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EFFECT OF $^2H_2O/H_2O$ REPLACEMENT ON THE DIELECTRIC STRUCTURE OF LIPID BILAYER MEMBRANES

H.G.L. COSTER, D.R. LAVER and B.P. SCHOENBORN *

Biophysics Laboratory, School of Physics, University of N.S.W., Sydney, NSW 2033 (Australia)

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Dielectric dispersion measurements were made on planar egg phosphatidylcholine and egg phosphatidylcholine/cholesterol bilayer membranes in H_2O and ^2H_2O electrolyte solutions. The properties of the dielectrically distinct substructural layers of these membranes, which can be characterised by this technique, were found to be insensitive to replacement of H_2O by ^2H_2O.

Neutron diffraction studies of lipid membrane preparations have greatly enhanced our knowledge of the molecular organization in these systems. Such studies have to a large extent relied on replacement of H_2O by ^2H_2O both to 'phase' the various orders in the diffraction patterns and to determine the degree of penetration of water into the bilayer (see, for example, Refs. 1–5).

In this method it is assumed that replacement of H_2O by ^2H_2O in itself does not affect the membrane structure although there is ample evidence that the presence of ^2H_2O in a medium does affect, for instance, the growth of bacteria (see, for example, Ref. 6).

In order to validate this crucial assumption we have carried out experiments to determine the dielectric substructure of planar lipid bilayer membranes made in ^2H_2O and H_2O solutions of 1 mM KCl.

The dielectric substructure of a membrane gives rise to interfacial polarizations at the boundaries of the various substructural layers. This manifests a dispersion with frequency of the overall capacitance and conductance of the membrane [7]. In lipid membranes this impedance dispersion due to the presence of regions or layers with distinctly different dielectric properties occurs at very low frequencies (0.001 to 1000 Hz). We have used measurements of the dispersion in capacitance and conductance to characterize the dielectric parameters of substructural layers in membranes of egg phosphatidylcholine and egg phosphatidylcholine + cholesterol in 1 mM KCl solutions in ^2H_2O and H_2O. The method requires precision (phase angle $\pm 0.02°$, impedance $\pm 0.3\%$), four-terminal, measurements of the impedance and phase angle of the membranes using a low frequency digital impedance spectrometer described previously [7–9].

The membranes were generated by forming a film of the lipids, either phosphatidylcholine or 2 : 1 phosphatidylcholine/cholesterol (dissolved in *n*-hexadecane), across a 1–2 mm aperture in a polycarbonate septum dividing two chambers which contained 1 mM KCl in either ^2H_2O or H_2O. All measurements were made on membranes which (i) were 'black' over the entire area of the aperture, (ii) were at least 1 h old and (iii) whose dielectric properties had stabilised (< 1% drift per

* On leave from Department of Biology, Brookhaven National Laboratory, Upton, NY, 11973, U.S.A.

hour). The measurements were made at 30°C.

The membrane substructure can, in the first instance, be divided into three dielectrically distinct regions, viz. the hydrophobic region (containing the acyl chains), the two acetyl regions (containing the carboxyl groups) and the two polar-head regions. In the present study it was possible to distinguish a further partition of each of the two polar-head regions. These two parts will be referred to as the polar-head A and polar-head B regions. One of these has a conductance of approx. 10 S/m^2 (part A) while the other has a conductance of 100 S/m^2 (part B); their capacitances, however, are very similar and thus their electrical time constants therefore differ by an order of magnitude. An example of the variation of the capacitance of phosphatidylcholine/cholesterol membranes with frequency is shown in Fig. 1. The points marked are the experimental values; the vertical bars (often smaller than the size of the symbols plotted) indicate the standard error for five runs. The full curve is a plot of the theoretical variation of the capacitance with frequency for a membrane composed of a multilayer sandwich of materials of different dielectric properties (i.e. having different electrical time constants). The dispersion in capacitance with frequency in this case arises from polarization effects at the interfaces of the dielectrically different layers (a Maxwell-Wagner dispersion).

The separate capacitances of various layers or regions of phosphatidylcholine membranes, derived from dispersion curves such as that shown in Fig. 1 (and the corresponding dispersion in the membrane conductance) in both ^2H_2O and H_2O solutions are shown in Fig. 2. It is immediately clear that the dielectric parameters of these two systems are very similar.

