Eur Biophys J (2008) 37:1051–1057
DOI 10.1007/s00249-008-0265-4

BIOPHYSICS LETTER

Localisation of partially deuterated cholesterol in quaternary SC lipid model membranes: a neutron diffraction study

Doreen Kessner · Mikhail A. Kiselev · Thomas Hauß · Silvia Dante · Siegfried Wartewig · Reinhard H. H. Neubert

Received: 13 August 2007 / Revised: 30 December 2007 / Accepted: 8 January 2008 / Published online: 23 January 2008

Abstract This letter presents our first results in using the benefit of selective deuteration in neutron diffraction studies on stratum corneum (SC) lipid model systems. The SC represents the outermost layer of the mammalian skin and exhibits the main skin barrier. It is essential for studying drug penetration through the SC to know the internal structure and hydration behaviour on the molecular level. The SC intercellular matrix is mainly formed by ceramides (CER), cholesterol (CHOL) and long- chain free fatty acids (FFA). Among them, CHOL is the most abundant individual lipid, but a detailed knowledge about its localisation in the SC lipid matrix is still lacking. The structure of the quaternary SC lipid model membranes composed of either CER[AP]/CHOL-D6/palmitic acid (PA)/cholesterol sulphate (ChS) or CER[AP]/CHOL-D7/PA/ChS is characterized by neutron diffraction. Neutron diffraction patterns from the oriented samples are collected at the V1 diffractometer of the Hahn-Meitner-Institute, Berlin, measured at 32°C, 60% humidity and at different D_2O contents. The neutron scattering length density profile in the direction normal to the surface is restored by Fourier synthesis from the experimental diffraction patterns. The analysis of scattering length density profile is a suitable tool for investigating the internal structure of the SC lipid model membranes. The major finding is the experimental proof of the CHOL localisation in SC model membrane by deuterium labelling at prominent positions in the CHOL molecules.

Keywords Stratum corneum lipids · Cholesterol · Neutron diffraction · Deuterium labelling

Abbreviations

CER[AP]	*N*-(α-Hydroxyoctadecanoyl)-phytosphingosine
PA	Palmitic acid
CHOL	Cholesterol
ChS	Cholesterol sulphate

Introduction

The stratum corneum (SC) represents the outermost layer of the mammalian skin and exhibits the main skin barrier (Elias 1983). For studying drug penetration through the SC, it is essential to know the SC internal structure and hydration behaviour on the molecular level. The SC intercellular lipid matrix is mainly formed by ceramides (CER), cholesterol (CHOL) and long-chain free fatty acids (FFA). Cholesterol is the most abundant individual lipid in the SC, accounting for approximately 25% of the SC lipid mass (Wertz and van den Bergh 1998). This appears to be a

D. Kessner · R. H. H. Neubert (✉)
Institute of Pharmacy,
Martin-Luther University Halle/Wittenberg,
Wolfgang-Langenbeck-Straße 4, 06120 Halle/Saale, Germany
e-mail: reinhard.neubert@pharmazie.uni-halle.de

M. A. Kiselev
Frank Laboratory of Neutron Physics,
Joint Institute for Nuclear Research, Dubna, Russia

T. Hauß · S. Dante
Hahn-Meitner-Institute, Berlin, Germany

T. Hauß
Physical Biochemistry, Department of Chemistry,
Darmstadt University of Technology, Darmstadt, Germany

S. Wartewig
Institute of Applied Dermatopharmacy, MLU Halle,
Halle, Germany

saturating level. Cholesterol is a ubiquitous membrane lipid and appears as a key component for SC barrier function, as in vitro studies revealed (de Kruyff et al. 1974; Takahashi et al. 1996; Sparr et al. 1999). In SC lipid research, it has been shown that for optimum barrier properties, the relative amount of cholesterol should be as large as possible, but not above the solubility of CHOL in the lipid bilayer. Otherwise, pure domains of crystalline CHOL are formed, which could cause a discontinuity in the lipid matrix (Subramaniam and McConnel 1987; Engblom et al. 1998; Norlén and Engblom 2000). However, the presence of crystalline cholesterol was shown not to affect the expected multilamellar periodicity of the lipid systems as revealed by Jager et al. (2004). Bouwstra et al. (1999) could show that at a high CHOL/CER ratio the lamellar lipid organisation is insensitive towards variations in the skin ceramide compositions. Summarising, much is known about the specificity of the interactions between CHOL and other membrane components, but little is known about the detailed structure of the bilayer they form. The work of McIntosh (2003) performing X-ray studies on skin lipid mixtures with long-chain ceramides extracted from porcine SC could contribute to the research about the localisation of CHOL in the SC lipid matrix. The formation of a long-periodicity phase of approximately 130 Å was observed. The electron density profile showed that this repeating unit contained two asymmetric bilayers between cholesterol is asymmetrically distributed.

