A Comparative SAXS Study of Stratum Corneum Lipid Organization in an Epidermal Cell Culture Model (ROC)

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Introduction

Cell cultured skin constructs are developed as an alternative to native skin for in vitro evaluations. In addition to robustness of the model, adequate barrier properties of the stratum corneum (SC) are of uppermost importance. For an epidermal cell culture model based on rat epidermal keratinocytes (REK organotypic culture, ROC), competent barrier properties of the SC could be shown in addition to the overall comparably simple cell culture conditions and high reproducibility of the model [1]. In our previous study, the lipid organization of ROC SC was investigated by synchrotron SAXS and thermal analysis of the separated SC in comparison to native and rat SC and results clearly indicate a high structural order of the SC lipids in the cell culture model with high similarity to human SC. However, in our previous experimental setup [2,3,4], changes of the hydration level of the SC sheets (e.g. drying out during heating) could not be excluded. In the present study, SC sheets with defined hydration level are tightly sealed in glass capillaries to overcome this problem and samples are studied over a wider s-range.

Materials and Methods

SC separation: ROC [2,3,4] and human epidermis (abdominal female skin obtained from plastic surgery, heat separation of the epidermis) was incubated with 0.1 % trypsin solution for at 4 °C for 24 h and at 37 °C for 1 h. Rat SC was obtained by trypsination (0.1 % trypsin solution) of excised, shaved abdominal rat skin (Wistar rats) for 3 days at 37 °C. The SC sheets were dried over silica gel under nitrogen atmosphere and light protection. Prior to use, SC sheets were stored over sodium bromide solution (27 % w/w) to ensure a hydration level of the SC of about 20 %. Differential scanning calorimetry (DSC): SC samples (hydration level 20 %) were accurately weighted into standard aluminum pans and measured in a DSC 1 calorimeter (Mettler Toledo). Samples were heated from 10 to 120 °C, cooled to 0 °C and heated again to 120 °C with a scan rate of 10 °C/min. Temperature was held constant for 60 s at 120 °C and for 500 s at 0 °C. Small angle X-ray scattering (SAXS): Measurements with synchrotron radiation (wavelength 0.15 nm) were done at the A2 beamline at HASYLAB/DESY. SAXS was recorded with a MarCCD165 2D detector (sample – detector distance was 1.684 m) in the s-range between 0.015 and 0.35 nm⁻¹ (s = 1/d is the scattering vector and d the spacing). Hydrated SC samples (hydration level 20 %) were filled into capillaries (outside ø 1.5 mm, wall thickness 0.01 mm, Hilgenberg GmbH) and equilibrated for at least 24 h over sodium bromide solution (27 % w/w) before sealing the capillaries. Samples were measured in a temperature range between 25 °C and 120 °C. Data was analyzed the A2tool software version 0.23a.

Results and Discussion

Overall, results of the present study are in reasonable agreement with the previous ones for the thermally untreated SC samples but reflections were more intense when measured in sealed capillaries (figure 1). The most predominant spacings were 63.2 ± 0.8 (n = 5), 61.5 ± 0.3 (n = 5) and 55.0 ± 0.5 (n = 6) Å for human, ROC and rat SC at 25 °C. For human and ROC SC, a very weak reflection was detected at smaller s-values which may indicate the presence of the so-called long periodicity phase (LPP) with a repeat distance (calculated by the second order reflection) of about 127 Å for human SC in good agreement with literature data [5].
Changes in the SAXS patterns upon heating human SC (Figure 1 A) could clearly be related to the different thermal phase transitions detected by DSC [2]. The alteration of the reflection at 0.0235 Å between 25 and 40 °C, for example, to the T1 (transition related to lipids on the skin surface and/or to a rearrangement from an orthorhombic to a hexagonal chain packing) and that at 0.0158 Å between 70 and 80 °C to the T2 (main transition of the intercellular lipids) thermal transition. Interestingly, a weak and broad reflection could be detected up to 100 °C. This reflection was not reversible after heating to 120 °C (e.g. not detectable in the repeated heating run) where the protein is denatured. This indicates that this reflection may be related to lipids covalently bound to the protein. The changes in the scattering patterns of ROC SC in dependence on temperature (Figure 1 B) were rather similar to those observed with human SC. Although no distinct thermal transitions corresponding to the T1 and T3 (transition related to lipids covalently bound to protein) could be detected for ROC SC in DSC, alterations in the SAXS patterns in these temperature ranges (e.g. between 25 and 40 °C and between 80 and 100 °C) were observed. Worth to note is also the appearance of a rather distinct reflection between 60 and 70 °C upon heating ROC SC (Figure 1 B), e.g. in the temperature range of the thermal transition of the intercellular lipids in ROC (at about 64 °C). A similar but more diffuse reflection could also be observed in human SC but at slightly lower s-values.

**Summary and Conclusion**

SAXS measurements of SC samples with defined hydration level in tightly sealed glass capillaries appears more suitable as changes in the SAXS patterns due to an alteration of the hydration level [5] can be avoided. The SAXS patterns of ROC stratum corneum was more close to that of human than that of rat SC. Alterations in the SAXS patterns in dependence on temperature may indicate similar lipid organization as in human SC albeit T1 and T3 thermal transitions could not clearly be detected for ROC SC in DSC so far.

**References**