl<sub>2</sub> enhanced, while EDTA reduced the rate of hydrolysis, indicating that the hydrolysis is due to a phospholipase A<sub>2</sub>.

**Key Words:** Stratum Corneum, Liposomes, Phospholipase

**INTRODUCTION**

The outer layer of human skin is the epidermis. It consists of keratinocytes in distinct states of differentiation. The outer layer of the epidermis is the stratum corneum (SC), which consists of terminally differentiated keratinocytes (corneocytes) embedded in multilamellar lipid layers. These multilamellar lipid layers consist mainly of ceramides, free fatty acids and cholesterol in almost equal amounts. To describe this architecture the 'brick-and-mortar' model was established 20 years ago. However, this model does not reflect the highly dynamic structure of the SC, which can respond to changes of the environment and hosts several hydrolytic enzymes: lipases, proteinases and glycosidases. The main function of the SC is the protection from uncontrolled water loss and to prevent the penetration of foreign substances (including drugs) into the skin. Mezei and co-workers [1] were the first to demonstrate that liposomes can be used to enhance the delivery of drugs into human skin. Approximately 10 years later Cevc [2] proposed a model and a mechanism for efficient delivery of lipophilic as well as hydrophilic drugs into deeper layers of skin using ultraflexible vesicles.

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The penetration of liposomes into the epidermis is affected by several factors, e.g. the lipid composition of the vesicles. The interaction of liposomal lipids with the multilamellar lipid layers of the SC can be predicted by a membrane fusion assay [3]. In addition the penetration of topically applied lipids can be studied using 3-dimensional epidermal cultures [4] as well as human skin. However, the fate of the liposomal lipids during the penetration into the epidermis is not known and difficult to determine. Evidence will be presented that phospholipases are present in the SC, which can hydrolyse topically applied lipids during penetration.

### MATERIAL AND METHODS

Human stratum corneum enzymes were extracted from sole scrapings by an incubation in 0.1 M KCl or 0.1 M HEPES (pH 7.3) at 4°C over night [5]. After centrifugation and sterile filtration the sample was lyophilised and stored at -18°C. PL A<sub>2</sub> activity was characterised with the fluorescently labelled phospholipids NBD-C<sub>6</sub>-PC (Molecular Probes, Eugene, OR) and NBD-C<sub>6</sub>-PE, NBD-C<sub>6</sub>-PS, NBD-C<sub>6</sub>-PA, NBD-C<sub>6</sub>-PG (Avanti Polar Lipids, Alabaster, AL). Measurements were performed in duplicates at 30°C using a Fluoroskan Ascent (Labsystems, Helsinki, Finland). The sample volume was 250 µl with a protein concentration of 0.25 mg/ml and a substrate concentration between 0 and 20 µg/ml.

The influence of temperature and ions on the rate of hydrolysis was determined with a Hitachi F-4500 fluorescence spectrophotometer. Excitation and emission wavelength were set to 465 nm and 543 nm respectively and the change in the fluorescence intensity was followed for 30 min.

Hydrolysis products were quantified by HPTLC. Liposomes were incubated for 20 h at room temperature with stratum corneum extract. After lipid extraction and chromatography with CHCl<sub>3</sub>/CH<sub>3</sub>OH/NH<sub>4</sub>OH (65/35/3) the plates were stained with CuSO<sub>4</sub> solution at 180°C for 6 min. The lipids were quantified with a Scanner II (Camag, Muttenz, Switzerland) and the software package Cats.

### RESULTS AND DISCUSSION

Liposomes were incubated with the aqueous extract of human plantar stratum corneum (SCE). HPTLC analysis demonstrated that the composition of the liposomes changed within 20 h. With increasing amounts of liposomes made of PL 90 or phosphatidylglycerol the amount of free fatty acids increased (Fig. 1). This indicates that a phospholipase A<sub>2</sub> (PL A<sub>2</sub>) is in the SCE. No hydrolysis occurred in liposomes made of phosphatidylserine and rigid phosphatidylcholine (DSPC). Therefore the PL A<sub>2</sub> has a substrate specificity for PC and PG and a preference for fluid membranes. The presence of PL A<sub>2</sub> activity in the viable part of the epidermis has been reported. Different PL A<sub>2</sub> isoenzymes have been

