Polymyxin B induces interdigitation in dipalmitoylphosphatidylglycerol lamellar phase with stiff hydrocarbon chains

J.L. Ranck and J.F. Tocanne*

Centre de Génétique Moléculaire, CNRS, 91190 – Gif sur Yvette and * *Centre de Recherche de Biochimie et de Génétique Cellulaire, 118, Route de Narbonne, 31077 – Toulouse Cedex, France*

Received 23 April 1982

Phosphatidylglycerol *Antibiotics interactions* *Interdigitation* *Hydrocarbon chains* *X-Ray diffraction*

1. INTRODUCTION

Polymyxins are broad spectrum antibiotics exhibiting activity against Gram-positive and Gram-negative bacteria, yeast and protozoa [1]. These antibiotics are known to cause a rapid permeability change of the cytoplasmic membrane resulting in a release of cellular materials [1]. Polymyxins are peptidolipids to which the presence of 5–6 2,4-diaminobutyric acid residues confers a net positive charge [2]. Recent studies of the interaction of polymyxins with lipid monolayers [3,4] and bilayers [5,8] has revealed a high and specific affinity of the polymyxins for acidic phospholipids. We demonstrate here that this antibiotic has the effect of inducing interdigitation of the hydrocarbon chains when interacting with dipalmitoylphosphatidylglycerol.

2. MATERIAL AND METHODS

rac-DPPG–Na salt was of synthetic origin [9]. The lipid was pure as indicated by thin-layer chromatography.

Polymyxin B sulphate was purchased from Sigma (St Louis). It was converted into the iodide form by titration with barium iodide. The precipitated bariumsulphate was removed by centrifugation; the clear aqueous supernatant was lyophilized and polymyxin B iodide obtained as a white microcrystalline powder.

Abbreviations: Rac-DPPG, racemic dipalmitoyl phosphatidylglycerol; PxB, polymyxin B

Polymyxin B/rac-DPPG complexes were obtained using the following procedure. The two components, in the desired molar ratio, were dissolved in a minimum chloroform/methanol (1/1, v/v) solution (50 mg/0.1 ml). Addition of acetone (5 ml) precipitated the peptidolipid complex as a white powder whereas sodium iodide remained soluble in the acetone phase. The precipitate was centrifuged and washed with acetone (5 ml) and finally dried in vacuo over phosphorus pentoxide. The 5/1, 7/1, 10/1, 20/1 and 25/1 rac-DPPG–PXB complexes prepared with this procedure were lysophosphatidylglycerol free, as revealed by thin-layer chromatography.

The X-ray diffraction experiments were done as in [10,11]. The interpretation of low and high angle diffraction pattern is described in [10,13].

RESULTS

3.1. *Dry rac-DPPG–PXB*

Five different samples rac-DPPG–PXB were studied, at molar (rac-DPPG/PXB) ratios 5/1, 7/1, 10/1, 20/1 and 25/1. At room temperature, the 5 samples fall into 3 classes. Two classes (5/1,7/1) show one lamellar phase (with the same spacing, $d=37.7$ Å) with stiff interdigitated chains; the organization of the chains is of type p6. The 10/1 sample contains two lamellar phases whose spacing are 37.7 and 56.6 Å, respectively. According to the high angle reflections, the hydrocarbon chains are stiff and the organization of the chains of type p6: it can be presumed that the chains are interdigitated in the

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

thinner phase*. The last two samples (20/1, 25/1) contain only one lamellar phase Lβ with spacing 56.7 Å and thus with non-interdigitating chains. The presence of sharp reflections at 4.12, 2.4 and 2.09 $Å^{-1}$ show that the chains are stiff and parallel, oriented at a right-angle to the plane of the lamellae [12] and organized according to the two-dimensional hexagonal lattice, type p6; this phase is similar to the one observed with rac-DPPG–NH_4 [13].

3.2. *Hydrated rac-DPPG–PXB*

The 5 samples, roughly at the same water concentration (see fig.1,2), can be sorted in two classes. With the two lowest rac-DPPG–PXB ratios (5/1 and 7/1) a lamellar phase Lα is observed at high temperature (41°C and fig.1). Between 41–0°C, the lamellar phase present is of type β [12] with stiff

* The lamellar repeat distance, d = 37.7 Å, is exactly that observed for dry rac-DPPG–Ach sample [13]

