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Observation of rippled dioleoylphosphatidylcholine bilayers by neutron diffraction

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Neutron diffraction data have been obtained from a rippled phase of multibilayer stacks containing mixtures of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE). In orientated bilayers neutron diffraction patterns show Bragg peaks near to the meridian and displaced from the usual long spacings. These off-axis reflections are assigned to a rippled phase and it is suggested it is formed as a transition phase between L_α and H_{II} phases.

Phospholipids, one of the major components of membranes, have long been the subject of investigation by a large number of techniques [1]. Of particular interest has been the ability of hydrated phospholipids to form a number of different ordered states including bilayer structures, ripples and hexagonal arrays. Diffraction techniques have been used to provide detailed conformational information of many of these structural phases. To date relatively little is known about rippled phases, although X-ray diffraction has given some information about the $P_{\beta'}$ ripple which occurs between the L_α and $L_{\beta'}$ phases [2].

Neutron diffraction has a distinct advantage over the use of X-rays in that the coherent neutron scattering amplitude of deuterons is considerably higher than that of protons. This means that in diffraction studies of specifically deuterated macromolecules the deuterons are easily identified in neutron scattering profiles. This not only facilitates the phasing of the diffraction data, but can also be used to determine the conformation of the molecules containing the deuterium labels. Such methods have been applied to the study of saturated fatty acyl chain phospholipid bilayers [3].

The phospholipids 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) were obtained from Sigma Chemical Company and confirmed to be single species by thin-layer chromatography. Orientated bilayer stacks were prepared by spreading approximately 40 mg of phospholipid in chloroform over a quartz microscope slide. The solvent was allowed to evaporate off under a stream of warm air and the sample was fully dried under vacuum for 24 h. The lipid was annealed for at least 4 h at 70°C and 100% humidity. Once fully hydrated, the samples were brought down to the experimental relative humidity of 57% by equilibrating for at least 30 min at 30°C over a saturated solution of sodium bromide.

Neutron diffraction data were collected using the D16 instrument at the Institut Max von Laue – Paul Langevin (I.L.L.), Grenoble, France. After equilibration each quartz slide containing its orientated bilayer sample was placed in the temperature and humidity controlled cell of the instrument. Temperature control was achieved by circulating water from a thermostat around a closed aluminium can containing the sample in an atmosphere over a saturated salt solution. The D16 detector receives scattered neutrons over an area that is approximately 9° horizontally by 4.5° vertically, with a resolution of 0.143° horizontally and 0.286° verti-

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Abbreviations: DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine.

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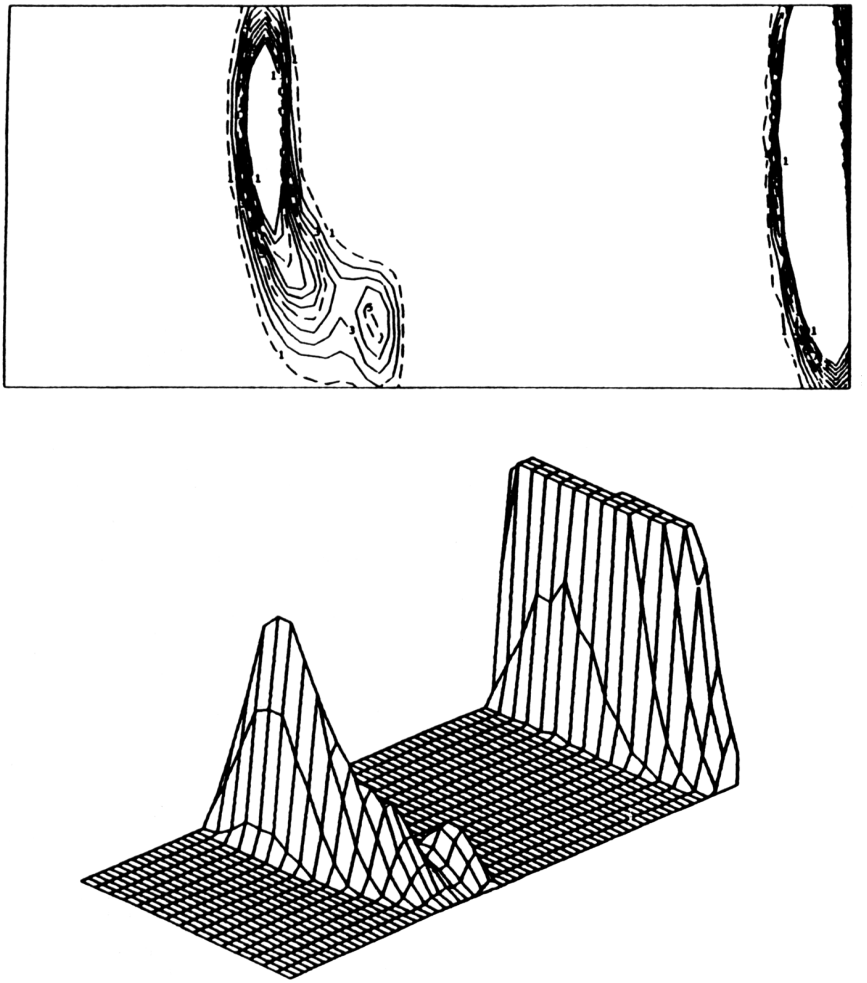

