Biochimica et Biophysica Acta, 979 (1989) 139–141
Elsevier

BBA 70595

BBA Report

Orientation of α-helical peptides in a lipid bilayer

John C. Huschilt, Barry M. Millman and James H. Davis

Biophysics Interdepartmental Group, Physics Department, University of Guelph, Guelph (Canada)

(Received 2 September 1988)

Key words: X-ray diffraction; Lipid bilayer; Membrane structure; Peptide–lipid interaction; Dipalmitoylphosphatidylcholine; Alpha-helical peptide

Samples of pure lipid (dipalmitoylphosphatidylcholine) and lipid containing short α-helical peptides were oriented and examined by X-ray diffraction, together with unoriented samples of pure peptide. X-ray reflections from the bilayer and the α-helices showed that the peptides had oriented in the bilayer with their helical axes perpendicular to the surface.

Lipids and proteins are the primary components of biological membranes. Most intrinsic membrane proteins contain large segments of hydrophobic α-helices which tend to be aligned with their α-helical axes perpendicular to the membrane surface. As part of a larger study of the incorporation of proteins and peptides into lipid bilayers [1–4], we have examined the orientation of short α-helical peptides into a model bilayer system. The peptides used have a hydrophobic core of 16 or 24 leucines with hydrophilic amino acids at either end and had been studied previously by a range of biophysical techniques [2–4]. It has been demonstrated by circular dichroism that these peptides form helices, probably α-helices, in non-polar solvents and in lipid vesicles in both gel and liquid-crystalline phases [2]. The length of the longer peptide (3.6 nm) is such that it can span the hydrophobic region of a bilayer [2]. The experiments reported here were undertaken to verify the orientation of the helices in the bilayer. Oriented bilayers, formed from mixtures of lipid and peptide were studied by low-angle X-ray diffraction. Specific reflections, identified from both bilayers and α-helices, were used to check the alignment of the bilayer surface and the helical axis.

The deuteriated and naturally hydrated 1,2-bis(perdeuterio)palmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was synthesized in this laboratory by the method described by Gupta et al. [5] and purified on a 1.5 m Sephadex LH 20 column. Analysis of the lipid by thin-layer chromatography showed no evidence of impurities. Its molar mass was 832 ± 9 g allowing for 2 ± 0.5 water molecules of hydration [6].

The peptide were synthesized on a Beckman solid-phase peptide synthesizer [2]. Their structures are $K_2GL_{16}K_2A$-amide (peptide-16) and $K_2GL_{24}K_2A$-amide (peptide-24). Amino acid analyses gave the following compositions: for peptide-16, G (1.02), A (1.10), K (3.94), L (15.94); for peptide-24, G (1.02), A (1.07), K (4.28), L (23.64). Their molar masses were, respectively. 2765 ± 100 and 3669 ± 100 g, including five acetate counter-ions and allowing for approximately ± 1 amino acid in the distribution of the masses of the final products.

Peptide/DPPC samples were made by mixing appropriate amounts of methanol solutions of peptide and DPPC and removing the methanol with a Buchi Rotavapor. Lipid : peptide mole ratios were 10 : 1 for peptide-16 and either 10 : 1 or 30 : 1 for peptide-24. Some samples were rehydrated from samples prepared from NMR studies [2].

Oriented specimens were prepared by a technique which was a modification of a method recommended to us by Dr. R.P. Rand (Dept. of Biological Sciences, Brock University, St. Catharines, Ontario, Canada). Specimens were prepared by placing a few drops of concentrated solution onto a thin slice of mica: approx. 1.5 cm^2. A weak jet of nitrogen gas was used to evaporate the solvent from each drop. In total, a few milligrams were deposited on a spot about 8 mm in diameter. To fully hydrate the sample, it was equilibrated for several days using a saturated water atmosphere in the dark at room temperature. Specimens were about 0.1 mm thick and appeared somewhat frosty. Pure peptide specimens were prepared similarly, but did not orient. Immediately before the X-ray exposure, the mica was gently bent into a curve (radius about 1.4 cm) and placed on an aluminium holder. During the exposure, the specimens

Correspondence: B.M. Millman, Biophysics Interdepartmental Group, Physics Department, University of Guelph, Guelph, Ontario, Canada N1G 2WI.

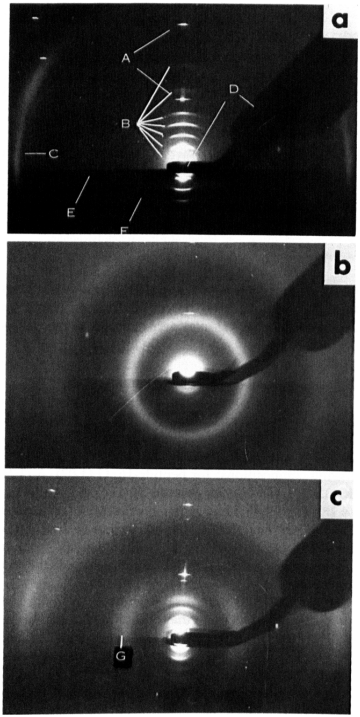

Fig. 1. X-ray diffraction patterns of pure, oriented DPPC (a), pure, unoriented peptide-24 (b), and oriented lipid/peptide-24 mixture (c). Original X-ray diffraction patterns are magnified 2.9 times.

were mounted in a darkened chamber at room temperature which was constantly flushed with water-saturated helium gas.

