Comparative X-ray diffraction study of diphytanoylphosphatidylcholine and -ethanolamine under different states of hydration

*D. R. Gauger, C. Selle, W. Pohle and M. H. J. Koch**

*Department of Biophysical Chemistry, Institute of Molecular Biology, Friedrich-Schiller University Jena, Winzerlaer Str. 10, 07745 Jena, Germany, *EMBL c/o DESY, Notkestr. 85, D 22603 Hamburg, Germany*

Project Number : NCS-99-6
EMBL Hamburg beam line : X-33

Diphytanoylphosphatidylcholine (DPhPC) has been used in studies of protein-lipid interaction and membrane channel activity because it has high bilayer stability and low ion leakage [1], and as transfection agent with high efficiency and low cell toxicity [2]. No L_α to gel phase transition was detected by differential thermal analysis for DPhPC in water over a temperature range from +120 to –120 °C [1].

In contrast to the observations made by thermal analysis, DPhPC superstructure is strongly influenced by the water content. As revealed in NMR and X-ray diffraction studies, it exhibits a pronounced polymorphism upon hydration [3,4]. Not as much is known about its ethanolamine analogue (DPhPE). Our previous FT-IR spectroscopic measurements had indicated very fluid states in the films of both lipids, and moreover structural rearrangements at about 80 % relative humidity (rh) in the DPhPC samples and near 0 % rh in both lipids.

We have used the beamline D1/2 in the HASYLAB at DESY to characterize phases of DPhPC and DPhPE (both purchased from Avanti Polar Lipids, Alabaster, AL, USA) in dependence on the state of hydration by means of small- and wide-angle X-ray scattering (SAXS, WAXS). The lipid films were prepared from chloroformic solutions (10 mg/ml) cast onto mica plates and measured at 100 or 0 % rh after equilibration times of at least one day. The sample cells are constructed such that water activity can be regulated via the surrounding gas volume by appending small vessels containing either P_2O_5 (0 % rh) or H_2O (100 % rh).

Fig. 1 A demonstrates how the SAXS patterns of DPhPC vary at 100 % (a) and 0 % (b) rh. At 100 % rh DPhPC exhibits the pattern typical of a lamellar phase: the s values of the first-, second- and third-order peaks correspond to a ratio 1:2:3. The resulting repeat distance is 5.18 nm, and the lamellae are fluid as revealed by WAXS. The phase adopted in the dry DPhPC sample is at present not assignable since only one peak appeared. Provided that it would be lamellar the pertinent repeat distance is 3.77 nm. In contrast to the results in [3] no H_{II} phase was observed for the fully hydrated film of DPhPC.

Fig. 1 B shows the diffractograms of DPhPE at 100 % (a) and 0 % (b) rh. The hydrated sample exhibits the typical pattern for a hexagonal phase with s ratios of $1:\sqrt{3}:2:\sqrt{7}$ and can be assigned to a H_{II} phase with a repeat distance between the centers of the adjacent cylinders of 5.39 nm. At 0 % rh the two peaks suggest the existence of a second H_{II} phase with a significantly smaller repeat length of 4.08 nm. There is no evidence for a ribbon phase as found before in dry samples of dioleoyl PE [5]. As might be expected for DPhPE, the space requirements of the isoprenoid chains speak in favour of the more “curved” H_{II} phase.

Altogether, the data for these isoprenoid lipids confirm that PE`s exhibit a stronger tendency to form nonlamellar phases than PC`s, as found previously for unsaturated chain lipids (W. Pohle et al., unpublished data). Moreover the repeat lengths of the diphytanoyl lipids are generally smaller than those found for their oleoyl counterparts (W. Pohle et al., unpublished data).

