Ion Diffusion Selectivity
in Lecithin-Water Lamellar Phases

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ABSTRACT The diffusion coefficients of Na⁺, Rb⁺, and Cl⁻ were determined in lecithin-water lamellar phases at 18°C as a function of phase hydration. Diffusion was measured within the phase with no transfer between phase and bulk aqueous medium. The relative diffusion coefficients of anion and cation depended strongly on phase hydration. At low water content, the diffusion coefficient of Cl⁻ was greater than that of Na⁺ or Rb⁺ whereas at high water content both cations diffused faster than the anion. The change in relative diffusion coefficient occurred at 0.24 g water/g phase (24% water). The possibility that a change in conformation of the lecithin polar head occurs at a phase water content of 24% is considered. The diffusion coefficients of all three ions decreased at the water content where the relative diffusion rates inverted. Freeze fracture and polarizing microscopy studies were carried out to obtain information on phase structure. The latter study indicated that a change in long-range organization of the phase occurred at 24% water. This change accounts for the decrease in the ion diffusion coefficients at this water content. The change in conformation of the choline phosphate group proposed as an explanation for the change in ion selectivity could lead to changes in long-range organization of the phase as a second order-effect.

INTRODUCTION

Ion transport across cell membranes, whether by carrier or through polar channels, probably involves the interaction of ions with polar regions of the membrane. Mesomorphic lipid-water phases provide a good model for the study of ion diffusion in a polar environment of known molecular structure. These systems permit the study of the diffusion of ions through an aqueous region in contact with polar groups. The dimensions of the aqueous region and the hydration of the polar groups can be varied by changing the phase water content. In this paper we present the results of measurements of the diffusion coefficients of Na⁺, Rb⁺, and Cl⁻ in lamellar lecithin (egg yolk)-water phases at
18°C as a function of phase water content. The molecular structure of this phase has been determined by X-ray diffraction studies (Reiss-Husson, 1967; Lecuyer and Dervichian, 1969) which have shown that the phase is lamellar in a temperature range from about 3 to 40°C and a range of water content of 0.1–0.4 g water/g phase. The phase is made up of stacks of lipid molecules, associated in lamellae, separated by water layers in contact with the polar groups, as visualized in Fig. 1. The diffusion of ions in this system occurs in the aqueous region of the phase, in a direction along the lipid leaflets. Our experimental conditions are such that the ions diffuse entirely within the phase and no transfer between phase and a bulk aqueous medium occurs.

The ion selectivity of cell membranes and model systems such as lipid black films has been extensively studied. It is not possible to determine from such studies to what extent the observed selectivity occurs in the membrane interior or whether it is primarily an interface effect such as one of adsorption to membrane surface charged groups. The experiments on ion diffusion in lecithin-water lamellar phases described here show that ion selectivity can arise simply due to differences in diffusion rates through aqueous regions in contact with polar groups. In spite of the absence of net charge in the system, there is a difference between the diffusion coefficients of Na⁺ and Cl⁻. The relative diffusion coefficients of these ions depend very strongly on phase hydration. The possibility that a conformational change of the lecithin polar head occurs at a specific phase hydration is examined in this paper.
MATERIALS AND METHODS

Lecithin was extracted from egg yolk according to the method of Singleton et al. (1965) and checked for purity by thin layer chromatography. The phases were prepared as follows. A measured volume of either distilled water or distilled water containing 0.1 M NaCl was added to a weighed amount of lecithin. The mixture was kept under nitrogen for several hours to initiate equilibration. It was then placed in a mechanical mixing device and stirred under partial vacuum to remove air and to complete the homogenization. The phase was then transferred to a syringe and kept for at least 24 h before use. Phase water content was determined by drying at 90°C.

The diffusion coefficients at 18°C were measured as described previously (Lange et al. 1974) in approximately 50 μl of the phase contained in a glass capillary. The diffusion coefficients of the ions were found to be independent of NaCl concentration in the phase over the range 0.05-1.0 M. 3HHO, 14C-urea, and 86Rb were obtained from C. E. A. (France) and 22Na, 36Cl, and 14C-benzene from the Radiochemical Centre, Amersham, England.