The aim of this study was to elucidate the detailed structure of a multilamellar model system formed by the most prominent components of the SC lipid matrix. Neutron diffraction was established as an attractive tool for studying the molecular arrangement (Kiselev et al. 2005; Kessner et al. 2006; Ruettinger et al. 2006). H_2O/D_2O exchange provides the possibility to solve the phase problems in Fourier synthesis that is regarded to be the crucial limitation in X-ray diffraction experiments.

Additionally, selective deuteration of significant positions in the molecules affords enhancement of the local contrast that normally is a very small disturbance to the structure. Using benefits of neutron scattering, the present study is the continuation of our research on SC lipid model matrix (Kiselev et al. 2005). As a first feature, they showed that neutron diffraction is an appropriate method for the investigation of the internal nanostructure of SC lipid model membranes, especially due to performing of the Fourier synthesis. Secondly, they observed a low hydration of approximately 1 Å of the intermembrane space at water excess. Therefore, a certain amount of applied CER[AP] assumes to exist at full extended conformation, whose existence was already described (Raudenkolb et al. 2005). CER[AP] at full extended conformation appears to create an extremely strong intermembrane attraction, which tighten neighbouring bilayers to a dense contact and decrease the water diffusion in the lateral direction (Kiselev et al. 2005). This finding recently lead to the suggestion of the *armature reinforcement model* representing an independent theoretical model that describes the SC lipid organisation (Kiselev 2007; Kessner et al. 2007). Thirdly, several experiments on above SC lipid model system varying in the content of CHOL were performed revealing that with increasing content of CHOL, the intensity of the maximum at approximately 12.8 Å in the neutron scattering length density profile increased (Kiselev et al. 2005). Therefore, the maximum at approximately 12.8 Å was related to CHOL, in particular to the position of the boundary A/B of the steroid nuclei as this region represents the lowest density of hydrogen atoms.

This work is a continuation of the work of Kiselev et al. (2005) on a SC lipid model matrix composed of CER[AP]/CHOL/palmitic acid (PA)/cholesterol sulphate (ChS). Although the most abundant free fatty acids (FFA) in human SC are the 22- and 24-carbon entities (Wertz and van den Bergh 1998), palmitic acid (C16) was employed. To prove the previously assumed position of cholesterol inside the oriented SC lipid model membrane, the original lipid composition from the work of Kiselev et al. (2005) has been retained unchanged. For this purpose, two different deuterated CHOL moieties were applied using the benefit of enhancing the local contrast by specific deuteration.

Experimental

The CER[AP] (Fig. 1) was generously provided by Degussa Care Specialities (Essen, Germany). CHOL (Fig. 2), ChS and PA were offered by Sigma-Aldrich (Taufkirchen, Germany). Cholesterol-D6 and cholesterol-D7 (Fig. 2) were purchased from Chemotrade (Leipzig, Germany). Quartz slides (Spectrosil 2000) were obtained from Saint-Gobain (Germany). For the analysis of the CHOL position, the quaternary systems CER[AP]/CHOL-D6/PA/ChS and CER[AP]/CHOL-D7/PA/ChS were studied, where the weight ratio was kept as 55/25/15/5% (w/w), respectively.