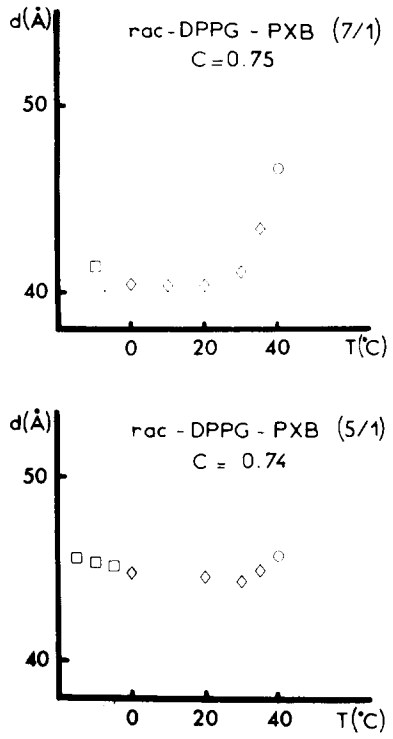

Fig. 1

Fig.1. Lamellar repeat distances, *d*, as a function of temperature. The symbols refer to the organization of the chains; liquid-like (○); two-dimensional lattice p6 (◆); two-dimensional lattice cmm (■). For the ordered conformation, open symbols indicate interdigitating chains.

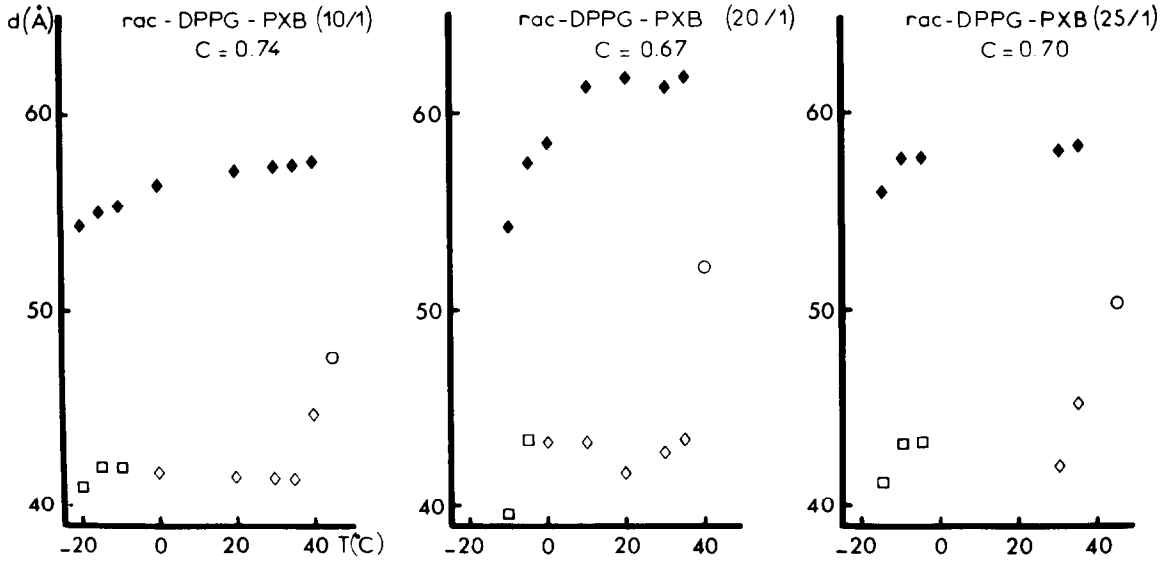

Fig.2. Lamellar repeat distances, *d*, as a function of the temperature. The symbols refer to the organization of the chains: liquid-like (○); two-dimensional lattice p6 (◆); two-dimensional lattice cmm (■). For the ordered conformations, open symbols indicate interdigitating chains, full symbols non-interdigitating chains.

interdigitated chains, organized according to the lattice type p6. At < 0–5°C, the packing became cmm-type (fig.1). At 45°C, the samples 10/1, 20/1 and 25/1 also contain a lamellar phase Lα (fig.2). Below 40°C, two lamellar phases are observed (fig.2) whose packing differs by ~ 20 Å. For the thinner phase, the chains are stiff and interdigitated. From 40–0°C the organization of the chains is of type p6 and of type cmm below – 5°C (down to – 15°C) (fig.2). The other lamellar phase also contains stiff and parallel chains: yet it is not easy to establish whether the chains are tilted with respect to the direction perpendicular to the lamellae, because the overlapping reflections prevents one checking whether the 4.1 $Å^{-1}$ reflection is sharp or diffuse [12].

4. DISCUSSION

The antibiotic polymyxin B, at molar ratios 5/1 and 7/1 has the effect of inducing interdigitation of the hydrocarbon chains in the lamellar phase of rac-DPPG. At higher polymyxin B content and below 41°C, two lamellar phases are present simultaneously; one with interdigitated chains, in which the rac-DPPG interacts with polymyxin B; the other, of type Lβ or Lβ′, apparently contains only rac-DPPG–NH_4 and water [13]. An inspection of the intensities of the reflections shows that the amount of interdigitated phase decreases with decreasing PXB content.

