Biochimica et Biophysica Acta, 641 (1981) 281—285

BBA Report

BBA 71500

LOCATION AND EFFECT OF PROCAINE ON LECITHIN/CHOLESTEROL MEMBRANES USING X-RAY DIFFRACTION METHODS

H.G.L. COSTER [a], V.J. JAMES [a], C. BERTHET [b] and A. MILLER [b]

[a] *Biophysics Laboratory, School of Physics, University of New South Wales, Kensington, N.S.W. 2033 (Australia) and* [b] *European Molecular Biology Laboratory, C.E.N.G., L.M.A. 85X, 38041 Grenoble Cedex (France)*

(Received August 4th, 1980)
(Revised manuscript received November 3rd, 1980)

Key words: Anesthetic; Lipid bilayer; Procaine; X-ray diffraction; Lecithin/cholesterol multilayer

Summary

X-ray diffraction studies were made on lecithin/cholesterol multilayers with very high water content and containing the local anaesthetic procaine. Narrow-angle diffraction experiments show that the procaine molecules are located with the uncharged aromatic amine group approx. 10 Å from the centre of the bilayer. The polar tertiary amine group of these molecules is almost certainly located in the polar head-group region of the membrane. Wide-angle diffraction experiments show that the incorporation of procaine molecules into such lipid membranes produces an approx. 30% increase in the spread of acyl chain separation, although the average spacing between the chains is slightly reduced.

X-ray studies were performed with (egg) lecithin/cholesterol multilayers prepared with excess water containing KCl electrolyte to a concentration of 10^{-3} M. The molecular ratio of the components in the multilayer preparations used were lecithin : cholesterol : water, 2 : 1 : 50. The large water content (with added KCl) was used to obtain a membrane system as least nominally resembling physiological conditions. It is known [1—3] that electrolyte concentration can strongly modulate (even reverse!) the effects of anaesthetic molecules (such as benzyl alcohol) on the dielectric substructure of bimolecular lipid membranes.

The effect of procaine was studied by adding this substance to the electrolyte at a concentration of $2 \cdot 10^{-6}$ M prior to mixing the electrolyte solution

with the lipids. This corresponds to a procaine/lecithin ratio of 10^{-6}. Disoriented multilayers of the lipid membranes were prepared by mechanically masticating the mixture.

To prevent drying-out or oxidation, the sample was mounted in a sealed cell which had mylar windows to allow the passage of X-rays through the sample. The X-ray camera was a mirror-monochromator [4] which isolated the $CuK\alpha_1$ wavelength with the best focussing in the meridional direction. The X-ray source was an Elliott rotating-anode generator GX20 (used with a helium-filled camera which incorporated the possibility of an accurately calibrated variable sample-film distance). Three diffraction patterns were obtained for each sample and the experiments were repeated with four samples to check consistency of the intensity patterns. The intensities were measured using an LC position-sensitive detector (designed and built by A. Gabriel at the E.M.B.L.) mounted in the meridional direction.

The intensities were scaled so that the adjusted intensity of the direct, unscattered, beam measured through a central pinhole after passing through the sample was the same for all samples. After scaling in this fashion the varia-