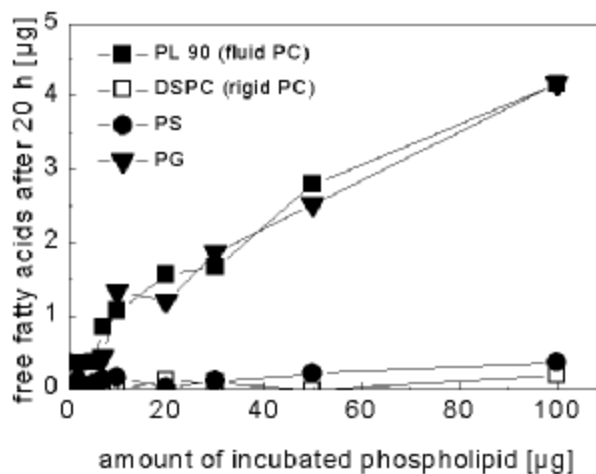


Fig. 1. Composition of liposomes 20 h after incubation with aqueous human stratum corneum extract. Phosphatidylcholine (PL 90) and PG were hydrolysed, indicated by an increasing amount of free fatty acids. PS and DSPC were not degraded.

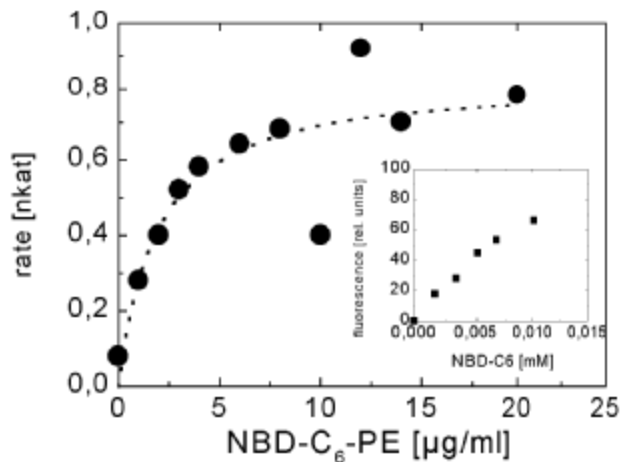


Fig. 2. Influence of the NBD-C<sub>6</sub>-PE concentration on the rate of hydrolysis. Each point represents the mean of 3 independent determinations. Inset: The fluorescence intensity increased with increasing concentration of the hydrolysis product NBD-C<sub>6</sub>.

characterised in keratinocyte cultures [6, 7] and recently also in human epidermis [8]. Mao-Qiang [9] showed that the PL A<sub>2</sub> activity is required for an intact permeability barrier.

In order to analyse the temperature dependence of the PL A<sub>2</sub> activity NBD-C<sub>6</sub>-PC was incubated with SCE and the rate of hydrolysis was determined at

different temperatures. A maximum was detected between 30 and 35°C, which is the temperature at skin surface.

Next we characterised the  $K_m$  and the  $v_{max}$  of the enzyme. Fig. 2 shows that the PL A<sub>2</sub> in the SCE follows the Michaelis-Menten kinetic. The  $K_{m(app)}$  and  $v_{max(app)}$  values were  $3.3 \pm 1.7 \mu\text{M}$  and  $0.8 \pm 0.1 \text{ nkat}$  for NBD-C<sub>6</sub>-PE. NBD-C<sub>6</sub>-PS and NBD-C<sub>6</sub>-PA showed no detectable hydrolysis. Similar results were obtained with non-labelled lipids (fig. 1).

Finally, the effect of other factors on the rate of hydrolysis were determined. After the addition of sodium desoxycholate in micellar concentration (2 mM) and CaCl<sub>2</sub> (20 mM) the rate of hydrolysis increased 2.4fold and 1.5fold, respectively. In contrast, 20 mM EDTA or CuSO<sub>4</sub> reduced the rate of hydrolysis to 55 % and 22 %, respectively.

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