CATION-INDUCED ORGANIZATION CHANGES IN A LIPID BILAYER MODEL MEMBRANE*

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SUMMARY Cation-induced changes in organization of the lipids in a bilayer model membrane were monitored using a spin label probe. The degree of organization of the lipids was found to be dependent on the concentration and valence of the cation as well as upon the presence of a net negative charge on the lipids. These results suggest that the influence of inorganic cations on biological membranes can take place via ion-induced organizational changes in the lipids.

Ions are known to have profound effects on biological membranes. In general, the molecular basis for these effects is obscure. The present communication indicates that inorganic cations can cause organization changes in a model membrane which consists of a stack of polar lipid bilayers, supporting thereby the view that similar changes may be important in biological systems.

The multibilayers were prepared by using a stream of wet nitrogen to evaporate a chloroform solution of lipids and spin label in a flat, quartz ESR cell, and drying the resultant film in a vacuum for two hours. Hydration was then carried out by keeping the lipid film in contact with the aqueous phase for a minimum of 20 minutes at 20-23°C. Unless stated otherwise, all ESR spectra were obtained after draining the cell. It has already

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been shown by X-ray diffraction (1,2) and spin label (3,4) studies that under appropriate conditions, notably after hydration, the lipids appear to order themselves into a stack of bimolecular lipid lamellae.

The sensitivity of the present technique is determined by the dependence of the nitroxide spin label ESR spectrum on the angles between the spin label and the applied magnetic field. Thus, if the phospholipid films are ordered, and if the spin label is constrained by the organized phospholipids so as to reflect this order, the separation between the hyperfine lines of the spin label ESR spectrum will depend upon the angle between the plane of the film and the applied magnetic field (3,4). The higher the degree of order, the greater will be the angular dependence. For perfect order one would expect maximum and minimum hyperfine splittings of 32 and 6 gauss, respectively, when the magnetic field is perpendicular and parallel to the plane of the lipid film*. If the cholestane spin label (Fig.1) undergoes rapid motion about its long axis the expected splittings would be 19 and 6 gauss, respectively (4). The rigidity of the steroid backbone of the cholestane spin label makes it an excellent monitor of the state of the phospholipid chains. The ESR spectra reported here were

Figure 1 Cholestane spin label (3-spiro-[2'-(N-oxyl-4,4'-dimethyl-oxazolidine]-cholestane).

* A detailed discussion of this phenomenon is given in a recent review (5).

obtained on a Varian E3 spectrometer with the magnetic field perpendicular and parallel to the plane of the lipid film.

Figure 2A is an example of the spectrum given by a lipid film where the spin label is in a highly-ordered state. The film was made using fraction 1 of the lipids of the white matter of beef brain (6) and was hydrated using 0.05 M NaCl. The hyperfine splitting constants (distance between the peaks) when the film is perpendicular and parallel to the magnetic field are 6.5 and 19 gauss, respectively, indicating that the labels are preferentially oriented with their long steroid axes perpendicular to the plane of the film (4), and with rapid rotation (> 73 MHz) occurring about this axis. This suggests that the lipids are in a lamellar phase with their long axes perpendicular to the planes of the lamellae, and that these lamellar planes are parallel to the surface on which they are supported. This is in accord with the results of X-ray studies which indicate that phospholipids in the presence of excess water usually form bimolecular lipid lamellae (7).

When the films were hydrated using lower concentrations of NaCl less spectral anisotropy (hence less molecular ordering) was apparent, as demonstrated by the smaller difference between the spectra obtained in parallel and perpendicular orientations (Fig.2B). The degree of ESR spectral anisotropy, measured as the ratio of the amplitudes of the peaks labelled D and E in Figure 2, varied with concentration and charge of cation as shown in Figure 3. The effectiveness of cations in increasing spectral anisotropy depended on charge and may be summarized as $Na^{+}=K^{+}=Li^{+}<Mg^{2+}=Ca^{2+}<La^{3+}<Th^{4+}$. Anions do not play a major role since identical degrees of anisotropy were produced by solutions of NaCl, NaCNS, Na_2SO_4, KCl and K_2CrO_4 of the same, low normality (1-50 mN). Also the effect is not due to osmotic phenomena since exposing a film hydrated with

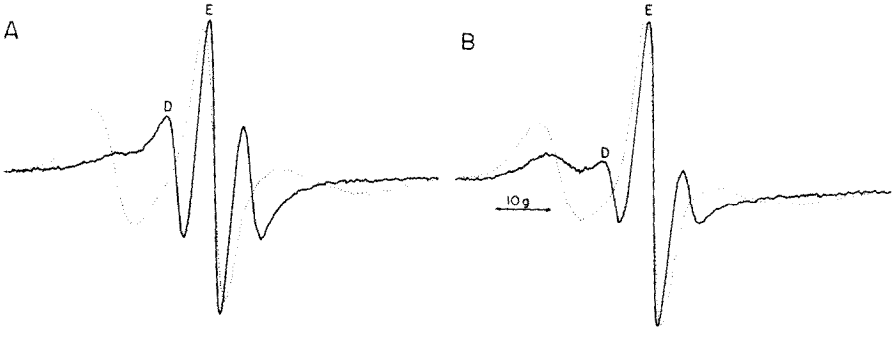

Figure 2 ESR spectra of cholestane spin label in film of bovine brain white matter lipids. A - film hydrated with 50 mM NaCl. B - film hydrated with 5 mM NaCl. Solid lines: film perpendicular to magnetic field. Dotted lines: film parallel to magnetic field.