RESULTS

Diffusion of Ions

The diffusion coefficients of Na⁺, Rb⁺, and Cl⁻, measured at 18°C in lecithin-water lamellar phases at different water contents are shown in Fig. 2. There are two striking aspects of these results. The diffusion coefficients of all the ions have a dependence on water content which shows a sudden change in the vicinity of 24% water. Moreover, the rates of diffusion of the different ions change order at this water content. We shall argue that structural changes are occurring at two levels. First, there must be a change at the molecular level which differentially changes the interactions determining the diffusion rates of the different ions. Second, there is probably a macroscopic change in the structure of the phase leading to changes in all the diffusion coefficients.

The diffusion coefficients of the ions and the ratios of these coefficients at different phase water contents, Φₖ (g water/g phase) are given in Table I. For phase water content Φₖ ≤ 0.24, Cl⁻ diffuses faster than Na⁺. An increase in water content of approximately 2% results in an inversion of the relative diffusion coefficients so that at Φₖ ≥ 0.26, Cl⁻ diffuses more slowly than Na⁺. In the range of water content where Cl⁻ diffuses faster than Na⁺, Rb⁺ diffuses more slowly than Na⁺. At higher water content Na⁺ and Rb⁺ diffuse at the same rate which is faster than Cl⁻. The change in relative diffusion coefficients of Na⁺ and Cl⁻ occurring at Φₖ = 0.24 is accompanied by a decrease in the diffusion coefficients by a factor of approximately 6 for Na⁺ and approximately 16 for Cl⁻.

At low phase water content, Na⁺ diffuses faster than Rb⁺. This difference can be discussed qualitatively along the lines of Eisenman’s (1961, 1962, 1965) theory of alkali cation equilibrium selectivity. The diffusion of an ion in the
phase depends on two factors, its interaction with fixed sites in the aqueous region of the phase, namely the choline phosphate groups of lecithin, and its interaction with water. In free solution Rb+ diffuses faster than Na+, in other words, Na+ interacts more strongly with surrounding water molecules than does Rb+. Assuming that the interaction of Na+ with water is also greater than that of Rb+ when the ions are diffusing in the hydration water of the phase, our result that the diffusion coefficient of Na+ is greater than that of Rb+ in phases of low water content implies that the interaction of Rb+ with the fixed sites in the aqueous region of the phase is stronger than that of Na+. The balance of ion-water and ion-site forces will then be such that Na+ diffuses faster than Rb+. With increasing phase water content, the importance of the ion-site interactions diminishes and the diffusion coefficient of Rb+ approaches that of Na+.

The relative strengths of the interactions of anion and cation with the sites can be assessed in a similarly qualitative manner. In free solution, Cl− and Rb+ diffuse at approximately the same rate. In the phase at low water content Cl− diffuses faster than Rb+ which indicates that the Rb+-site interaction is stronger than the Cl−-site interaction. At high phase water content, Rb+ diffuses faster
TABLE I
DIFFUSION COEFFICIENTS OF Na⁺, Rb⁺, AND Cl⁻ IN LECITHIN-WATER PHASES AT DIFFERENT PHASE WATER CONTENTS