CER[AP]

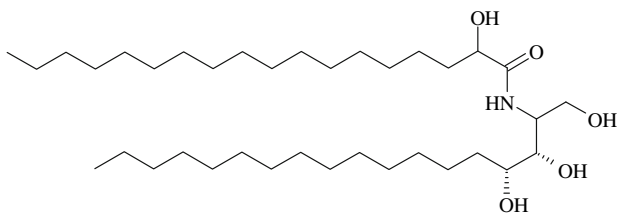
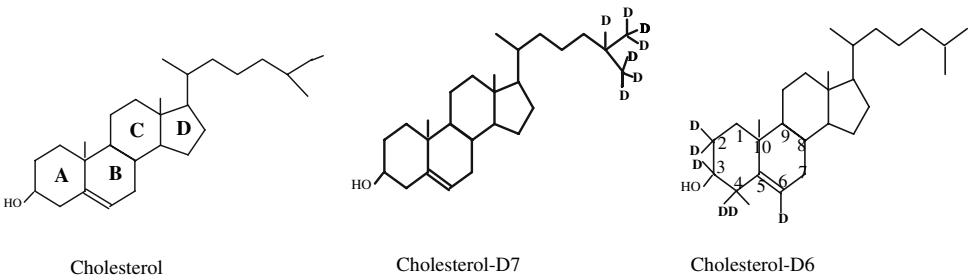


Fig. 1 Chemical structure of CER[AP]



Cholesterol Cholesterol-D7 Cholesterol-D6

Fig. 2 Chemical structures of Cholesterol, Cholesterol-D7 and Cholesterol-D6

Neutron diffraction patterns from the sample were collected at the V1 diffractometer of the Hahn-Meitner-Institute, Berlin, located at a cold neutron source ($\lambda = 4.517$ Å) with a sample-to-detector distance of 101.8 cm. The two-dimensional position-sensitive ^{3}He detector (20 cm × 20 cm area, 1.5 mm × 1.5 mm spatial resolution) was used. Diffraction patterns were recorded as sample rocking curves for the case of the CER[AP]/CHOL-D7/PA/ChS model membrane. The diffraction pattern was recorded as θ–2θ scan from 0 to 30° for the case of the CER[AP]/CHOL-D6/PA/ChS model system. Both samples were equilibrated for 12 h in a chamber at fixed humidity of 60%, kept by a saturated salt solution of sodium bromide, and at a temperature of 32°C prior to measurements. Three types of contrast [$H_2O/D_2O = 92/8$, $H_2O/D_2O = 80/20$ and $H_2O/D_2O = 50/50$ (w/w)] were used. Neutrons interact with atomic nuclei rather than electrons as in the case of X-rays, and neutron scattering lengths are of the same order of magnitude for all elements, including hydrogen (Bacon 1972]. Some isotopes, however, may have different coherent scattering lengths and in particular the values for hydrogen and deuterium are −3.741 and 6.671 fm, respectively. This is a relatively large difference (Schoenborn and Nunes 1972), which can be used for differential labelling and/or contrast variation.

When the neutron diffraction pattern from an oriented lipid bilayer system shows sufficient diffraction orders, the neutron scattering length density profile across the bilayer $\rho_s(x)$ can be reconstructed by Fourier transformation according to Eq. 1:

$$\rho_s(x) = \frac{2}{d}\sum_{h=1}^{h_{max}} F_h \cos\left(\frac{2\pi h x}{d}\right) \quad (1)$$

where F_h is the structure factor of the diffraction peak of order h and d is the lamellar repeat distance. The absolute value of the structure factors $|F_h| = \sqrt{hI_h}$ is given by the integrated intensity of the hth diffraction peak I_h. The signs of the structure factor have the values +1 or −1 for centrosymmetric bilayers, which can be determined by H_2O/D_2O exchange. The procedure describing the evaluation of neutron diffraction data is described elsewhere (Worcester and Franks 1976; Wiener and White 1991; Kiselev et al. 2005).

Results and discussion

It is reasonable to use the non-deuterated membrane CER[AP]/CHOL/PA/ChS as reference. The neutron scattering length density $\rho_s(x)$ of this membrane with the composition ratio 55/25/15/5 (w/w) at 60% humidity, $T = 32$°C and H_2O/D_2O ratio 92/8 (w/w) is shown in Fig. 3 (Kiselev et al. 2005).

The neutron diffraction pattern for the composition CER[AP]/CHOL-D7/PA/ChS (55/25/15/5% w/w) is presented in Fig. 4 for the case of 60% humidity, H_2O/D_2O ratio 92/8 (w/w) and 32°C. Five orders of neutron diffraction could be detected due to the low sample mosaicity. The membrane repeat distance $d = 45.05 \pm 0.04$ Å was calculated from the peak positions in a linear regression procedure.