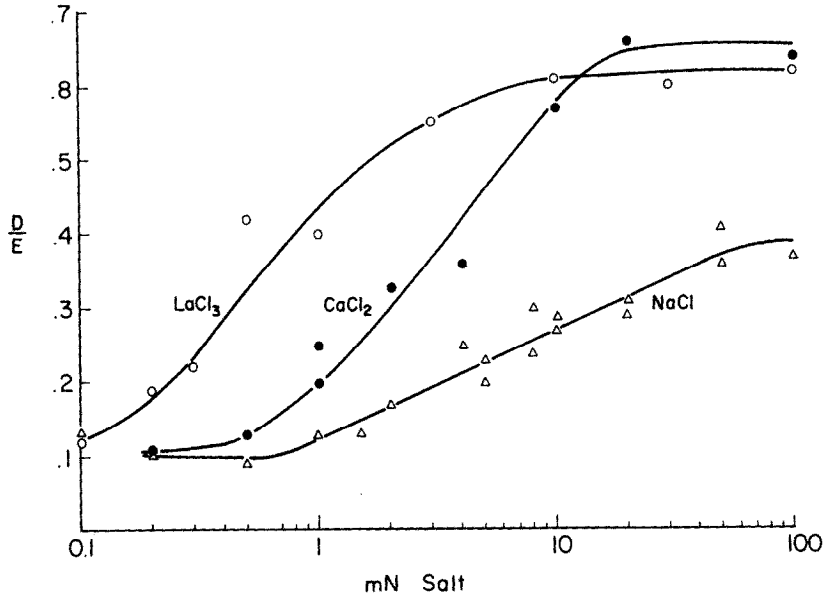

Figure 3 Variation of ordering of the cholestane spin label as a function of cation charge and concentration. The amplitude ratio D/E is used as a measure of anisotropy. Films made using bovine brain white matter lipids.

LiCl to an equiosmolar solution of $CaCl_2$ (6 and 12 ideal millios-molar) increased spectral anisotropy and vice versa. Furthermore, hydrating the films with 0.10 M sucrose did not cause greater anisotropy than distilled water.

The role of lipid charge in producing ESR spectral anisotropy was demonstrated in experiments using egg lecithin. Chromatographically pure egg lecithin has no net charge at the pH of the experiments (5.7-6.8) (8). The angular dependence of ESR spectra of films formed using this material plus 25 mole per cent cholesterol were independent of the presence of ions. However, such sensitivity appeared when dicetyl phosphate (K & K Laboratories, Plainview, New York), (10 mole percent) was included. Films formed from the brain lipid preparation would be expected to bear a net negative charge in solutions of low salt concentration since the preparation contains, in addition to cholesterol and other neutral lipids, acidic lipids such as phosphatidyl serine, cardiolipin and sulfatides (6).

Under physiological conditions, the concentration of divalent cations ($\sim$ 3 mM) is always lower than that of monovalent cations ($\sim$ 140 mM). Therefore it was of interest to see if the degree of ordering produced by a concentration of cations close to the physiological value would be increased if a small amount of Ca^{2+} or Mg^{2+} were added. Such increases were readily observable in experiments using brain lipids with 100 mM Na^+ plus or minus 2mM Ca^{2+}.

One interpretation of our data is the following. When hydrated with solutions of higher salt concentration the lipids form a stack of bimolecular leaflets oriented with their planes parallel to that of the surface on which they are formed. The polar lipids are oriented with their long axes perpendicular to the plane of the leaflets and the spin label, because of its

rigidity, is constrained in the same orientation. Cations interact with monolayers of anionic lipids to decrease the surface charge density; the effectiveness of a given concentration of cation in this respect increases with its charge (9). Therefore, the surface charge density of the lamellae increases with decreasing salt concentration and hence, the anionic residues of the lipids are forced apart and the film expands. This expansion, which has been shown to occur in phospholipid monolayers (9), relaxes the constraints on the included spin labels, allowing a lower degree of order to exist, and results in decreased spectral anisotropy.

A number of models for the intralamellar motions and organization of the lipids can be constructed to account for the variation in spectral anisotropy with expansion in the lamellar plane. At the present stage of development in the interpretation of the ESR spectra it is difficult to distinguish between the various possibilities. However, the salient point at present is that the spectra suggest that inorganic cations can affect the way in which lipids are organized in a lamellar structure. The significance of this finding is that it is consistent with postulates that ion-induced changes in membrane structure are crucial to some membrane processes (10,11). It is expected that subsequent studies using the oriented film technique will be able to shed additional light on the detailed nature of ion-induced lipid structural changes and their roles in biological as well as model systems where ions affect the parameters of membrane processes (e.g., references 12,13,14).

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