Chemistry and Physics of Lipids 163 (2010) 42–50

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Impact of the long chain ω-acylceramides on the stratum corneum lipid nanostructure. Part 1: Thermotropic phase behaviour of CER[EOS] and CER[EOP] studied using X-ray powder diffraction and FT-Raman spectroscopy

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ARTICLE INFO

Article history:
Received 17 March 2009
Received in revised form 12 October 2009
Accepted 26 October 2009
Available online 10 November 2009

Keywords:
Stratum corneum
Ceramide [EOS]
Ceramide [EOP]
X-ray powder diffraction
FT-Raman spectroscopy

ABSTRACT

The stratum corneum (SC), the outermost layer of the mammalian skin, is the main skin barrier. Ceramides (CERs) as the major constituent of the SC lipid matrix are of particular interest. At the moment, 11 classes of CERs are identified, but the effect of each single ceramide species is still not known.

Therefore in this article, the thermotropic behaviour of the long chain ω-acylceramides CER[EOS] and CER[EOP] was studied using X-ray powder diffraction and FT-Raman spectroscopy.

It was found that the ω-acylceramides CER[EOS] and CER[EOP] do not show a pronounced polymorphism which is observed for shorter chain ceramides as a significant feature. The phase behaviour of both ceramides is strongly influenced by the extremely long acyl-chain residue. The latter has a much stronger influence compared with the structure of the polar head group, which is discussed as extremely important for the appearance of a rich polymorphism. Despite the strong influence of the long chain, the additional OH-group of the phyto-sphingosine type CER[EOP] influences the lamellar repeat distance and the chain packing. The less polar sphingosine type CER[EOS] is stronger influenced by the long acyl-chain residue. Hydration is necessary for the formation of an extended hydrogen-bonding network between the polar head groups leading to the appearance of a long-periodicity phase (LPP). In contrast, the more polar CER[EOP] forms the LPP with densely packed alkyl chains already in the dry state.

1. Introduction

The outermost layer of the skin, the stratum corneum (SC), is the major barrier both to dermal and to transdermal delivered drugs and plays a key role in the skin barrier integrity (Wertz and van den Bergh, 1998). This superficial layer consists of dead cells, the corneocytes, filled with keratin, which are embedded in a complex matrix of multilamellar organized lipids (Elias, 1983). The unique lipid composition is one important feature of the SC. The major lipid classes are ceramides (CER), cholesterol, and long chain free fatty acids (Gray and Yardley, 1975; Gray and White, 1978). It is generally accepted that ceramides as the main constituents play a predominant role in maintaining the barrier function (Holleran et al., 1991; Coderch et al., 2003). Therefore, it becomes apparent that increasing interest is focussed on this particular class of lipids. To date, 11 ceramide subclasses have been detected in the SC lipid matrix (Mizutani et al., 2009).

Ceramides consist of a long chain fatty acid bound to the amino-group of a long chain di- or trihydroxy sphingoid base (sphingosine, phyto-sphingosine, and 6-hydroxysphinganine). The acyl residue of ceramides can be hydroxylated at the α-position or at the end of the hydrocarbon chain (ω-position) (Fig. 1).

Up to now, a detailed picture of the molecular organization of lipids in the SC, in particular the function of each ceramide subclass, has not been fully elucidated. It is clear that a profound knowledge of the physical properties of the SC lipids and of their interactions is essential for a deeper understanding of the impact of each ceramide species on the barrier function of the SC.

The physical properties of SC lipids were studied using a variety of experimental techniques such as small angle X-ray diffraction (Friberg and Osborne, 1985; Bouwstra et al., 2000), differential scanning calorimetry (Bouwstra et al., 1989; Shah et al., 1995; Wegener et al., 1996), electron microscopy (Swartzendruber et al., 1989) and vibrational spectroscopy as Fourier transform infrared (FT-IR) (Moore et al., 1997; Lafleur, 1998; Mendelsohn and Moore, 1998; Chen et al., 2000) and FT-Raman spectroscopy (Neubert et

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doi:10.1016/j.chemphyslip.2009.10.007