From the integrated peak intensity I_h, the absolute values of the structure factor $|F_h| = \sqrt{hI_h}$ (h = diffraction order) were derived (Nagle and Tristram-Nagle 2000). Isotopic substitution of H_2O by D_2O was used to evaluate the sign of the structure factors (Worcester and Franks 1976; Wiener and White 1991). The mixed quaternary

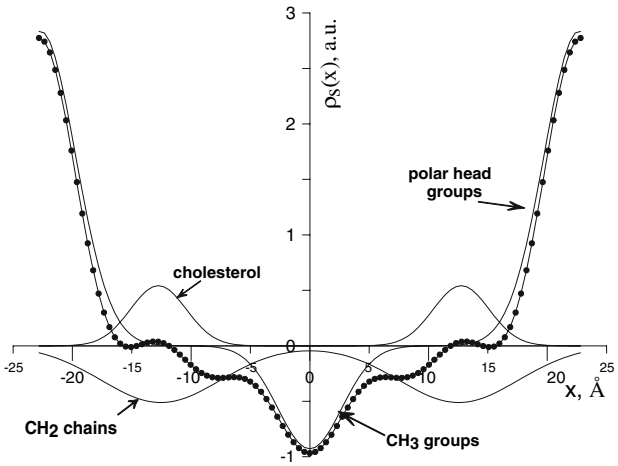


Fig. 3 The neutron scattering length density $\rho_s(x)$ of the CER[AP]/CHOL/PA/ChS membrane with composition 55/25/15/5 (w/w) at 60% humidity, $T = 32$°C, and H_2O/D_2O 92/8 (w/w) content (*dots*) and fitting curve (*solid line*). *Arrows* mark the four components: CH_3 groups, CH_2 chains, cholesterol and polar head groups. *Source:* From Kiselev et al. (2005) with permission from the Springer

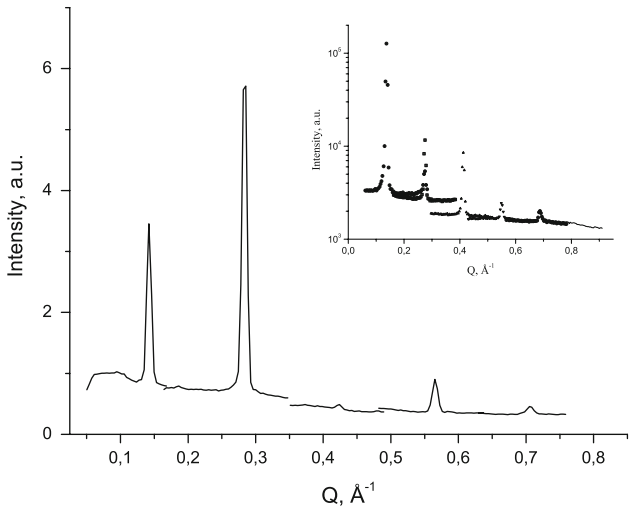


Fig. 4 Neutron diffraction pattern of CER[AP]/CHOL-D7/PA/ChS versus neutron diffraction pattern of CER[AP]/CHOL/PA/ChS (*inset*) [*Source:* From Kiselev et al. (2005) with permission from the Springer] ($T = 32$°C, 60% humidity, H_2O/D_2O 92/8 w/w). The differences in the relative heights of the diffraction peaks demonstrate the sensitivity of the method with respect to the label position

system was measured at H_2O/D_2O ratios of 92/8, 80/20 and 50/50 (w/w), respectively. The sign of structure factors can be decided from the slope of the F_h dependence on the D_2O concentration (Franks and Lieb 1979). For the CER[AP]/CHOL-D7/PA/ChS multilamellar membrane, the signs of the structure factor were determined as −, +, −, + and + for the diffraction orders 1, 2, 3, 4 and 5, respectively. The neutron scattering length densities (in arbitrary units) across the bilayer $\rho_s(x)$ were restored by Fourier synthesis (Nagle and Tristram-Nagle 2000) for these membranes at H_2O/D_2O ratio of 92/8, 80/20 and 50/50 (w/w). The Fourier profile calculated at a H_2O/D_2O ratio of 92/8 (w/w) is presented in Fig. 5. At this contrast, water has zero scattering length and only the membrane is detected. The two maxima of the $\rho_s(x)$ at the edges of the profiles correspond

