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CATION DIFFUSION SELECTIVITY IN A PORE MODEL

THE PHOSPHATIDYLCHOLINE/WATER LAMELLAR PHASE

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Summary

The diffusion coefficients D (cm^2/s), of four monovalent cations K^+, Na^+, Rb^+ and Cs^+ and of Ca^{2+} have been measured in phosphatidylcholine/water lamellar phase as a function of phase hydration and temperature and in the presence of divalent cations. Diffusion rates vary strongly with phase hydration, between 10^{-7} and 10^{-6} cm^2/s for monovalent and 10^{-8} and 10^{-7} for Ca^{2+}. The activation energies obtained are relatively small (5—10 kcal/mol). As the phase water content increases, a series of diffusion sequences is obtained, corresponding to the sequences predicted by Eisenman's theory of alkali ion equilibrium selectivity.

This diffusionnal selectivity, which depends exclusively upon non-equilibrium parameters (mobility) within the hydrophilic path is discussed in respect to current theories of pore selectivity.

Introduction

For many years, the idea that ions penetrate membranes through specific polar pores has been widely accepted, although these discrete pathways have not been clearly identified in biological systems. Extensive studies on natural and synthetic antibiotics have contributed a great deal to the understanding of the possible organization and functioning of these ionic pathways, but our picture of these pores in biological membranes still falls short of a detailed molecular description.

Mesomorphic phosphatidylcholine/water lamellar phases [1,2] appear to be a very useful physico-chemical model of the particular milieu in which ion movements proceed in these biological pathways: the hydrophilic layer of the lamellar phases constituted by the hydrated choline phosphate groups offers a specific molecular organization of dipoles in a very restricted space, a few ang-

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ströms wide. Depending upon the hydration level, water molecules are present either in a strongly bound state, or in a free liquid state [3].

Moreover, at variance with other model systems such as black films or liposomes, lamellar phases allow experimental measurements of diffusion rates alongside the bilayer planes in this particular hydrophilic path, without any interference of partition phenomenon between the hydrophilic medium studied and bulk aqueous solution. This is an important distinction between these measurements and those carried out on biological or model membranes. It is not possible to determine from such permeability experiments to what extent the observed selectivity occurs in the membrane interior or whether it is primarily an interface effect. Generally, selectivity of such pores is ascribed to the interface step, considering that the diffusion process within the pore is quite comparable to that in bulk water [4—6].

From our experiments in phosphatidylcholine/water phases it is clear that such is not the case and that diffusion in narrow hydrophilic paths is very complex and many give rise in itself to selectivity. It has been shown [7—9], that the diffusion of hydrophilic solutes in these systems is strongly dependent upon the water content. The evolution of diffusion rates with this parameter is not monotonic but exhibits very sharp breaks; a prominent maximum of diffusion of all classes of solutes is constantly observed at around 21% water, and a minimum around 28%—30% water. This behaviour cannot be ascribed to the variation of the thickness of the aqueous layer which increases continuously with the water content as shown by X-ray diffraction measurements [1,2]. It has to be related in the first place to the state of water in the hydrophilic region. The data obtained consonantly by various techniques such as water sorption isotherms [10], differential calorimetry [11], NMR [3] and ESR [12, 13], show that in a phosphatidylcholine/water lamellar phase, 10—11 water molecules per polar head are strongly bound as the primary hydration shell (corresponding to 21 g water per 100 g phase). Beyond these 10—11 water molecules, an equal amount of water, participating to choline phosphate hydration (as the second hydration shell [10]), is in an intermediate situation, characterized by its isotropic movement and its rapid exchange with the water of the primary hydration shell [3]. Free water would be present only above 20 water molecules per polar head (corresponding to 30 g water per 100 g phase). The variations of the diffusion rates reflect this situation. Moreover, the second factor involved is [7,8,13] a probable change in the conformation of the choline phosphate dipoles (due to their hydration) from a bent configuration (the dipole being approximately parallel to the plane of the bilayer below 21% water) to an extended configuration (the dipole then being perpendicular to the plane of the bilayer above 21% water).

The selectivity for various hydrophilic solutes and ions seems to be controlled by these parameters.

In this paper, we present the results of measurements of the diffusion coefficients of alkali monovalent cations in egg-yolk phosphatidylcholine/water lamellar phases at 25°C as a systematic function of hydration and temperature and in presence of divalent cations. The possible relevance of some of the mechanisms of ionic selectivity in these systems to permeability processes in biological pores will be discussed.