The corresponding substructural parameters for phosphatidylcholine/cholesterol membranes are given in Fig. 3. Again the parameters for the various layers in ^2H_2O and H_2O solutions are very similar. The only difference between the ^2H_2O and H_2O systems is a very slight and probably insignificant decrease in the value of the capacitance of one part (B) of each of the two polar-head regions in ^2H_2O. This part of the polar-head region is the one with the highest conductance (94 ± 12 S/m^2

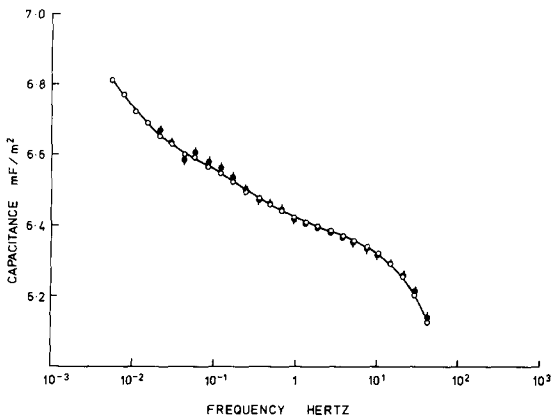

Fig. 1. The variation of the capacitance of phosphatidylcholine/cholesterol bi-molecular membranes with frequency at low frequencies in 1 mM KCl electrolyte solutions of H_2O (○) and ^2H_2O (●). The vertical bars (sometimes not discernible) represent the standard errors for five runs. The full curve is the theoretical variation of capacitance with frequency for a multilayer sandwich membrane composed of dielectrically different substructural layers. The fitting of this theoretical curve to the experimental data (capacitance and conductance vs. frequency) allows a determination of the capacitances and conductances of the individual substructural regions (see Figs. 2 and 3).

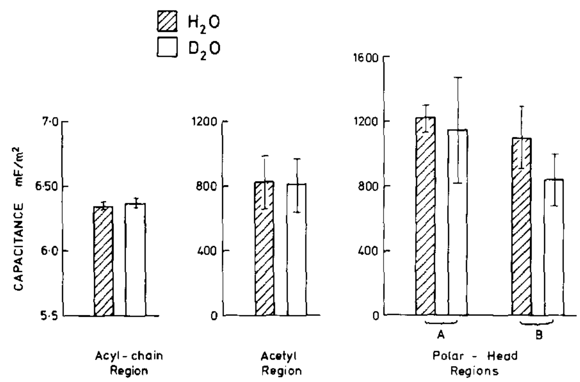

Fig. 2. The capacitances of the individual, dielectrically distinct, substructural layers of planar phosphatidylcholine bilayer membranes in 1 mM KCl solutions of H_2O and ^2H_2O. Two separate regions (designated 'A' and 'B') with electrical time constants which differed by an order of magnitude) could be distinguished in each of the polar-head regions, in addition to the central acyl-chain region and the two acetyl regions. The vertical bars indicate standard errors for an average of four runs on each of four or five separate membranes. It is clear that within the experimental variations the substructure in H_2O and ^2H_2O is essentially the same.

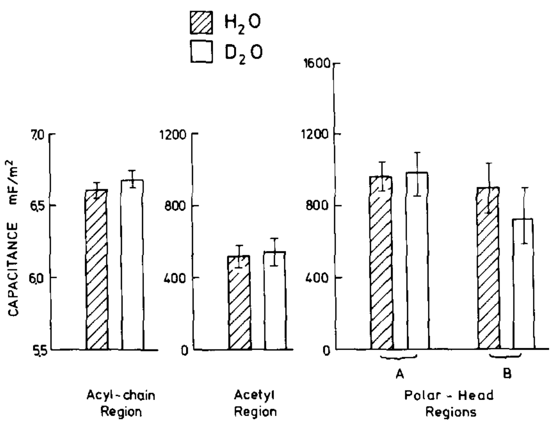

Fig. 3. The capacitances of the substructural layers in phosphatidylcholine/cholesterol bilayers in 1 mM KCl solutions of H_2O and ^2H_2O. As for the phosphatidylcholine bilayers, the dielectric substructure for both the H_2O and ^2H_2O systems are very similar. The only difference that can be discerned at all (but is probably not significant) is a slightly decreased value of the capacitance of the polar-head B region in ^2H_2O. This region has a time constant of approx. one-tenth that of region A. Note that cholesterol normally gives rise to a small increase in the capacitance of this region in H_2O (cf. Fig. 1).

for region A vs. 9.5 ± 1.4 S/m^2 for region B). It should be noted that the capacitance of this region is normally somewhat increased by the addition of cholesterol (cf. Figs. 2 and 3). The very slight difference in the second polar-head capacitance in the ^2H_2O and H_2O systems, however, is not associated with any significant changes in the conductance of this layer (94 ± 12 S/m^2 in H_2O vs. 72 ± 14 S/m^2 in ^2H_2O).

There appeared to be no change at all in that part of the polar-head region (A) which had the lower conductance when ^2H_2O was substituted for H_2O.

The general conclusion reached from the present study is that replacement of H_2O by ^2H_2O has essentially no effect on the dielectric substructure of phosphatidylcholine and also phosphatidylcholine/cholesterol membranes and should not affect the fidelity of the structure determined by neutron diffraction methods.

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