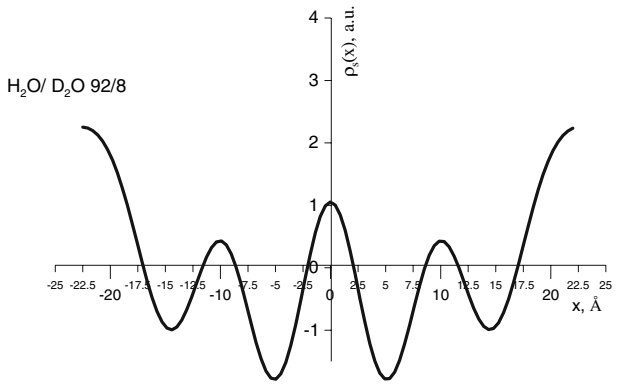


Fig. 5 The neutron scattering length density $\rho_s(x)$ of CER[AP]/CHOL-D7/PA/ChS membrane with the composition of 55/25/15/5% (w/w) at 60% humidity, $T = 32$°C and a ratio of H_2O/D_2O 92/8 (w/w)

to the position of the polar head groups of the lipid bilayer because of the positive coherent scattering length of nuclei forming the polar groups. The maximum of $\rho_s(x)$ at the center of the bilayer corresponds to the position of the CD_3 groups of CHOL-D7 molecules because of the positive value of the coherent scattering length of deuterium. The calculated Fourier profile does not reveal a region of intermembrane space, which is typical for phospholipid membranes (Worcester and Franks 1976; Franks and Lieb 1979; Gordeliy and Kiselev 1995). The distance d_{ph} between the maxima belonging to polar head groups equals the membrane repeat distance d, which indicates that the thickness of the intermembrane space is smaller than the resolution in the neutron diffraction experiments. This feature is in line with the finding of Kiselev et al (2005), that the membrane thickness is $d_m = d_{ph} = d$ and that the thickness of the water layer is $d_w \approx 0$ Å at 60% humidity.

As it is generally known, the values of the coherent scattering lengths of hydrogen (−3.741 fm) and deuterium atoms (6.671 fm) are quite different. The neutron scattering length density profile of the non-deuterated sample (Fig. 3) revealed a maximum at 12.8 Å, which was attributed to the boundary A/B of the steroid nuclei of the cholesterol molecule exhibiting a minimum density of hydrogen atoms (Fig. 2). In contrast, the neutron scattering length density profile of the model system containing CHOL-D7 (Fig. 5) shows a relative maximum in the center of the membrane. It is known that the centre of the membrane is formed by CH_3 groups. For the case of CHOL-D7 in the studied sample, the CH_3 groups of the alkyl chain residue was substituted by CD_3 groups. Because of the positive coherent scattering length of deuterium, a maximum is pronounced, indicating that the alkyl chain residue of CHOL-D7 is localised in the center of the membrane.

A further step in the study of the CHOL positioning, the same procedure as described earlier, was done for the CER[AP]/CHOL-D6/PA/ChS model system (spectra not shown). The difference plot between the neutron scattering length density profile of the sample containing CHOL-D6 and the sample containing non-deuterated CHOL is shown in Fig. 6. Thereby, the influence of the H-D exchange on the projection of the neutron scattering density onto the line normal to the multilayer surface was followed. The maximum is shifted from 12.8 Å (non-deuterated sample) to 17.3 Å (deuterated system). The difference of 4.5 Å corresponds to the distance between the molecular region with the lowest density of hydrogen nuclei (maximum in neutron scattering length density profile of the non-deuterated sample) and the molecular region with the highest density of deuterium nuclei (maximum in the neutron scattering length density profile of the deuterated sample). For the sake of interpretation, it has to take into account that the interspace of two sp^3-hybridised carbon atoms equals 1.54 Å (Fox and