| Φw          | Na⁺       | Rb⁺       | Cl⁻  | Rb  | Cl  |
|-------------|-----------|-----------|------|-----|-----|
|             | D × 10⁶ cm² s⁻¹ |          |      |     |     |
| 0.13        | 0.08      | 0.05      | 0.12 | 0.63| 1.50|
| 0.14        | 0.44      | 0.30      | 0.56 | 0.68| 1.27|
| 0.16        | 0.74      | 1.1       |      |     |     |
| 0.16        | 0.37      | 0.30      | 0.57 | 0.80| 1.54|
| 0.17        | 0.85      | 0.77      |      | 0.30|     |
| 0.18        | 1.3       | 2.0       |      |     | 1.54|
| 0.22        | 1.1       |           |      |     |     |
| 0.23        | 0.85      | 0.86      | 1.4  | 1.0 | 1.65|
| 0.24        | 0.92      | 0.76      | 1.3  | 0.83| 1.41|
| 0.25        | 0.23      |           |      |     |     |
| 0.26        | 0.19      | 0.19      | 0.13 | 1.0 | 0.68|
| 0.27        | 0.35      |           | 0.20 |     | 0.57|
| 0.29        | 0.37      |           | 0.17 |     | 0.46|
| 0.30        | 0.49      | 0.47      | 0.32 | 0.96| 0.65|
| 0.30        | 0.35      |           | 0.13 |     | 0.37|
| 0.35        | 0.93      | 0.92      | 0.35 | 1.0 | 0.38|

At each water content, diffusion coefficients were determined in quintuplet for each ion. Standard errors of the above diffusion coefficients are 3-5%.

than Cl⁻ so in this case the Cl⁻-site interaction must be stronger than the Rb⁺-site interaction.

In free solution, Cl⁻ diffuses faster than Na⁺, the same order is observed for diffusion of these ions in phases at Φw ≤ 0.24. Nothing can be inferred about the relative strengths of interaction of Cl⁻ and Na⁺ with the sites at low water content, but there is no contradiction with the inference, obtained from the comparison of diffusion coefficients of Rb⁺ and Cl⁻ at Φw < 0.24, that the cation-site interaction is stronger than the anion-site interaction at low water content. However for Φw > 0.24, Cl⁻ diffuses more slowly than Na⁺, indicating that in this range of water content, the interaction of the site with the anion is greater than its interaction with the cation, as was deduced from the comparison of Rb⁺ and Cl⁻ diffusion at high phase water content.

The discrimination between anion and cation observed in this system is interesting in view of the fact that lecithin is a zwitterion and there is no net charge present in the aqueous region of the phase. However, the choline phosphate group is a dipole, with the positively charge ammonium group separated from the negatively charged phosphate group by a distance which is of the order of 10 Å when the choline phosphate is fully extended. The interaction of a diffusing ion with the electric field in the vicinity of the dipole will
depend on the sign and the size of the ion and will be different for the three ions studied.

Our diffusion measurements have shown that the interaction of Rb+ with the dipole is stronger than that of Cl- for \( \Phi_w < 0.24 \) whereas the interaction of Cl- with the site is stronger than that of Rb+ for \( \Phi_w > 0.24 \). This sudden change in relative strengths of interaction suggests that some change in the configuration of the dipole occurs at phase water content of 24%. A change in the conformation of the choline phosphate group at this water content clearly could lead to the great change in the nature of its interaction with diffusing ions, suggested by the diffusion data.

In the above analysis, a separation was made between ion-water and ion-site interactions. This separation is not strictly valid given that the water in the phase is organized around the lecithin polar groups. However, similar conclusions concerning the mechanism leading to the abrupt selectivity changes observed may be reached without invoking such a separation.

At low phase water content, Cl- diffuses faster than Na+ whereas at high water content the reverse is true. This change can only be due to a change in the electric field in the vicinity of the dipole, which is different for ions of opposite sign. This implies that there has been a change in the conformation of the dipole.

The possibility of different configurations of the lecithin polar head has been discussed by Sundaralingam (1972) and it is reasonable that the favorable configuration depends upon the state of hydration of the polar group. Various authors (Elworthy, 1961; Chapman et al., 1967) have measured the amount of water of hydration associated with lecithin. A value of approximately 20% has been obtained by differential scanning calorimetry (Chapman et al., 1967), a method which does not distinguish between different hydration shells. The change in conformation of the lecithin polar head group suggested by our diffusion measurements might reasonably be correlated with the saturation of the hydration requirements of lecithin.

A macroscopic change in phase structure could give rise to a change in the diffusion coefficients but could not account for the variations in ion selectivity discussed above. Two approaches have been taken to investigate the possibility that such macroscopic changes occur with variations in phase water content. First the diffusion coefficients of two hydrophilic nonelectrolytes, water and urea and of the hydrophobic molecule, benzene, have been measured in the phase as a function of water content. Second we have carried out structural studies by freeze fracture and polarizing microscopy.