X-ray diffraction patterns were obtained using a double-focussed Frank's camera [7] with an X-ray beam about 0.1 × 0.5 mm. Sample-to-film distances were between 3.2 and 4.2 cm. Kodak direct-exposure film (DEF-5) was used and exposures ranged from 1 to 6 days. Reflection spacings were measured on an optical comparator and were calibrated using orders of the 1.00 nm mica spacing.

Fig. 1 shows X-ray diffraction patterns from pure lipid (a), pure protein (b) and a lipid/protein mixture (c). Taken as a group, these patterns confirm that the peptides were oriented in the bilayer with the helical axes perpendicular to the bilayer surface. All X-ray patterns show sharp reflections from the mica substrate at spacings corresponding to 1.000 and 0.5000 nm (A), a backstop shadow (D) and a shadow from the curved mica sheet (E and F) which reduced the intensity of the X-ray reflections below the backstop.

In Fig. 1a, sharp reflections are seen along the meridional axis (that axis perpendicular to the mica surface), corresponding to order 1 to 5, 7 and 9 of an oriented multi-bilayer system of thickness 6.15 ± 0.02 nm (averaged from measurements of the first to fifth orders: B on Fig. 1a). This spacing compares favorably with those from multilamellar dispersions of lipids. In addition, a moderately sharp but arced reflection (C) is seen on the equator (that axis parallel to the mica surface) at 0.44 nm, which arises from oriented tails of the lipid molecules and is characteristic of a lipid gel phase.

Fig. 1b shows an X-ray diffraction pattern from non-oriented peptide-24. Two fairly diffuse rings are seen: the inner and brighter one at a spacing of 1.09 nm and the outer one at a spacing of 0.49 nm. This pattern is consistent with an α-helical structure: the reflections correspond to the 1.0 nm equatorial (i.e. perpendicular to the helix axis) and 0.5 nm meridional (i.e. parallel to the helix axis) reflections from α-helical proteins arising from the diameter and pitch, respectively; of the α-helices.

Fig. 1c shows an X-ray diffraction pattern from an oriented lipid/protein-24 mixture in a mole ratio of 10:1. Similar, but weaker, patterns were seen at a mole ratio of 30:1 and with peptide-16. In all cases, reflections from both peptide and lipids were seen and were oriented. Bilayer reflections are seen on the meridian in Fig. 1c, similar to those of Fig. 1a, corresponding to the first five orders of a bilayer periodicity of 6.18 nm. This spacing is comparable to spacings from pure lipid bilayers. A strong, arced reflection was seen on the equator at 0.43 nm arising from a gel phase of the lipid tails. An additional, diffuse reflection at 1.10 ± 0.01 nm was seen on the equator [G] which matched the α-helical reflection in Fig. 1b and corresponds to the helical diameter. This reflection is confined within 25° of the equator, which indicates that the helical axes are aligned to within 25° of a perpendicular to the bilayer surface. The 0.5 nm reflection which is expected on the meridian cannot be unambiguously identified in this X-ray pattern, partly because it is diffuse and much fainter than the 1.0 nm reflection, and partly because it cannot be clearly distinguished (on such patterns) from rings or arcs associated with the lipid tails. There is, however, intensity on the meridian at about 0.5 nm in these patterns, suggesting that this reflection is probably present.

Because of the amino acid sequence of the peptide used [2], it was anticipated that the leucine core would orient parallel to the hydrophobic lipid tails in the bilayer. The hydrophilic terminal residues of the peptides were expected to be positioned near the hydrophilic lipid headgroups. Because of their lengths, the peptides could then span the bilayer like an intrinsic protein. Any mismatch of the lengths of the corresponding hydrophobic parts of the molecules was expected to result in a longitudinal stretch or compression of the lipid tails, in a tilt of the peptide, or in some other conformational change which would compensate for the length difference by minimizing the free energy of the molecular configuration. Indeed, the 'Mattress Model' of Mouritsen and Bloom [8] and the design of these experiments [1] were based on these expectations. The X-ray diffraction patterns confirm that the peptides form α-helices, which orient with their helical axes perpendicular to the lipid bilayer (to within 25°).

The authors are grateful to the National Science and Engineering Research Council of Canada: J.C.H. for a scholarship, B.M.M. and J.H.D. for grant support.

References

1 Davis, J.H., Hodges, R.S. and Bloom, M. (1982) Biophys. J. 37, 170–171.
2 Davis, J.H., Clare, D.M., Hodges, R.S. and Bloom, M. (1988) Biochemistry 22, 5298–5305.
3 Huschilt, J.C., Hodges, R.S. and Davis, J.H. (1985) Biochemistry 24, 1377–1386.
4 Morrow, M.R., Huschilt, J.C. and Davis, J.H. (1985) Biochemistry 24, 5396–5406.
5 Gupta, C.M., Radhakrishnan, R. and Khorana, H.G. (1977) Proc. Natl. Acad. Sci. USA 74, 4315–4319.
6 Albon, N. and Sturtevant, J.M. (1978) Proc. Natl. Acad. Sci. USA 75, 2258–2260.
7 Elliott, G.F. and Worthington, C.R. (1963) J. Ultrastruct. Res. 9, 166–170.
8 Mouritsen, O.G. and Bloom, M. (1984) Biophys. J. 46, 141–153.