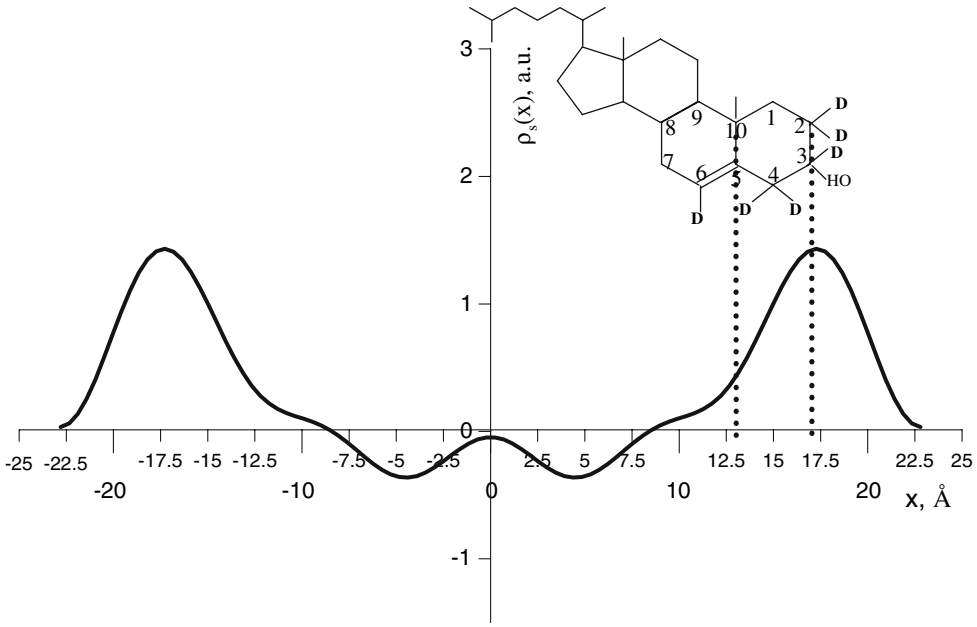


Fig. 6 Projection of neutron scattering density of CHOL onto the line (*x*-axis) normal to the multilayer surface

Whitesell 1994), which means that the distance between three carbon atoms equals approximately 4.5 Å.

It is not possible to see the exact position of a single atom, but the projection of the neutron scattering length onto the *x*-axis allows estimating the proposed localisation of the labelled molecular groups. Consequently, the position of CHOL molecules in the SC model system could be shown experimentally.

During discussions about the cholesterol position inside a SC lipid model system, the phase behaviour of the lipids applied has also to be taken into account. It can be probably expected that the phase behaviour of the lipids affects the position of the CHOL molecules, but from the presented experimental data, it cannot be answered. From a current point of view, it can be assumed that the lipids are in gel phase, as during heating procedures, phase transitions similar to the melting process of alkyl chains were detected. But because of the lack of further experimental evidences, it would be very useful to perform differential scanning calorimetry (DSC), Raman spectroscopy and wide angle X-ray scattering (WAXS) studies on these lipid mixtures to elucidate their phase behaviour.

Our studies on localising the cholesterol position in a quaternary SC lipid model matrix can be summarised as three major findings.

(1) By studying the CER[AP]/CHOL/PA/ChS model membrane as a reference system, the position of the CHOL molecules was roughly attributed to the maximum at 12.8 Å, which assumes to represent the axis through the molecule with the lowest density of hydrogen atoms conforming to the boundary A/B of the steroid nuclei.

(2) The investigation of the CER[AP]/CHOL-D7/PA/ChS model membrane revealed a maximum density of deuterium in the center of the neutron scattering length density profile, indicating that the deuterium-labelled alkyl chain residue of CHOL-D7 is placed in the center of the membrane.

(3) The calculation of the difference plot between the neutron scattering length density profile of CER[AP]/CHOL-D6/PA/ChS and the one of CER[AP]/CHOL/PA/ChS revealed a shift of the maximum from 12.8 Å (non-deuterated system) to 17.3 Å (deuterated system). The distance of 4.5 Å corresponds to the distance between the region with the lowest density of hydrogen atoms (maximum in non-deuterated sample) and the region with the highest density of deuterium atoms (maximum in the deuterated sample). Based on these experimental results, we propose the schematic presentation for the localisation of cholesterol in the SC lipid model system as depicted in Fig. 7.

CHOL appears to be immersed in the hydrocarbon chain region of the bilayer. This arrangement maximizes the hydrophobic interactions with the alkyl chains from CER and FFA and agrees with other investigations (Worcester and Franks 1976; Franks and Lieb 1979) indicating the position of cholesterol in lipid membranes by neutron diffraction.

A lot of other studies focusing on the localisation of CHOL molecules were performed on membranes containing mostly cholesterol and phospholipids by numerous experimental techniques like X-ray scattering, NMR Spectroscopy and dynamic light scattering (Huang et al.