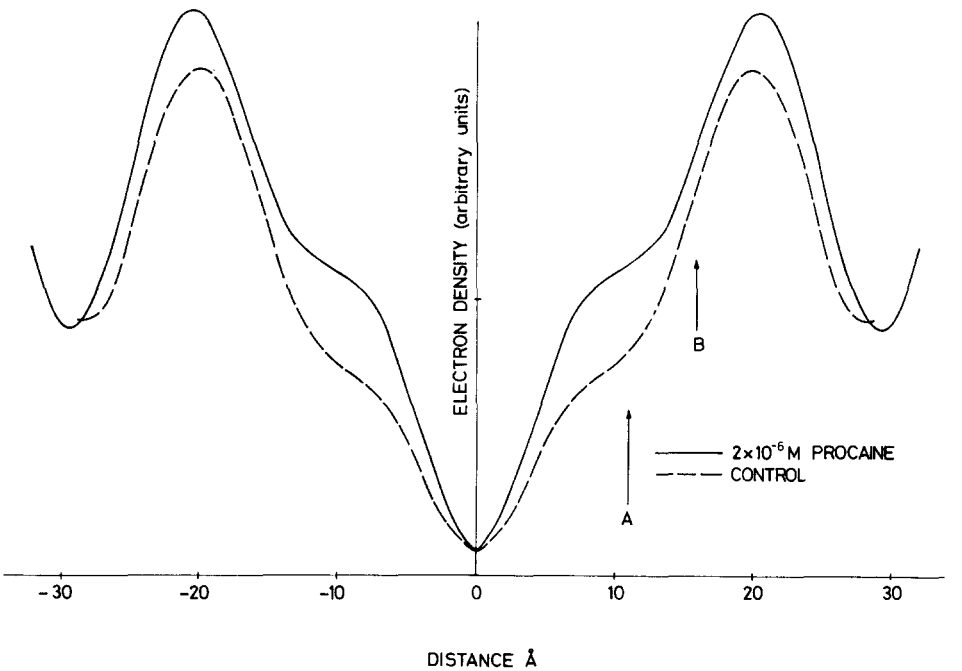

Fig. 1. Electron-density profiles for lecithin/cholesterol membranes prepared with excess 10^{-3} M electrolyte solutions with procaine at $2 \cdot 10^{-6}$ M (———) and without procaine (- - - - -). The relative intensities for the two systems shown were scaled so that the central (unscattered) X-ray beam after passing through the sample had the same (adjusted) intensity. The phases for the four orders used to generate these profiles were — — + —. The inclusion of procaine molecules led to large changes in the hydrocarbon region of the membranes (arrow A). Arrow B indicates the boundary of the hydrophobic region determined by subtraction of the phosphate-to-carbonyl (C_α) group distance (estimated by Büldt et al. [7] in neutron diffraction studies of dipalmitoyl phosphatidylcholine membranes) from the position of the high-density phosphate peaks in the profiles. This also corresponds closely to the point of inflection in the electron-density profiles in that region.

tions in the intensities (for each of the four diffraction orders) in the different samples were all less than ±2%.

The electron-density structures of the lipid membranes with an without procaine, reconstructed from the diffraction pattern using the diffraction lines up to four orders, are shown in Fig. 1.

The phases assigned were those obtained using a very basic block model of the membrane and correspond to those previously published by others (e.g., see Ref. 5). With these phases the plot of the structure factors as a function of the reciprocal Bragg spacing yielded a smooth curve without extraneous or unexpected peaks incompatible with the known morphology of these membranes. It is clear that the effect of procaine is to enhance the electron-density profile, very markedly, in the region (indicated by arrow A (in Fig. 1) between 7 and 14 Å from the centre of the bilayer.

The mean ratio of lecithin to total procaine added to the sample was 10^6 : 1 (the ratio must be in excess of this in the lipid membranes themselves as a finite amount of the anaesthetic must remain in the aqueous phase). Differences in the mean electron densities of procaine and lipids alone are therefore unlikely to affect significantly the mean electron densities of the bilayers. The latter, of course, could be affected by modulation of the membrane structure induced by procaine.

The lipid bilayer membrane is known to consist of a hydrophobic region sandwiched between two more hydrophilic, polar regions. The precise delimiting boundaries of these regions are difficult to define. In the corresponding electron-density profiles of such membranes one could for simplicity (although perhaps a little arbitrarily) define the boundaries of the central hydrophobic region to be at points of inflection in the electron-density profile leading into the polar head-group region.

This yields a value of 32 Å for the thickness of the hydrophobic region. Alternatively, we could make use of the values determined by Büldt et al. [7] of the distance between the carbonyl region (C_α) and the phosphate group in dipalmitoyl phosphatidylcholine membranes above their transition temperature (using neutron diffraction). Assuming that the peak intensities in the electron-density profiles of the membranes correspond to the high-density phosphate groups, it is then possible to calculate the boundary of the hydrocarbon region. This was the method adopted by McIntosh et al. [8] and with our profiles yields values for the thickness of the hydrocarbon regions of 33 Å for the control membranes and 34 Å for the membranes with $2 \cdot 10^{-6}$ M procaine.