**Diffusion of Nonelectrolytes**

The diffusion coefficients of water and urea as a function of phase water content are shown in Fig. 3. The curves have the same general form as that obtained in the case of the diffusion of ions: a rapid increase of the diffusion
coefficient with phase water content until $\Phi_w \sim 0.24$, at which point the diffusion coefficient decreases considerably. It can be concluded that the abrupt decrease in the diffusion coefficients at $\Phi_w \sim 0.24$ reflects a structural change in the phase affecting the diffusion of all hydrophilic molecules.

Certain types of structural change in the phase would also be reflected in the diffusion of a hydrophobic molecule through the lipidic region of the phase. In particular, an increase in the tortuosity of the lamellae would affect molecules diffusing in the aqueous or lipidic regions of the phase to a comparable extent. For this reason, the diffusion coefficient of benzene was determined as a function of phase water content. The results are shown in Fig. 4. It can be seen that the diffusion coefficient of benzene also decreases at $\Phi_w \sim 0.24$, however the change is by a factor of only 2 compared to decreases by factors of approximately 4 for water, 5 for urea, 6 for Na$^+$, and 16 for Cl$^-$. The smallest decrease in diffusion coefficient observed, namely that for benzene, must give an upper bound on the effect of an increase in tortuosity which could be occurring at 24% water. Such an increase in tortuosity would contribute to the drop in the diffusion coefficients observed for hydrophilic molecules, however the much greater effect observed for hydrophilic molecules than for benzene must be attributed to a property of the aqueous channels.

The extremely rapid increase in diffusion coefficient with phase water content between $\Phi_w = 0.10$ and 0.20 observed in the case of the ions, water and urea does not occur for benzene and must also reflect a property of the aqueous channels. This effect can be explained in terms of steric hindrance due to the presence of the choline phosphate groups in the aqueous region of
the phase. A possible configuration (A) has each choline phosphate group bent over such that its terminal positively charged group is juxtaposed with the negatively charged group of the lecithin molecule adjacent to it on the same leaflet. At the opposite extreme from this is the configuration (B) where each choline phosphate group is fully extended, perpendicular to the plane of the bilayer leaflet. The steric hindrance of the aqueous channel to molecules diffusing through it is much greater in the latter situation than in the former. A cooperative change in the configuration of the polar groups from A to B could account for the sudden large decrease in the diffusion coefficients of hydrophilic molecules. When the polar groups are in configuration A, the fractional cross section of the aqueous channel unencumbered by these groups increases rapidly with phase water content, which accounts for the initial rapid increase of the diffusion coefficients with \( \Phi_w \). A change in the configuration of the polar heads from A to B could also give rise to the electric field changes underlying the inversion of ion selectivity.

X-ray studies (Reiss-Husson, 1967; Lecuyer and Dervichian, 1969) have shown that the lecithin-water phase is lamellar from about 10 to 45\% water at 18\(^\circ\)C and no discontinuity in repeat distance is observed in this range of water content. Calculations of aqueous channel thickness have been made from the measured repeat distances. These calculations were made assuming continuity of the partial specific volumes of water and lecithin in the phase and therefore predict that the aqueous channel thickness is continuous with phase water content. It is extremely likely that a change in polar head configuration at a particular phase water content would give rise to a sudden change in aqueous channel thickness at this water content. However, a change in polar head configuration and the organization of the surrounding water would also give rise to a discontinuity in the partial specific volume of the aqueous region of the phase and therefore the calculations which predict continuity of aqueous channel thickness are in no way contradictory with our observations.
FIGURE 5. Freeze fracture electron micrographs of lecithin-water phases at (a) 11% and (b) 28% water content. Specimens were fractured in a Balzer machine at $-150^\circ\text{C}$. Magnification $\times 93,000$. 
FIGURE 6. Polarizing microscope pictures of lecithin-water phases at (a) 15% and (b) 28% water content. Magnification $\times 500$. 
Structural Studies

Preliminary observations of phase structure have been made by freeze fracture and polarizing microscopy studies. The former technique revealed that the organization of the phase is essentially the same below and above a water content of 24%. The structure is perfectly lamellar in both regions of water content, with some increase in the tortuosity of the lamellar leaflets at high water content. Typical pictures of the phase are shown in Fig. 5.