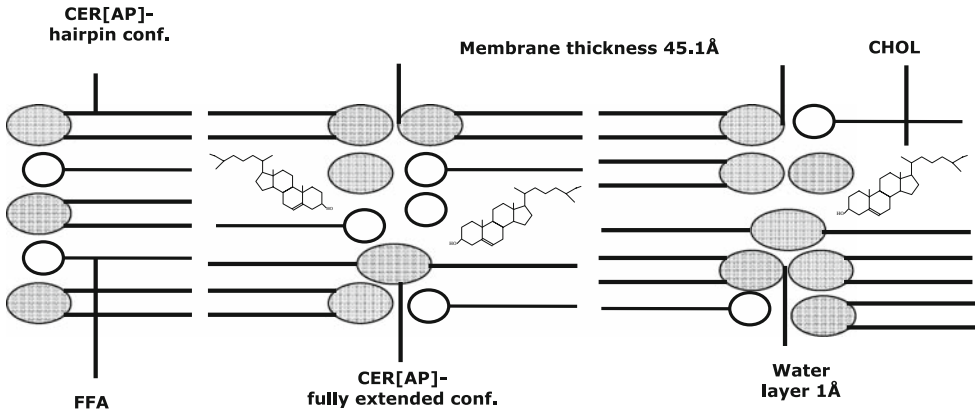


Fig. 7 Schematic presentation of SC lipid model system composed of CER[AP]/CHOL/PA/ChS

1999; Brzustowicz et al. 2002; Bach and Wachtel 2003). Summarising them, it can be stated that the proposed arrangement of CHOL in the SC lipid matrix is comparable to the one in other membrane systems, e.g. in phospholipid bilayers. However, it has to be mentioned that SC lipid model systems do not contain phospholipids, as they are not a constituent of the SC lipid matrix. As mentioned above, there are a lot of studies on skin lipids contributing to the interactions between CHOL and other membrane components, but little is known about the architecture of the bilayer they form.

Finally, it has to be emphasised that the present neutron diffraction study on three SC model systems varying in the degree and in the position of deuteration of the CHOL moiety provided three clear results that can be used for the determination of the CHOL position inside the lipid bilayer. This arrangement is reasonable and in accordance with other proposed models. It underlines the use of neutron diffraction in combination with selective deuteration in lipid research and contributes insights into SC lipid model systems. The experimental method of neutron diffraction combined with the experimental tool of deuterium labelling is well established in the field of phospholipid membranes research (Worcester and Franks 1976; Franks and Lieb 1979; Gordeliy and Kiselev 1995; Nagle and Tristram-Nagle, 2000). It is the intention of the present work to progress the application of neutron scattering on SC lipid model systems as well as to broaden its use by introducing deuterium labelling as a tool to localise single membrane components.

Summary and final remarks

In conclusion, the purpose of this study was to elucidate the molecular arrangement of the most abundant individual SC lipid, namely cholesterol, in a quaternary SC lipid model system by neutron diffraction. Therefore, the benefit of selective deuteration inducing local contrast enhancement should be used in particular. By applying two specifically deuterated CHOL derivatives, the position of CHOL inside the hydrocarbon chain region could be detected. Further, it can be stated that deuterium labelling at prominent positions in the molecules is a suitable tool for investigating the internal structure of SC lipid model membranes.

Acknowledgments We acknowledge the Hahn-Meitner-Institute, Berlin, for the use of the V1 diffractometer and for financial assistance. Further we would like to thank the Graduiertenförderung des Landes Sachsen-Anhalt for funding.

References

Bach D, Wachtel E (2003) Phospholipid/cholesterol model membranes: formation of cholesterol crystallites. A review. BBA 1610:187–197

Bacon GE (1972) Neutron diffraction. Clarendon Press, Oxford

Brzustowicz MR, Cherezov V, Caffrey M, Stillwell W, Wassall SR (2002) Molecular organization of cholesterol in polyunsaturated membranes: microdomain formation. Biophys J 82:285–298

Bouwstra JA, Dubbelaar FER, Gooris GS, Weerheim AM, Ponec M (1999) The role of the ceramide composition in the lipid organisation of the skin barrier. Biochim Biophys Acta 1419(2): 127–136

de Kruyff B, van Dijck PWM, Demel RA, Schuijff A, Brants F, van deenen LLM (1974) Non-random distribution of cholesterol in phosphatidylcholine bilayers. Biochim Biophys Acta 356:1–7