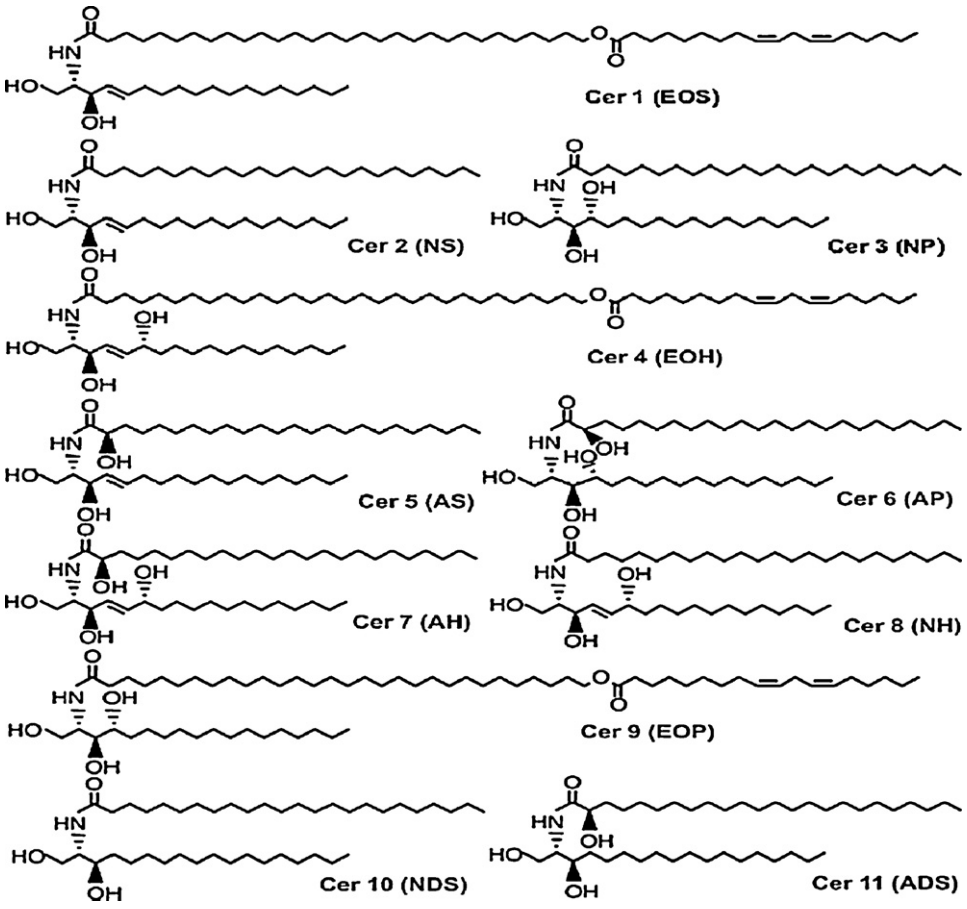

Fig. 1. Chemical structure and nomenclature of ceramides of the SC. A = α-hydroxy fatty acid; EO = ester-linked ω-hydroxy acid; N = non-hydroxy fatty acid; P = phytosphingosine; S = sphingosine; H = 6-hydroxysphingosine; D = dihydro according to Mizutani et al. (2009).

al., 1997; Wegener et al., 1997; Wartewig et al., 1998; Raudenkolb et al., 2003b).

Summarizing, a rich polymorphism appears to be a characteristic feature of ceramides and seems to be important for the function of the SC lipid matrix. At physiological temperatures, all crystalline phases of ceramides exhibit lamellar structures with highly ordered hydrocarbon chains. Among the different phases of the ceramides, those with fully extended molecules packed in a double layer with either their two chains parallel oriented or stretched out to opposite direction of the head group are thought to be of particular relevance for the molecular architecture of the SC lipid matrix. The different behaviour of the ceramide head groups may be an important factor for the skin barrier function.

Unfortunately, data about the physicochemical properties of the acylceramides CER[EOS] and CER[EOP], which seem to be crucial for the integrity of the SC lipid matrix, are lacking so far because the availability of these compounds is limited.

From X-ray diffraction studies on some SC lipid model systems containing CER[EOS] it was concluded that CER[EOS] is a prerequisite for the formation of the long-periodicity phase (LPP) with 13 nm (Bouwstra et al., 2000; McIntosh et al., 1996; McIntosh, 2003; Jager et al., 2005). In order to determine the influence of the head group structure, CER[EOS] was partially replaced by CER[EOP] which contains an additional hydroxyl group at the sphingoid backbone. It can be stated that the head group structure has a remarkable influence on the behaviour and structure of the SC lipids. The substitution of CER[EOS] by CER[EOP] results in a reduction of the LPP and in a phase separation which was attributed to the larger and more hydrophilic head group of CER[EOP] (Jager et al., 2004).

However, the existence of the 13 nm lamellar repeat pattern in stratum corneum *in vivo* is currently under discussion. Apart from some conventional electron micrographs (Swartzendruber et al., 1989), the 13 nm repeat unit has been observed in some SAXD studies (White et al., 1988; Bouwstra et al., 1991; Hatta et al., 2001), while it has not been confirmed in other SAXD studies (Garson et al., 1991) or in cryo-transmission electron microscopy studies on native hydrated epidermis samples (Al-Amoudi et al., 2005) or in neutron diffraction studies on hydrated stratum corneum (Charalambopoulou, 2004).

In the work of Kessner et al. (2008), the analysis of neutron diffraction patterns of a CER[EOS]/CER[AP]/CHOL model membrane implies that one CER[EOS] molecule penetrates from one membrane layer into an adjacent layer. A 13 nm periodicity phase has not been observed under the experimental conditions used in these experiments. CER[EOS] can be arranged inside a phase with a repeat distance of 4.5 nm which is predominantly formed by short-chain CER[AP] with distinct polarity.

In the present work, we report a comprehensive analysis of the thermotropic behaviour of CER[EOS] and CER[EOP] by means of X-ray powder diffraction and FT-Raman spectroscopy.

2. Materials and methods

2.1. Materials

CER[EOS] and CER[EOP] were generously provided by Evonik Goldschmidt GmbH (Essen, Germany). In order to increase chemical purity above 96%, the substances were treated using a MPLC technique on a silica gel column with a chloroform/methanol