Materials and Methods

Phosphatidylcholine was extracted from egg-yolk according to the method of Singleton et al. [14] and checked for purity by thin layer chromatography.

The phases were prepared as described previously [8] in distilled water containing 10^{-2} M of chloride salts (no significant dependence of diffusion coefficients on salt concentration between 10^{-5} and 10^{-1} M was observed).

Water concentration, w (g water/g phase), was determined by drying at 100° C until constant weight.

The diffusion coefficients at 25° C were measured as described previously [15] on a macroscopic scale (cm). At each water content diffusion coefficients were determined, at least 5 times, for each ion with a standard error varying from 2.5% to 5%.

^{42}K and ^{45}Ca were obtained from C.E.A. (France), ^{22}Na, ^{36}Rb, ^{134}Cs and ^{36}Cl, from the Radiochemical Centre, Amersham (U.K.).

Results

(1) Effects of phase water content on ion diffusion

The diffusion coefficients D ($cm^2\ s^{-1}$), of two monovalent ions ($^{36}Cl^-$ and $^{22}Na^+$) and of a divalent cation ($^{45}Ca^{2+}$) are plotted as a function of phase water content, w, in Figs. 1 and 2, respectively. The three diffusion curves appear to be very dependant upon the amount of water present in the phase, the main feature being, as already shown for water and non-electrolytes [9], the presence of a prominent maximum occuring at $\acute{w} = 0.21$. However the diffusion rates between $w = 0.21$ and $w = 0.28$ decrease by a factor of 8 for Na^+, 11 for Cl^-, 2.5 for Ca^{2+} as compared to the factor of 3 obtained for ^3HHO. It is clear that the magnitude of the variation depends upon the nature of the solute, that is their possible interactions within the hydrophilic pathway of the lamellar phases, and not only upon their size. As already stated in the introduction, this behavior has been accounted for in the complete reorganization of the hydration structure of the pathway at 22% water, involving a cooperative change in the configuration of the choline phosphate dipole. It may be seen that below 21% water, Cl^- diffuses faster than Na^+, the reverse being true above 30% water, reflecting the change in the interactions of the diffusing ions with the dipoles.

Another point to be made is that the diffusion in a rigidly organized medium containing only "bound" water molecules, such as the hydrophilic path of the lamellar phase, is a relatively fast process. In the case of Na^+ the diffusion rates measured are only about ten times slower than in bulk water (D^+_{Na} in water = $1.32 \cdot 10^{-5}\ cm^2\ s^{-1}$ [16]. The difference is even smaller considering that in the lamellar system, the geometrical "tortuosity" has to be taken into account; this has been evaluated as being approx. 2 as compared to bulk diffusion [7].

The diffusion rates for Ca^{2+} are 1 order of magnitude lower than for Na^+. For instance at 20% water, the ratio $D^+_{Na}/D^{2+}_{Ca} = 13.6$ instead of 2, in bulk water [17]. This difference cannot be accounted for by steric factor only. (At 20%, the aqueous channel is approximately 20 Å wide, which is large, compared to both ionic radii.)

If this difference is ascribed to interaction factors only, this would indicate

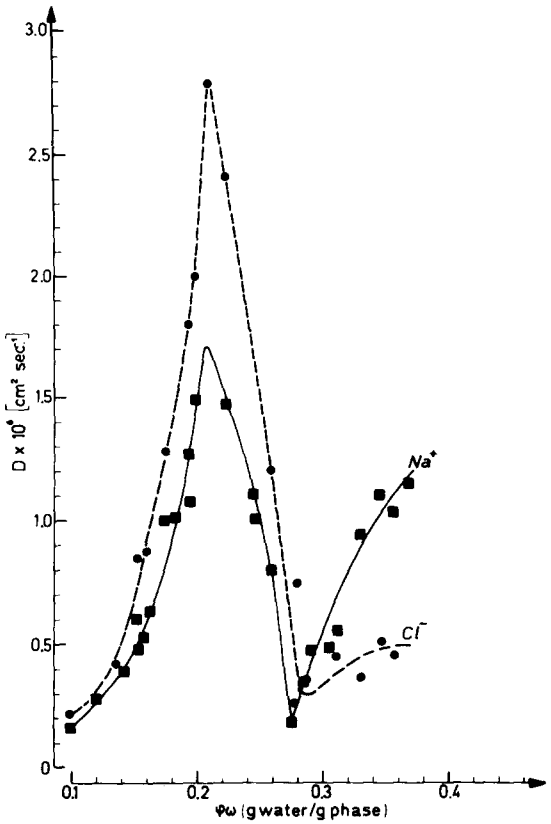

Fig. 1. Diffusion coefficients of Na^+ (■) and Cl^- (●) as a function of phase water content (φ_w) in phosphatidylcholine/water lamellar phases (T = 25° C).