Samples of phase contained between two glass slides were viewed in a polarizing microscope. An abrupt change in the appearance of the phase was observed at 24%, the size of the perfectly oriented zones decreasing considerably at this water content. A characteristic picture of the phase on each side of the transition is shown in Fig. 6. The black regions of the photographs correspond to areas of perfect orientation. The nature of the defect lines or boundaries of the monocrystals is not understood but is the same in both cases. In carrying out the polarizing microscope studies it was observed that, for water content below 24%, the number of defects could be considerably decreased by compressing the phase between the glass slides or by applying a shearing force. This effect was much less apparent in phases with water content above 24% which could be related to the independent observation that the viscoelastic properties of the phase undergo a sudden change at 24% water content. Above this water content the phase is considerably more viscous than below. No quantitative study of this effect has been made.

The two techniques used to obtain structural information, freeze fracture and polarizing microscopy, yield results which are difficult to fit together. Information is obtained on the scale of a tenth of a micron by the former technique and on the scale of tens of microns by the latter technique. However, both techniques indicate that the nature of the phase is similar below and above 24% water. The defects seen by polarizing microscope are the same in both regions of water content although their number has increased at high water content.

The nature of the structural change is not understood. The change is manifest in two ways. The anion-cation selectivity of the system reverses abruptly and the long-range order of the system changes. Both these phenomena occur at the same water content and it is likely that they are related. The change in conformation of the choline phosphate group proposed as an explanation for the change in ion selectivity could lead to the changes in long-range order as a second-order effect.

1 The optical studies of the texture of lecithin-water phases are due to M Kleman (Laboratoire de Physique des Solides, Université de Paris Sud) and M. Veyssié (Laboratoire de Physique de la Matière Condensée, Collège de France, Paris). More detailed results will be published elsewhere.
**Effect of Cholesterol**

As discussed above, the conformation of the polar head group is probably dependent upon its state of hydration. Lamellar phases can be prepared with the lipid fraction composed of equimolar quantities of cholesterol and lecithin. There are half as many choline phosphate groups in the aqueous region of this system compared to the pure lecithin phase. In this system the diffusion coefficient of water was found to have the same kind of dependence on phase water content, namely a rapid increase followed by an abrupt decrease. The decrease occurred at a water content of between 8 and 10 mol water/mol lipid, compared to the value of approximately 13 mol water/mol lipid (Φw = 0.24) found in the case of the lecithin-water phase. The presence of cholesterol decreases the amount of water of hydration in the phase and causes the transition to occur at a lower water content. This result indicates a connection between the saturation of the hydration requirements of the polar heads and the occurrence of the transition. However, since the amount of water of hydration of the lecithin-cholesterol mixture is not known, no quantitative statement can be made.

**Comparison with Previous Results**

The results presented in this paper were obtained using phases prepared by a technique different from that of our initial studies (Rigaud et al., 1972). The diffusion coefficients of water and benzene given here are not the same as those obtained previously. For either method of phase preparation, the diffusion coefficients obtained as a function of water content are reproducible. Therefore, in principle, either technique could be used to provide a phase with which to study the variations of parameters such as those studied in this paper. However, for the reasons given below, we believe that the method used to prepare the phases described in this paper is superior and that the diffusion coefficients reported here are a true reflection of fundamental lipid-water interactions and structure.

In the previous preparation method possibilities existed for both chemical and structural inhomogeneities on a macroscopic scale in the phase, possibilities which have been eliminated in the new technique. The use of organic solvents to prepare phases has been eliminated. The method by which the solvent was removed previously (Rigaud et al., 1972) could have led both to residual chloroform inhomogeneously distributed in the phase and an inhomogeneity in phase water content. Greatly improved mechanical mixing techniques to combat inhomogeneity were used in the new method.

