Vol. 100, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 15, 1981 Pages 105-110

THE STATE OF THE LIPIDS IN THE PURPLE MEMBRANE OF HALOBACTERIUM CUTIRUBRUM AS SEEN BY ^{31}P NMR

Irena Ekiel*, Derek Marsh*[+], Barry W. Smallbone[‡], Morris Kates[‡] and Ian C.P. Smith*

Division of Biological Sciences*,
National Research Council of Canada,
Ottawa, Canada K1A 0R6

Department of Biochemistry[‡],
University of Ottawa,
Ottawa, Canada K1N 9B4

Received March 23,1981

SUMMARY

The purple membrane from Halobacterium cutirubrum and aqueous dispersions of its principal phospholipid, phosphatidylglycerophosphate, were studied by ^{31}P NMR at 121.5 MHz. The spectra manifest separate powder patterns for the mono- and di-esterified phosphate groups, the latter having the larger chemical shift anisotropy. Spectral simulation was used to derive accurate values of the chemical shift anisotropy. Contrary to some earlier reports, there is no evidence for a lipid phase transition in the membrane or the isolated lipids over the range 5 to 60°C; at all temperatures the lipid is in a bilayer arrangement. Combination with protein and neutral lipids in the purple membrane leads to reduced amplitude and rate of motion of the phosphate moieties of the major phospholipid.

INTRODUCTION

The purple membrane of the halophilic bacteria H. halobium and H. cutirubrum is presently the subject of intense interest due to its unique photoactivated proton pumping function and to its relatively simple chemical composition (1,2). Only one protein, bacteriorhodopsin, and one type of lipid alkyl chain, glycerol ether-linked phytanyl groups, are present,

[+]NRCC Visiting Scientist, 1980: permanent address, Max Planck Institute for Biophysical Chemistry, D-3400 Göttingen, West Germany. Supported in part by N.A.T.O. Grant RG 108.80 to D.M. and I.C.P.S. and by the Natural Sciences and Engineering Research Council (M.K. and B.W.S.).

0006-291X/81/090105-06$01.00/0

although there are several lipid head group classes represented (3). There are conflicting reports in the literature with respect to a phase transition of the membrane lipids over the temperature range 5 to 60°C (4-9). We have therefore sought to resolve this question using ^{31}P NMR of the intact membrane and of the isolated major phospholipid, phosphatidylglycerophosphate. The spectra are of a novel complex type. The absence of a discontinuity in their temperature dependence demonstrates the lack of a lipid phase transition. The spectral lineshapes indicate a reduction of lipid mobility on incorporation into the membrane, and a bilayer state of the lipids under all conditions.

MATERIALS AND METHODS

Purple membrane (PM), and the phosphatidylglycerophosphate (PGP) therefrom, were prepared as described previously (10,11). PGP was studied in excess water after dispersing 50 mg in 1.5 ml water by vortex mixing. ^{31}P NMR spectra were obtained from slurries of centrifuged membranes on a Bruker CXP-300 spectrometer at 121.5 MHz, using a solenoid detection coil and high power ^{1}H decoupling. The decoupler was cycled to avoid sample heating. Computer simulations of the powder spectra were made according to the formulation of Seelig (12), with a variable angular-dependent linewidth. Calorimetry was performed on a high sensitivity Microcal MC-1 instrument on aqueous membrane dispersions of 5 mg/ml.

RESULTS AND DISCUSSION

The 121.5 MHz ^{31}P NMR spectrum of the purple membrane at 15°C is shown in the upper trace of Figure 1. It has a peculiar shape, and at low signal-to-noise ratio could be incorrectly attributed to the presence of lipid in a hexagonal phase. However, since the major phospholipid is PGP (85% of total phospholipid, ref. 3) which contains a phosphate monoester as well as a diester, the shape could be due to contributions with different effective chemical shift anisotropies (CSA) from the two types of phosphate moiety in a bilayer arrangement. This is more clearly seen in the spectra of dispersions of PGP, Figure 2. This interpretation is substantiated by simulations of the spectra, lower traces of Figure 1, in which good agreement is obtained using CSA values of -18.5 ppm and -61 ppm for the mono- and diester species, respectively, in a bilayer arrangement. Attribution of CSA values of -40 to -60 ppm to the phosphodiester moiety is consistent with the values found for other such systems