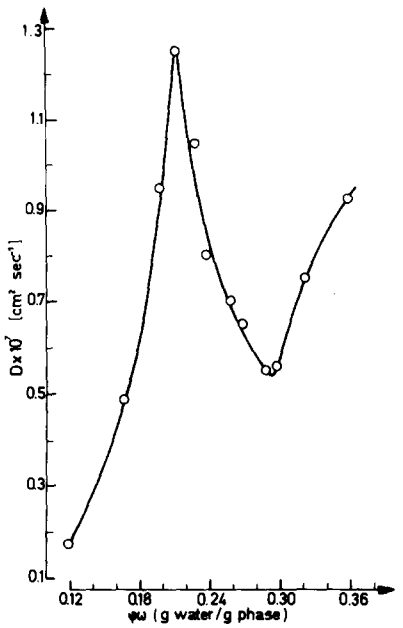

Fig. 2. Diffusion coefficients of Ca^{2+} as a function of phase water content (φ_w) in phosphatidylcholine/water lamellar phases (T = 25° C).

that Ca^{2+} interacts with the choline phosphate groups approx. 6 times stronger than Na^+. This fact opens the possibility of competitive diffusion experiments between mono- and divalent ions.

(2) Effects of temperature

The apparent activation energies for Na^+ diffusion in the system were estimated at different phase water contents. The mean values of D are given for different temperatures in Table I.

Arrhenius plot of these values yielded apparent activation energies varying from 10.6 kcal/mol at low-phase water content (φ_w = 0.11) to 4.6 kcal/mol at high phase water content (φ_w = 0.36). These values, significantly higher than the value of 4.39 kcal obtained by Wang [18] for the activation energy of Na^+ diffusion in bulk water, seem to indicate a weak binding between cations and the choline phosphate group binding sites (similar experiments give an apparent activation energy of 12 kcal for Rb^+ diffusion at φ_w = 0.11 instead of 3.95 kcal in pure water). This means that although selective, the binding of monovalent cations to phosphate groups is weak. Binding to a high field strength site of the

TABLE I

EFFECTS OF TEMPERATURE ON Na^+ DIFFUSION COEFFICIENTS IN PHOSPHATIDYLCHOLINE/WATER LAMELLAR PHASES AT DIFFERENT PHASE WATER CONTENTS

Apparent activation energies were deduced from Arrhenius plots.

Phase water content φ_w (g water/g phase)	$D \times 10^6$ ($cm^2\ s^{-1}$)			E_A (kcal/M)
	$T = 13°C$	$T = 25°C$	$T = 40°C$	
0.11	0.10	0.20	0.43	10.6
0.18	0.49	0.80	1.19	6.6
0.24	0.89	1.26	1.80	4.7
0.36	0.86	1.20	1.65	4.6

type postulated for glass electrode would make diffusion through a channel extremely slow. Binding energy between Na^+ and such a site is approx. 180 kcal/mol [19] which means that Na^+ combines almost irreversibly with the site and certainly could not dissociate from it rapidly enough to be compatible with the relative high values of diffusion rates obtained in this study.

(3) Diffusion selectivity sequences for monovalent alkali cations

The diffusion coefficients of the monovalent alkali cations K^+, Na^+, Rb^+ and Cs^+ at different phase water contents are represented in Table II.

