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Direct observation of the ion distribution between charged lipid membranes

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1. INTRODUCTION

Numerous investigations have demonstrated that the behavior of biological membranes is influenced by the electrostatic interactions between charged lipid head groups and ions in the adjacent water layer. There are several experimental difficulties which have hindered a quantitative experimental investigation of this problem since a direct observation of the electrostatic potential or the ion distribution has not been possible. In this study we tried to solve the problem by performing neutron diffraction experiments on dispersions of the negatively charged lipid DPPG (1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol) and water, containing the cation TMA^+ (tetramethylammonium), in which 12 hydrogens can be replaced by deuterons. The difference density profile of 2 samples with deuterated and undeuterated ions yields their distribution to a resolution of 10 Å. This method has the advantage of not introducing any change in the chemistry of electrostatic interactions into the system. Our results are a first direct experimental test on the more than 70-year-old Gouy-Chapman theory of the diffuse double layer [1].

2. MATERIALS AND METHODS

The experiments were performed on a multilamellar bilayer system which yields considerable resolution due to the one-dimensional periodicity. We attempted to have a rather high amount of water incorporated between the bilayers to reduce the electrostatic interaction of adjacent membranes and to observe the variation in the ion density of TMA over a large distance in the water layer. On the other hand, a high degree of swelling decreases the resolution in the diffraction pattern. Therefore, a compromise in both parameters was necessary. The sample preparation was as follows: unoriented samples were made from Fluka $DPPG^-$-NH_4^+ and 0.2 M TMA^+-Cl^- solution using 3 different mixtures of deuterated and nondeuterated TMA (100, 50, 0%). In a specially developed procedure, the 10-times diluted electrolyte was added to the lipid to allow for homogeneous swelling behavior, which does not easily happen, if the final concentration is used (as checked by X-ray diffraction). After several stages of water evaporation and mixing a final concentration of 46 wt% was selected, resulting in a bilayer spacing of 101 Å and 5 orders of lamellar diffraction. This method of 'post-swelling concentration' has been found to be a useful procedure to avoid the development of different periodicities and to establish the complete introduction of the electrolyte into the interlamellar space. For the purpose of a first experiment of this kind, a rather high electrolyte concentration was used in order to

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have significant differences between the spectra of the deuterated and non-deuterated preparations. A concentration of one TMA$^+$ per 8 lipids or 1.5 deuterium per lipid molecule was used.

Neutron diffraction was carried out at the Institute Laue-Langevin in Grenoble on the D16 diffractometer working on a monochromatic beam of 4.52 Å wavelength. Squared Lorentzians were fitted to the intensities of the 5 orders of diffraction to reduce errors from background noise and small overlaps. After correction by the common Lorentz-factor (h^2) for powder samples the structure amplitudes were derived as given in table 1. The sample with a mixture of 50% deuterated and undeuterated TMA was solely used to insure that no phase changes occurred dependent on deuteration [2]. To determine the scattering density profile of the centro-symmetric structure along the bilayer normal it is sufficient to determine the signs of the amplitudes. For symmetry reasons the 32 possibilities of assigning the 5 structure amplitudes reduce to 8. Knowing the density profile of similar lipid bilayers at a much higher resolution, it was easy to select the correct signs (table 1) by comparison of the Fourier transforms.

3. RESULTS AND DISCUSSION

Fig.1A presents the resulting neutron density profiles of the DPPG-electrolyte system for deuterated (broken line) and undeuterated (full line) ion preparations. The lipid region remains almost unchanged while in the electrolyte area the density is significantly higher for the deuterated ions. The difference of the 2 profiles, which is 5-times expanded in fig.1B, shows the TMA label distribution at a resolution of 10 Å. Several tests for the reliability of this result were made. The membrane thickness of DPPG can be derived from extrapolation of membrane spacing vs water content from X-ray studies [3] giving 57 Å thickness at zero water content including the cation. The precisely known water content of 46 wt% in our preparation fits well with our density profile. Furthermore, the distance between the maxima in the ionic label concentration at 10 Å resolution has been found from the difference Patterson function to be 38 Å using no phase information. This distance is identical to that in fig.1B. The X-ray wide-angle reflection at 42 Å indicates that the bilayers are in the $L_{\beta'}$ phase at room temperature. Knowing the thickness of the bilayer we can exclude the phenomena of interdigitation [4] of the paraffin chains under our experimental conditions.