Fig. 1. Contour and 3-dimensional plots of area around the second order lamellar diffraction peak of rippled DOPC bilayers with 10% (mol) DOPE at 30° C and 57% relative humidity.

cally. Each recorded diffraction pattern consisted of nine or ten well defined peaks. Once the position of these peaks had been established, the detector was set up to perform a series of scans in which the area around the predicted position of each diffraction peak was scanned a number of times. The scans were then added together taking into account the angular offset of each to give a simulated θ-2θ scan.

The full width half height of the diffraction peaks, often called the mosaic spread, was determined for each sample by aligning the detector with the fourth order (2θ), and scanning the sample (θ) through the optimum diffraction angle for this peak three times, with increasingly fine resolution. Each scan covered a smaller angular spread and was centred on the diffraction peak. In this manner the value for the background level could be obtained at several degrees from the peak itself and an accurate measurement of its full width half height could be performed.

The data that were collected by the Digital Equipment Corporation PDP 11/123 and 11/34 computers controlling the data collection process were transferred to the main computer of the I.L.L. The different scans of each run were collated by the computer to give a simulated continuous plot of intensity versus scattering angle.

The alignment and sensitivity of each pixel of the detector was not perfectly balanced, so a correction was applied to each channel of the data to allow for this. The response of each pixel was determined by using the detector to record the incoherent neutron scattering from a vial of water. This incoherent scattering is independent of angle so each pixel of the detector effectively received the same signal. With this information it was possible to derive a correction factor of each pixel of the detector. Another correction was necessary to allow for the background of incoherent radiation upon which the diffraction pattern was superimposed. The background level of incoherent radiation under each diffraction peak was determined by measuring the count rate in the channels immediately adjacent to the peak and interpolating across its base. This value was then subtracted from the channels under the peak.

The software used to control the data collection process was also capable of determining the angular position of the centre of each peak in terms of 2θ. However, the accuracy of this value was dependent upon the initial alignment of the neutron beam, detector and sample. This angular information is used to calculate the exact D spacing of each sample, and an accuracy greater than that of the machine offset is desirable. For this reason an iterative refinement technique was used to calculate both the true offset and the lattice parameters.

Fig. 1 shows an example of the off-meridional Bragg reflection in contour and simulated 3-d plots. Such

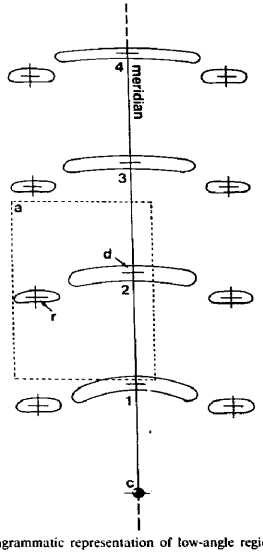

Fig. 2. Diagrammatic representation of low-angle region of lamellar diffraction pattern of DOPC/DOPE rippled phase. (a) area shown in Fig. 1. (c) centre of reciprocal lattice. (d) lamellar diffraction peak on meridian. (r) off-axis ripple diffraction peak.