Elias PM (1983) Epidermal lipids, barrier function, and desquamation. J Invest Dermatol 80(Suppl):44s–49s

Engblom J, Engström S, Jönnson B (1998) Phase co-existence in cholesterol-fatty acid mixtures and the effect of the penetration enhancer azone. J Control Release 52:271–280

Fox MA, Whitesell JK (1994) Organische chemie. Spektrum Verlag, Heidelberg

Franks NP, Lieb WR (1979) The structure of lipid bilayers and the effects of general anaesthetics. An x-ray and neutron diffraction study. J Mol Biol 133:469–500

Gordeliy VI, Kiselev MA (1995) Definition of lipid membrane structural parameters from neutronographic experiments with the help of the strip function model. Biophys J 69:1424–1428

Huang H, Buboltz JT, Feigenson GW (1999) Maximum solubility in phosphatidylcholine and phosphatidylethanolamine bilayers. BBA 1417:89–100

Jager MW, Gooris GS, Dolbnya IP, Ponec M, Bouwstra JA (2004) Modelling the stratum corneum lipid organization with synthetic

lipid mixtures: the importance of synthetic ceramide compositions. Biochim Biophys Acta 1684:132–140

Kessner D, Kiselev M, Ruettinger A, Neubert R, Dante S, Hauss T (2006) Investigation of deuterium labeled stratum corneum lipid model membranes, HMI experimental report

Kessner D, Ruettinger A, Kiselev MA, Wartewig S, Neubert RHH (2007) Properties of ceramides and their impact on the stratum corneum structure: a review, part II: stratum corneum lipid model systems. Skin Pharmacol Appl Skin Physiol 21:58–74

Kiselev MA, Ryabova NY, Balagurov AM, Dante S, Hauss T, Zbytovská J, Wartewig S, Neubert R (2005) New insights into the structure and hydration of a stratum corneum lipid model membrane by neutron diffraction. Eur Biophys J 34:1030–1040

Kiselev MA (2007) Conformation of ceramide 6 molecules and chain-flip transitions in the lipid matrix of the outermost layer of mammalian skin, the stratum corneum. Crystallogr Rep 52:525–528

McIntosh TJ (2003) Organization of skin stratum corneum extracellular lamellae: diffraction evidence for asymmetric distribution of cholesterol. Biophys J 85:1675–1681

Nagle JF, Tristram-Nagle S (2000) Structure of lipid bilayers. Biochim Biophys Acta 1469:159–195

Norlén L, Engblom J (2000) Structure-realted aspects on water diffusivity in fatty acid-soap and skin lipid model systems. J Control Release 63(1/2):213–226

Raudenkolb S, Wartewig S, Neubert RHH (2005) Polymorphism of ceramide 6. A vibrational spectroscopic and X-ray powder diffraction investigation of the diastereomers of *N*-(-hydroxyoctadecanoyl)-phytosphingosine. Chem Phys Lipids 133:89–102

Ruettinger A, Kiselev M, Otto D, Dobner B, Neubert R, Dante S, Hauss T (2006) Influence of the fatty acid chain length to the structure of a stratum corneum model membrane, HMI experimental report

Schoenborn BP, Nunes AC (1972) Neutron scattering. Annu Rev Biophys Bioeng 1:529–552

Sparr E, Ekelund K, Engblom J, Engström S, Wennerström H (1999) An AFM study of lipid monolayers. II. The effect of cholesterol on fatty acids. Langmuir 15(20):6950–6955

Subramaniam S, McConnel M (1987) Critical mixing in monolayer mixtures of phospholipids and cholesterol. J Phys Chem 91:1715–1718

Takahashi H, Sinoda K, Hatta I (1996) Effects of cholesterol on the lamellar and the inverted hexagonal phases of dielaidoylphosphatidylethanolamine. Biochim Biophys Acta 1289:2009–216

Wertz PW, van den Bergh B (1998) The physical, chemical and functional properties of lipids in the skin and other biological barriers. Chem Phys Lipids 91:85–96

Wiener MC, White SC (1991) Fluid bilayer structure determination by the combined use of X-ray and neutron diffraction. Biophys J 59:162–173

Worcester DL, Franks NP (1976) Structural analysis of hydrated egg lecithin and cholesterol bilayers. II. Neutron diffraction. J Mol Biol 100:359–378