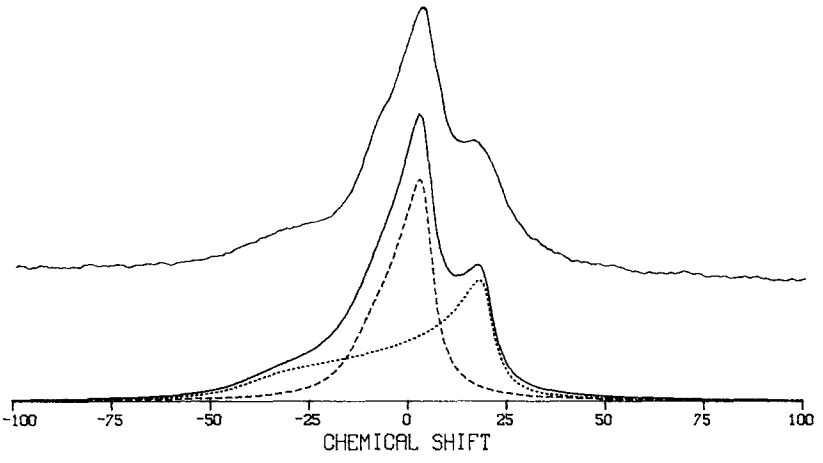

Figure 1. (Upper) ^{31}P NMR spectrum (121.5 MHz) of the purple membrane from Halobacterium cutirubrum obtained with high power ^{1}H decoupling, at 15°C; spectral width 125 kHz, acquisition time 6 ms, recycle time 1 s, pulse width 45°, decoupler on 4 μs before acquisition and during acquisition but off during remainder of cycle, Fourier transform of 16 K data points after zero filling. Chemical shifts are expressed with respect to that of 70% phosphoric acid in a sealed capillary.
(Lower) Computer simulation of the above in terms of two axially-symmetric powder patterns characterized by effective chemical shift anisotropies of -61 and -18.5 ppm; the angular-independent and -dependent linewidths were 300 and 200 Hz, and 350 and 250 Hz, for the phosphomonoester and phosphodiester, respectively. The broken curves are the powder patterns for the two types of phosphate ester present.

(12). The simulations also provide a more accurate measure of the effective CSA, which is difficult to obtain directly from the spectra. The temperature dependence of the PGP spectra is shown in Figure 2, and those of the derived CSA for PGP and the PM in Figure 3.

The shapes of the spectra for PGP and PM at all temperatures investigated are consistent with a lamellar arrangement of the lipids, and the CSA values are those expected for a liquid-crystalline state of the lipids (12). The temperature dependence of the CSA values for both PGP and the PM shows no evidence of discontinuities, Figure 3, arguing against the occurrence of a lipid phase transition over the range 5-60°C. The high sensitivity differential scanning calorimetry scans for both PGP and the PM over the range 5 to 70°C also show no evi-

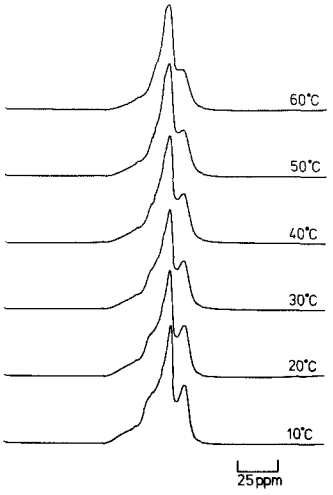

Figure 2. ^{31}P NMR spectra of phosphatidylglycerophosphate, isolated from the purple membrane, dispersed in excess water, at the indicated temperatures; spectral width 50 kHz, acquisition time 4 ms, recycle time 0.5 s, pulse width 45°, with continuous ^{1}H broad band decoupling, Fourier transform of 16 K data points after zero-filling.

dence for a lipid phase transition, in agreement with the earlier studies of Chen et al. (4) and Jackson and Sturtevant (7,8), but in disagreement with the conclusions of Degani et al. (9).

The effective CSA values for the PM are significantly larger than those for dispersions of PGP, the differences being of lesser magnitude for the phosphomonoester than for the diester. This indicates a smaller ampliture of motional averaging for the phosphate moieties in the presence of membrane protein and neutral lipids. In addition, the greater linewidths in the PM suggest slower motion.