Both methods of estimating the thickness of the hydrocarbon regions yield similar values, although both are a little larger than the value of 27 Å determined from very low frequency capacitance dispersion measurements [1,3,6] on single planar bimolecular lipid membranes with this lecithin/cholesterol ratio in 10^{-3} M KCl electrolyte.

The present study shows that $2 \cdot 10^{-6}$ M procaine with 10^{-3} M KCl tends to increase slightly the thickness of the hydrophobic region. Similar results (i.e., about a 2 Å increase in thickness on addition of procaine) were obtained with dielectric measurements made in this laboratory [3] on single planar bimolecular lipid membranes of this composition in 10^{-3} M KCl.

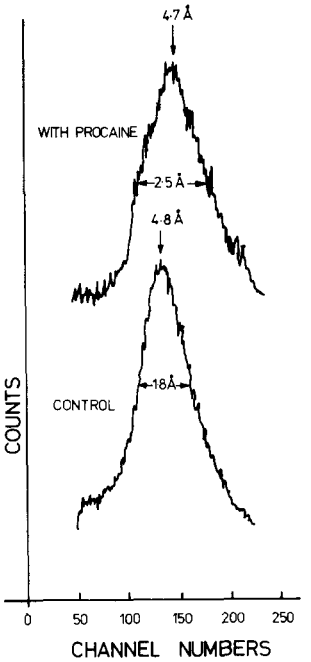

Fig. 2. The effect of procaine at $2 \cdot 10^{-6}$ M on the wide-angle X-ray diffraction patterns arising from the acyl chains of the lecithin/cholesterol bilayer lipid membranes. Procaine slightly reduced the average spacing but increased markedly the spread in the spacing; the latter, deduced at the half-intensity points, is indicated on the profiles. In the horizontal scale (channel numbers), 50 channels ≡ 0.1133 units of sin 2θ (where θ is the diffraction angle).

The wide-angle diffraction patterns with and without procaine, which arise from the acyl chains, are shown in Fig. 2. The effect of procaine is to reduce very slightly the average (projected) Bragg spacing for the chains *. (from 4.8 to 4.7 Å) and also to increase the relative spread (determined at the half-intensity points in the peaks) by approx. 30%. It would thus appear that in the presence of procaine the dynamic range of motion of the acyl chains of the lipids is enhanced (or the chains are more disordered).

The procaine molecule has a polar (tertiary amine) group at one end. This group is almost certainly located in the polar head-group region of the membrane. The non-polar part of the molecule would penetrate into the bilayer interior. This would place the aromatic amine group of 9—11 Å from the centre of the lipid bilayer membranes (about 5—7 Å into the hydrophobic phase from the polar head-group region).

Indeed, this is precisely the region where procaine most strongly affects the electron-density structure of the lipid bilayers.

---

* The Bragg spacing of 4.8 Å for the acyl chains in the control sample is somewhat larger than that frequently reported in the literature for lecithin liposomes. This could be due to systematic errors in the calibration of the position-sensitive detector at the wider angles, although checks made on the calibration using the position of the fourth-order diffraction revealed no such error. Our system is that of lecithin/cholesterol and it may also be that the presence of KCl changes the packing of the acyl chains from that usually seen in samples with no electrolyte.

Preliminary experiments in this laboratory using neutron diffraction methods with deuterated procaine also confirm the results presented here [9].

We are grateful to E.M.B.L., Grenoble, for the use of their facilities. We also thank J. Sedita, A. Gabriel, J.M. Bois, D. Tochetti and the members of the E.M.B.L. Biochemistry group for help with camera construction, detector, electronics, data collection and sample preparation, respectively.

References

1 Coster, H.G.L., Laver, D.R. and Smith, J.R. (1980) in Bioelectrochemistry (Kyzer, H. and Gutman, F., eds.), pp. 331—352, Plenum Press, New York
2 Ashcroft, R.G., Coster, H.G.L., Laver, D.R. and Smith, J.R. (1980) Biophys. J., submitted
3 Ashcroft, R.G. (1979) Ph.D. Thesis, University of New South Wales
4 Huxley, H.E. and Brown, W.J. (1967) J. Mol. Biol. 30, 383—434
5 Torbet, J. and Wilkins, M.H.F. (1976) J. Theor. Biol. 62, 447—458
6 Coster, H.G.L. and Smith, J.R. (1974) Biochim. Biophys. Acta 373, 151—164
7 Büldt, G., Gally, H.U., Seelig, A., Seelig, J. and Zaccai, G. (1978) Nature 271, 182—184
8 McIntosh, T.J., Simon, S.A. and MacDonald, R.C. (1980) Biochim. Biophys. Acta 597, 445—463
9 Coster, H.G.L., James, V.J. and Knott, R.B. (1979) Aust. Soc. Biophys. 3, 5A