The observations reported here and in [13] can be discussed within the framework of the biological significance of the polymorphic transitions of lipid–water systems. This is a long-standing problem [14,15,21], which has been revived recently by some ^{31}P NMR and electron microscopic observations of intact membranes and extracted lipids [16–19]. The structural transition induced by choline, acetylcholine and polymyxin B, involve a remarkable decrease of the thickness of the hydrocarbon layer: since this is the insulating layer in membranes such shrinkage may be presumed to produce large permeability changes. Yet interdigitation has been observed so far under conditions which are unlikely to prevail in physiologically active membranes, namely with lipids containing two identical saturated chains and in phases with stiff chains. From the physiological standpoint, a more interesting possibility is that the primary effect of PXB (as well as choline and acetylcholine) is to increase the area/polar group of DPPG at the lipid–water interface, interdigitation being a peculiar effect of that area expansion, observed only with some lipids and at low temperature. Indeed, the area/polar group is a critical parameter for the stabilization of the different phases of lipids [20–22], and local alterations of that parameter are likely to induce local perturbations of the lipid bilayer (the hexagonal and the cubic phases are well documented examples of such perturbations) which in turn may upset permeability and other physiological properties of membranes.

ACKNOWLEDGEMENTS

We thank Dr V. Luzzati for many fruitful discussions and help in preparing this manuscript and J. C. Dedieu for help in the preparation of the figures.

REFERENCES

[1] Storm, D.R., Rosenthal, K.S. and Swanson, P.E. (1977) Annu. Rev. Biochem. 46, 723–763.
[2] Teuber, M. and Miller, I.R. (1977) Biochim. Biophys. Acta 467, 280–289.
[3] El Mashak, E.M. and Tocanne, J.F. (1980) Biochim. Biophys. Acta 596, 165–179.
[4] Rosenthal, K.S., Swanson, P.E. and Storm, D.R. (1976) Biochemistry 26, 5783–5792.
[5] Hartmann, W., Galla, H.J. and Sackmann, E. (1978) Biochim. Biophys. Acta 510, 124–139.
[6] Sixl, F. and Galla, H.J. (1979) Biochim. Biophys. Acta 557, 320–330.
[7] Galla, H.J. and Trudell, J.R. (1980) Biochim. Biophys. Acta 602, 522–530.
[8] Sixl, F. and Galla, H.J. (1980) Biochem. Biophys. Res. Commun. 94, 319–323.
[9] Sacré, M.M., Hoffman, W., Turner, M., Tocanne, J.F. and Chapman, D. (1979) Chem. Phys. Lipids 25, 69–83.
[10] Ranck, J.L., Keira, T. and Luzzati, V. (1977) Biochim. Biophys. Acta 488, 432–441.
[11] Ranck, J.L. (1980) Thesis, Doctorat d'Etat, Université Paris-Sud.
[12] Tardieu, A., Luzzati, V. and Reman, F.C. (1973) J. Mol. Biol. 75, 711–733.
[13] Ranck, J.L. and Tocanne, J.F. (1982) FEBS Lett. 143, 171–174.
[14] Luzzati, V. and Tardieu, A. (1974) Annu. Rev. Phys. Chem. 25, 79–94.

[15] Ranck, J.L., Mateu, L., Sadler, D.M., Tardieu, A., Gulik-Krzywicki, T. and Luzzati, V. (1974) J. Mol. Biol. 85, 249–277.
[16] Cullis, P.R. and de Kruyff, B. (1979) Biochim. Biophys. Acta 559, 399–420.
[17] De Kruyff, B., Cullis, P.R. and Verkleij, A.J. (1980) Trends Biochem. Sci. 5, 79–81.
[18] De Kruyff, B. and Cullis, P.R. (1980) Biochim. Biophys. Acta 601, 235–240.
[19] De Kruyff, B. and Cullis, P.R. (1980) Biochim. Biophys. Acta 602, 477–490.
[20] Luzzati, V. and Husson, F. (1962) J. Cell Biol. 12, 207–219.
[21] Luzzati, V. (1968) in: Biological Membranes (Chapman, D. ed) vol. 1, pp. 71–123, Academic Press, London, New York.
[22] Luzzati, V., Gulik-Krzywicki, T., Tardieu, A., Rivas, E. and Reiss-Husson F. (1969) in: Molecular Basis of Membranes Function (Testeson, D.C. ed) pp. 79–93, Prentice-Hall, Englewood Cliffs NJ.