The smaller effective CSA values for the phosphomonoester relative to those of the phosphodiester moiety in both PGP and the PM could arise from two sources. Firstly, the CSA tensors for phosphomonoesters have a lesser anisotropy in the rigid limit (12). Secondly, as the monoester is located at the periphery of the headgroup region of PGP, a greater amplitude of motional averaging might be expected. Further insight into the relative contributions of these two sources can be gained by consideration of the CSA reported for dipalmitoyl phosphatidylcholine (-47 ppm) and the order parameters for the phosphate moiety

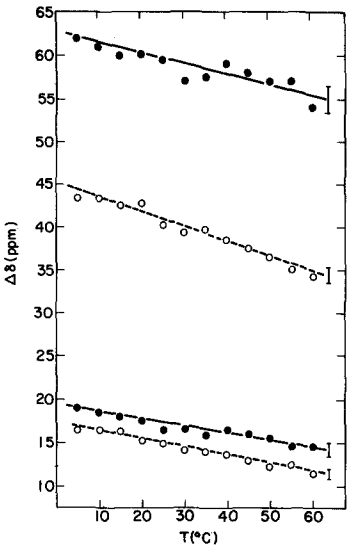

Figure 3. Temperature dependence of the effective ^{31}P chemical shift anisotropies ($\Delta\delta$) for the phosphodiester (upper data) and phosphomonoester (lower data) moieties in the purple membrane (solid circles) and phosphatidylglycerophosphate (open circles) in excess water. The anisotropies were taken from computer simulations of the spectra. The bars on the right sides of the curves are estimates of the precision of the measurements.

derived from the CSA and from the order parameters for other regions of the head group obtained by ^{2}H NMR (13). The similarity of the CSA for the phosphodiester moiety of PGP and the PM to that above suggests that a similar ordering tensor applies ($S_{11} = 0.12$, $S_{33} = -0.5$). If this were to apply also to the phosphomonoester, a CSA of -18 to -20 ppm would be expected. The observed value of -17 ppm agrees well with this, implying that the different effective CSA observed for the two phosphate moieties is mainly due to the smaller intrinsic CSA of the phosphomonoester. Confirmation of this could be made by studies of specifically-deuterated PGP.

CONCLUSION

The present ^{31}P NMR and calorimetry data show no evidence for a lipid chain phase transition in either PGP or the PM. The greater CSA values and linewidths in the ^{31}P NMR spectra of the PM suggest a lesser amplitude and slower rate

of motion due to interaction of PGP with bacteriorhodopsin and the neutral lipids of the PM. Under all conditions used here the lipids are in a bilayer arrangement.

ACKNOWLEDGMENT

We are grateful to Mrs. A. Joyce for the calorimetric data, to Mr. M.G. Taylor for advice on the use of his ^{31}P powder pattern simulation program, and to Mrs. P. Fejer for culturing the halophilic bacteria.

BIBLIOGRAPHY

1. Stoeckenius, W. (1980) Acc. Chem. Res. 13, 337-344.
2. Stoeckenius, W., Lozier, R.H. and Bogomolni (1979) Biochim. Biophys. Acta 505, 215-278.
3. Kates, M. and Kushwaha, S.C. (1978) in Energetics and Structure of Halophilic Organisms, S.R. Caplan and M. Ginzburg, eds., Elsevier/North Holland, Amsterdam, pp. 461-480.
4. Chen, J.S., Barton, P.G., Brown, D. and Kates, M. (1974) Biochim. Biophys. Acta 352, 202-217.
5. Plachy, W.Z., Lanyi, J.K. and Kates, M. (1974) Biochemistry 13, 4908-4913.
6. Lanyi, J.K., Plachy, W.Z. and Kates, M. (1974) Biochemistry 13, 4914-4920.
7. Jackson, M.B. and Sturtevant, J.M. (1978) Biochemistry 17, 911-915.
8. Jackson, M.B. and Sturtevant, J.M. (1978) Biochemistry 17, 4470-4474.
9. Degani, H., Bach, D., Danon, A., Garty, H., Eisenbach, M. and Caplan, S.R. (1978) in Energetics and Structure of Halophilic Organisms, S.R. Caplan and M. Ginzburg, eds., Elsevier/North Holland, Amsterdam, pp. 225-232.
10. Kushwaha, S.C., Kates, M. and Martin, W.G. (1975) Can. J. Biochem. 53, 284-292.
11. Kates, M., Yengoyan, L.S. and Sastry, P.S. (1965) Biochim. Biophys. Acta 98, 252-268.
12. Seelig, J. (1978) Biochim. Biophys. Acta 515, 105-140.
13. Seelig, J. and Gally, H.-U. (1976) Biochemistry 15, 5199-5204.