ANNOUNCEMENT — REFERENCE LIST

References should be brought together at the end of a paper, under the heading References. The names of all authors must be given. **The numbering of the references should be in order of citation in the text, not in alphabetical order.** The list of references should always be given on a separate sheet of the typescript, **double- or triple-spaced** throughout, and should be clearly distinguishable from footnotes. Authors are responsible for the accuracy of their references and should check whether every reference in the text appears in the list of references and vice versa.

Biochimica et Biophysica Acta implements the recommendations of the Committee of Editors of Biochemical Journals of the International Union of Biochemistry concerning presentation of reference lists. The recommended styles for citation of journals [1] (abbreviated according to the Bibliographic Guide for Editors and Authors *), books [2] and multi-author books [3] are given in the following examples:

1 Pispa, J.P. and Buchanan, J.M. (1971) Biochim. Biophys. Acta 247, 181—184
2 Dixon, M. and Webb, E.C. (1964) Enzymes, 2nd edn., pp. 565—567, Longmans Green, London
3 Brachet, J. (1967) in Comprehensive Biochemistry (Florkin, M. and Stotz, E.H., eds.), Vol. 28, pp. 23—54, Elsevier, Amsterdam

Note that first and last page numbers are to be included in the reference list.

Unpublished work and work in preparation should not be listed in the References but should be mentioned in the text as follows: (Spector, R., Jr. and Glimcher, J., unpublished data). Reference to a '**private** communication' should not be made. A '**personal** communication' should be referred to only when permission of the person who made the personal communication is obtained; it should be mentioned in the text, not in the list of references.

Reference to a paper as 'in the press' implies that it has been accepted for publication and therefore evidence (such as a photocopy of the notice of acceptance from the journal concerned) should accompany the submitted typescript. The year quoted for such a reference should be the best estimate, revised, if necessary, at proof stage; it should be included in the list of references, the title of the journal being followed by the words 'in the press'; for example:

4 Smith, J.P. and Jones, J.E. (1979) J. Biol. Chem., in the press

Further detailed information on reference lists and the preparation of papers for publication in Biochimica et Biophysica Acta can be found in Biochim. Biophys. Acta 582, 1—20 and in the revised (1979) edition of the brochure *Information for Contributors to Biochimica et Biophysica Acta*, which is available free of charge from the BBA Editorial Secretariat (P.O. Box 1345, 1000 BH Amsterdam, The Netherlands).

* Bibliographic Support Division, Chemical Abstracts Service, The Ohio State University, Columbus, OH 43210, U.S.A.

ANNOUNCEMENT

KEY-WORD SYSTEM OF INDEXING

Authors are requested to select a maximum of six key words (or short phrases) and include them with the submitted typescript. These will be used in the compilation of the annual cumulative index and will be printed on the title page of the published article. (Authors should note that American spelling will be used.)

Important factors of the investigation as a whole should be selected as key words and general terms like enzyme, rat, spectroscopy, etc. should be avoided. In some cases, the general term can be used when qualified; e.g. synaptic membrane, salicylate transport, etc. The key words should be chosen so that the combination of these (as they will appear in the published index) will give the reader sufficient direct information as to the relevance of a given article to his/her particular field.

Example:
Title: Oestrogen-induced synthesis of riboflavin-binding protein in immature chicks. Kinetics and hormonal specificity

Key words: Riboflavin-binding protein; Estrogen induction; Hormonal specificity; Clomiphene; (Immature chick)

The submitted list may be amended by the editorial office to ensure that index entries are consistent throughout the cumulative index. If the list appears with words in parentheses, this indicates that these words are appropriate to the article, but are not of sufficient indexing value; these words will not appear as individual index entries, but will be printed each time a key-word index entry appears.