The phases studied here have been well characterized. In addition to the structural studies described above, an X-ray diffraction study was carried out
and showed that a single lamellar phase was present at each of the water contents used.

**DISCUSSION**

The observed inversion in relative diffusion coefficients of cation and anion has been ascribed to a change in the configuration of the lecithin polar head. This is the most interesting explanation and perhaps the most likely one, but other possibilities must be considered.

The monocrystals and defect lines which separate them could provide two diffusion channels with different properties. If Cl\(^-\) diffuses faster than Na\(^+\) in the monocrystals and the reverse is true in the defects, then an increase in the number of defects could lead to the observed inversion of the diffusion coefficients. However, as both ions diffuse in the aqueous regions of the phase, such an effect is unlikely.

The structural change at 24% water, evident from the polarizing microscope pictures, could correspond to a folding up of the lipid lamellae to form closed structures. Such a change is consistent with the available X-ray data and with our freeze fracture studies, provided that the dimensions of the closed structures are sufficiently large.

Closed structures must be separated by regions which are either aqueous or lipidic and therefore the diffusion through the phase of either benzene or water would involve crossing a barrier in which the molecule has very low partition. This process would give rise to a high activation energy of diffusion. We have found that the activation energy for both benzene and water diffusion is low (less than 6 kcal/mol) which means that the crossing of a lipidic barrier in the case of water and of an aqueous barrier in the case of benzene is not an important part of the diffusion process.

A change from open to closed lamellar structures would be accompanied by a change in the tortuosity of the system. Such a structural change could explain the abrupt decrease in the diffusion coefficients of all molecules, but cannot alone account for the fact that Cl\(^-\) diffusion decreases by a factor of 16, whereas Na\(^+\) diffusion decreases by only a factor of 6.

The data presented here points unambiguously to a profound structural change occurring at 24% phase water content, a change which has never been observed by X-ray studies. Whatever the precise nature of the structural change, it must contain information about the interaction between lecithin and water and is not a trivial phenomenon.

While other possible explanations exist, it is likely that the differences in the diffusion coefficients of the three ions studied here arise from differences in the interactions of the ions with water and polar groups interior to the lamellar phase. The differences cannot be attributed to interface phenomena reflecting partition into the phase. This is an important distinction between these meas-
urements and those carried out in cell membranes and various model systems, where a separation of the observed selectivity into interfacial, as opposed to membrane interior phenomena, is extremely difficult.

Lecithin is a zwitterion and hence there is no net charge present in the aqueous region of the phase. In spite of the absence of net charge, the diffusion rates of Na\(^+\) and Rb\(^+\) are different in the system, as are the diffusion rates of Rb\(^+\) and Cl\(^-\). This selectivity is due to differences in the interactions of the different ions with the electric field in the vicinity of the choline phosphate group. A change in phase water content of approximately 2\% causes an inversion in the relative diffusion rates of anion and cation. This effect is attributed to a change in conformation of the choline phosphate groups and is indicative of the sensitivity of the diffusion rates of ions to the conformation of the group.

The ion selectivity of cell membranes is generally greater than that manifested by this simple model system. However, it must be noted that at a phase water content of 24\%, the critical water content, the aqueous channels are approximately 23 Å wide. Many of the diffusing ions are probably not close enough to the dipole to interact with it significantly. It is likely that the difference in diffusion coefficients of the different ions would be much greater in the absence of this dilution effect.

The mechanism leading to the change in relative diffusion rates of anion and cation brought about by a small change in phase composition could be of relevance to ion transport in excitable membranes. The effect observed in the model system suggests the possibility of much greater changes occurring under different conditions. For example, partition of different ions into the membrane could be differentially altered by a conformational change of charged groups interior to the membrane. Such an effect could greatly enhance the selectivity of the system observed in our study.

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