Table 1

Experimental structure factors of lamellar reflections of DPPG $L_{\beta'}$ phase, 46 wt% electrolyte content, 101 Å spacing

h	$F(h)_{\text{deut}}$	$F(h)_{\text{nondeut}}$
1	+ 7.3 ± 0.2	+ 9.8 ± 0.2
2	− 22.2 ± 0.3	− 19.6 ± 0.3
3	− 15.0 ± 0.7	− 16.5 ± 0.8
4	+ 6.1 ± 0.4	+ 5.4 ± 0.4
5	+ 13.2 ± 0.9	+ 13.6 ± 1.0

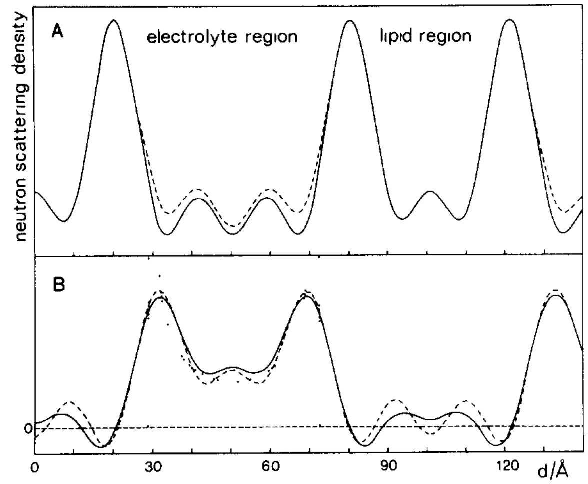

Fig.1. (A) Neutron scattering density profiles of DPPG$^-$-NH$_4^+$ with 0.2 M TMA$^+$-Cl$^-$: dispersion with (——) nondeuterated TMA, (– – –) deuterated TMA. (B) The difference of the profiles in A, 5-times enhanced (——); TMA distribution according to the exact solution of the Gouy-Chapman theory (···); Gouy-Chapman distribution calculated for the experimental resolution (– – –).

For a quantitative interpretation of the observed ion distribution the Gouy-Chapman double-layer theory would be an adequate first approximation. Therefore we have calculated the TMA distribution as predicted by the exact solution of this theory [5]. The parameters involved in this calculation are the following: From a cross-section area of 48 Å per DPPG molecule [3] a surface charge density of 0.34 C/m^2 was obtained. The dielectric constant of the water was assumed to be 80. Using these values the electric potential near the lipid surface decreases rapidly towards the centre of the water layer. Thus a modification of the theory due to an overlap was negligible. Finally, from the decay of this potential the ion distribution (dotted line, fig.1B) was determined. The observed and calculated distributions were scaled to the same area corresponding to the constant amount of TMA. The observed distribution is broadened due to the limited resolution. Therefore the Gouy-Chapman distribution should be plotted at the same resolution to obtain a direct comparison (broken line, fig.1B). In a fit of the first 5 orders of the observed and calculated structure factors a membrane thickness of 58 Å was found, in agreement with the value of Watts et al. [3].

Besides other assumptions [5] in the Gouy-Chapman theory, no variation of the dielectric constant in space is made. From data with higher resolution a profile of the dielectric permittivity would also be obtained. It is important to note that measurements of the dielectric constant by alternating external electric fields can only give an average value with respect to the direction of the applied field. Immobilized water does not contribute much to this value. Therefore a low dielectric constant has been determined from such measurements [6]. The dielectric constant which enters into the Gouy-Chapman theory and determines the observed ion distribution as well results from the material between charges which alters the strength of the Coulomb forces between them. Therefore, the dielectric constant as resulting from diffraction experiments yields information about the internal electric field near charges and is therefore sensitive to the microscopic structure between ions. Hence the value of 80 in the dielectric constant used in the Gouy-Chapman theory is not in conflict with much lower values obtained from dielectric measurement [6].

At 10 Å resolution the Gouy-Chapman theory fits quite well to the observed distribution. Obviously our resolution is too low to illuminate specific interactions in the so-called 'Stern layer' near the lipid head group region [7].

Nevertheless, the location of the highest ion concentration can be determined with an accuracy of ± 1.5 Å which is a very reliable value compared to other methods. Considering the influence of cations on integral membrane proteins and lipid properties, the distance of these maxima (across the membrane) defines a new thickness of biological importance.

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