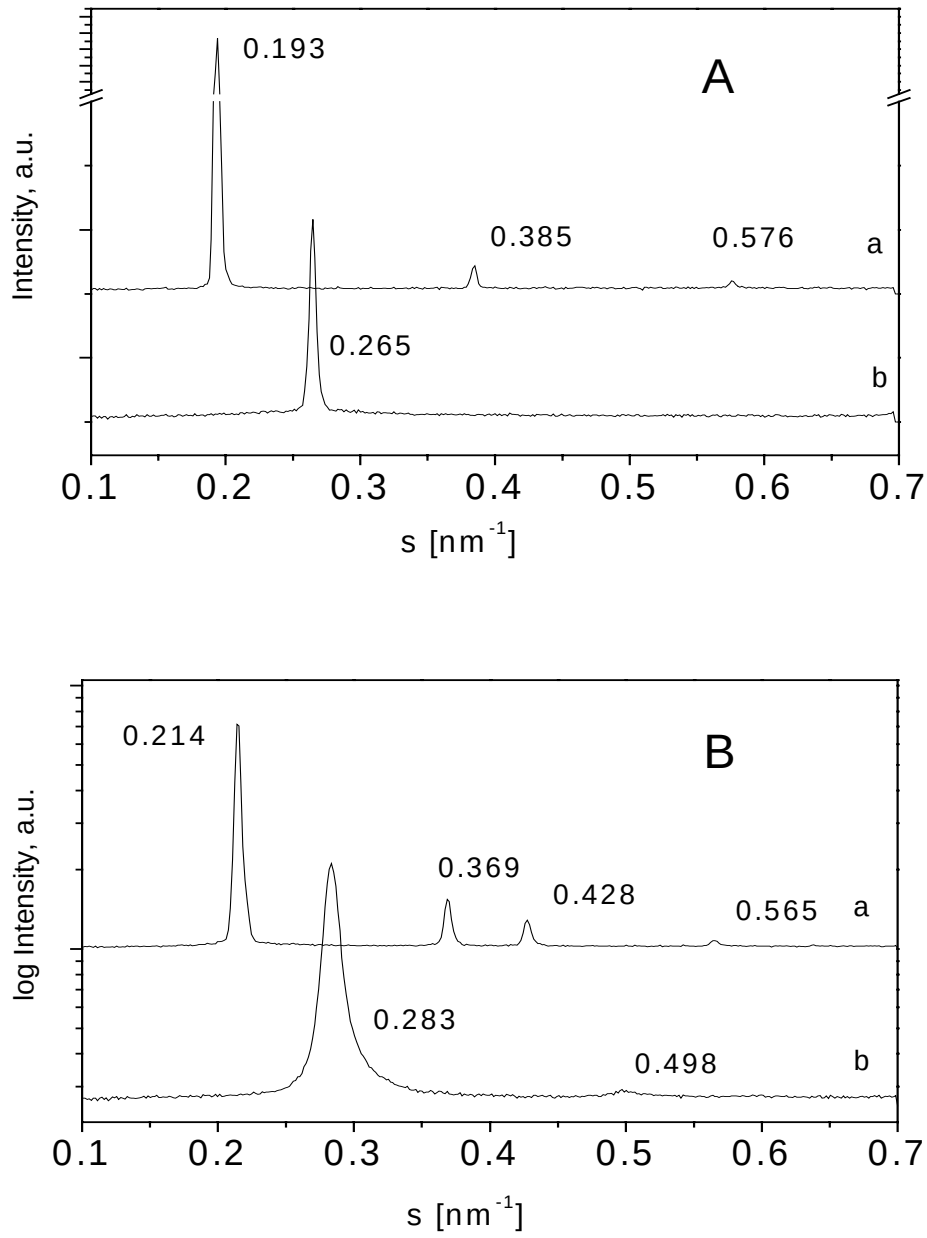

Fig.1: SAXS patterns obtained at 26 °C for films of DPhPC (A) and DPhPE (B) stored at 100 % rh (a) and 0 % rh (b) for more than 24 hours before doing measurements. Exposure time to the synchrotron radiation was 3 minutes. Data analysis was done by using the SAPOKO/OTOKO program.

References

[1] Lindsey, H., Petersen, N. O. & Chan, S. I., BBA 555, 147-167 (1979)
[2] Andreopoulos, D. & Kasi, L. P., J., Microencapsul. 14, 427-436 (1997)
[3] Hsieh, C.-H.,Sue, S.-C.,Lyu, P.-C. & Wu, W.-G., Biophys. J. 73, 870-877 (1997)
[4] Wu, Y., He, K., Ludtke, S. J. & Huang, H. W., Biophys. J. 68, 2361-2369 (1995)
[5] Pohle, W. & Selle, C., Chem. Phys. Lipids 82, 191-198 (1996)