off-meridional diffraction peaks were strongest at the sides of the first two lamellar peaks, with intensities approxiamtely proportional to intensities of the on-axid peaks. Fig 2 illustrates this area of the diffraction pattern, with the area sampled by the detector in Fig. 1 outlined. The extremely low mosaic spread of these samples made it necessary to introduce an offset into the source-sample-detector line to emphasise the ripple reflection on one side of the equator at the expense of its mirror image across the meridian. This tilt was approximately 3°. Iterative refinement of the angles of each order of diffraction with respect to a reciprocal lattice gave values for the bilayer spacing of 4.80 nm and 4.49 nm for samples containing 10% (mol) and 30% (mol) DOPE, respectively. The corresponding ripple wavelenghts were 6.6 nm and 5.7 nm. The mosaic spread of the bilayers containing 10% DOPE was 0.8°, and 1.2° for the bilayers with 30% DOPE.

A comprehensive characterisation of the rippled phase has been made in recent X-ray studies of the same system [4]. The relationship between ripple wavelength and bilayer repeat distance in this present study

indicates that this phase is essentially the same structure as that characterised by X-ray diffraction. This rippled phase occurs as a transition form between the L_α bilayers and H_{II} hexagonal states. The fact that the rippled phase was only observed in diffraction from samples containing COPE, and not in samples of pure DOPC, does not necessarily indicate that the rippled state is primarily caused by the addition of DOPE. The X-ray studies have indicated that there is a threshold value for the bilayer repeat of 4.8 nm below which the ripple occurs, and that this threshold is independent of the DOPE content of the bilayer. The bilayer repeat is itself determined by the amount of DOPE present. At a relative humidity of 57% the bilayer repeat is above 4.8 nm for the pure DOPC bilayers, and only drops below this value in the bilayers containing DOPE.

The neutron data do, however, supply some extra information on the rippled phase. This takes the form of the determination of mosaic spread in the rippled and non-rippled phases, a measurement which is not always possible or practical to make with X-ray diffraction. The mosaic spread of an orientated sample is influenced by a number of factors: the wavelength spread, the size of the diffracting units, the beam size, the finite length of the specimen, the stacking order in the specimen and increasing scattering angles [5,6]. If the neutron beam parameters and sample thickness for a series of specimens are kept constant, the mosaic spread may be used as a measure of the macroscopic order of the sample on the meiroscope slide; the smaller the value, the less the degree of disorder of the bilayers. The observed values of about 0.8° for 10% DOPE and 1.2° for 30% DOPE correlate well with values obtained for non-rippled bilayers of DOPC-DOPE [7]. This can be taken to indicate that the onset of the ripple is both uniform throughout the sample and that the rippled phase itself has a very low mosaic spread.

The observations described here demonstrate that neutron diffraction is a technique well suited to the study of phospholipid-water systems. To date it has been used to study headgroup and saturated fatty acyl chain conformation of a number of phospholipid bilayers. The present study has shown that the methods work equally well for non-bilayer systems, making the potential for studies of these systems enormous.

References

1 Knight, C.G. (ed.) (1981) Liposomes: From Physical Structure to Therapeutic Applications, Elsevier/North Holland Biomedical Press, Amsterdam.
2 Alecio, M.R., Miller, A. and Watts, A. (1985) Biochim. Biophys. Acta 815, 139.
3 Buldt, G., Gally, H.U., Seelig, A., Seelig, J. and Zaccai, G. (1978) Nature 271, 182.
4 Bradshaw, J.P., Edenborough, M., Sizer, P.J.H. and Watts, A. (1989) Biochim. Biophys. Acta 987, 104–110.
5 Blaurock, A.E. and Nelander, J.C. (1976) J. Mol. Biol. 103, 421.
6 Schwartz, S., Cain, J.E., Dratz, E.A. and Blasie, J.K. (1975) Biophys. J. 15, 1201.
7 Bradshaw, J.P., Edenborough, M., Sizer, P.J.H. and Watts, A. (1989) Biochemistry, submitted.