There are two striking aspects of the results: first, the ability of choline phosphate head groups to distinguish among such chemically similar cations as K^+, Na^+, Rb^+ and Cs^+; second, the dependance of relative diffusion coefficients of monovalent cations upon the phase water content. This variation with φ_w is rapid at low water content (3 successive different sequences between 0.12 and 0.20, each one existing only within a narrow water range). Between 21 and 35% only one sequence is obtained, followed by another beyond 40%. As the

TABLE II

DIFFUSION COEFFICIENTS AND DIFFUSION SELECTIVITY SEQUENCES OF MONOVALENT ALKALI CATIONS AT DIFFERENT PHASE WATER CONTENTS ($T = 25°C$)

Phase water content φ_w (g water/g phase)	Diffusion coefficients *				Sequences of diffusion
	Na^+	K^-	Rb^+	Cs^+	
0.135	0.35	0.60	0.30	0.26	$D^+_K > D^+_{Na} > D^+_{Rb} > D^+_{Cs}$
0.142	0.47	0.85	0.39	0.33	
0.160	0.56	1.05	0.63	0.48	$D^+_K > D^+_{Rb} > D^+_{Na} > D^+_{Cs}$
0.175	0.94	1.50	1.06	0.75	
0.195	1.11	2.00	1.30	1.14	$D^+_K > D^+_{Rb} > D^+_{Cs} > D^+_{Na}$
0.250	0.75	1.6	1.73	1.10	
0.310	0.53	0.90	1.56	0.85	$D^+_{Rb} > D^+_K > D^+_{Cs} > D^+_{Na}$
0.350	1.05	1.75	1.92	1.45	
1.410	1.30	2.40	3.00	2.44	$D^+_{Rb} > D^+_{Cs} > D^+_K > D^+_{Na}$

* At each water content, the diffusion coefficients of the four ions were measured using the same phase preparation.

phase water content is increased, the following sequences are observed:

$D_K > D_{Na} > D_{Rb} > D_{Cs}$
$D_K > D_{Rb} > D_{Na} > D_{Cs}$
$D_K > D_{Rb} > D_{Cs} > D_{Na}$
$D_{Rb} > D_K > D_{Cs} > D_{Na}$
$D_{Rb} > D_{Cs} > D_K > D_{Na}$

These sequences correspond neither to the sequence of the free solution mobilities, hydration energies, ionic radii, nor calculated hydrated radii. However, it must be noted that by increasing the phase water content we tend to obtain the sequence of the free solution diffusion coefficients, i.e. $D_{Cs} > D_{Rb} > D_K > D_{Na}$ and that partial results obtained at very low water content ($\varphi_w = 0.05$, that is at the lower limit of the range of existence of the lamellar phase) indicate a sequence in which D_{Na} ($= 0.07 \cdot 10^{-6}$ cm^2 s^{-1}) $> D_K$ ($= 0.05 \cdot 10^{-6}$ cm^2 s^{-1}).

These characteristics could be discussed in terms of interactions in the aqueous pathway, along the lines of Eisenman's theory [19,20] of alkali ion equilibrium selectivity. Eisenman proposed that two factors are involved in the cationic selectively: the free energy of interaction of the cation with water on one hand, and with anionic sites on the other. However, in addition to the equilibrium affinity such as binding constant between sites and cations, our diffusion measurements contain a non-equilibrium component such as mobility, which involves also selectivity.

The difficulties of interpretation arise from the fact that we do not know to what extent selectivity in these lamellar systems involves non-equilibrium or equilibrium parameters, i.e. which is the more important between equilibrium selectivity or mobility selectivity. To a better understanding of the alkali cation-site interactions we have experimented competitive reactions with divalent cations such as Ca^{2+}, Mg^{2+} and Ba^{2+}. The effect of divalent cations on the monovalent cationic diffusion may be a measure of purely equilibrium affinity uninfluenced by non-equilibrium factors such as mobilities [21].

(4) Effect of divalent cation

(a) At low-phase water content ($\varphi_w \leqslant 0.20$) the results are complex, as shown in Fig. 3A—D. The presence of $CaCl_2$ in the phase causes a decrease in the diffusion rates of anions (Cl^-), cations (Na^+, Rb^+) by a factor of about 1.6 (around 50%) and has no action on non-electrolyte diffusion rates (ethylene glycol and glucose were tested). On the contrary, the presence of $MgCl_2$ in the phase promotes an increase in the diffusion of all these solutes (ions as well as non-electrolytes). Finally, it should be noted that $BaCl_2$ has no action on the ionic diffusion rates.

At low-phase content, the important of the first hydration shell around the polar head has been already stressed: it may be thought that as water penetrates and expands the zwitterionic lattice of the choline phosphate group a continuous hydrated structure forms not only around each dipole, but between dipoles. In this region, each water molecule added participates in further H-bond formation without disturbing, but on the contrary, stabilizing and

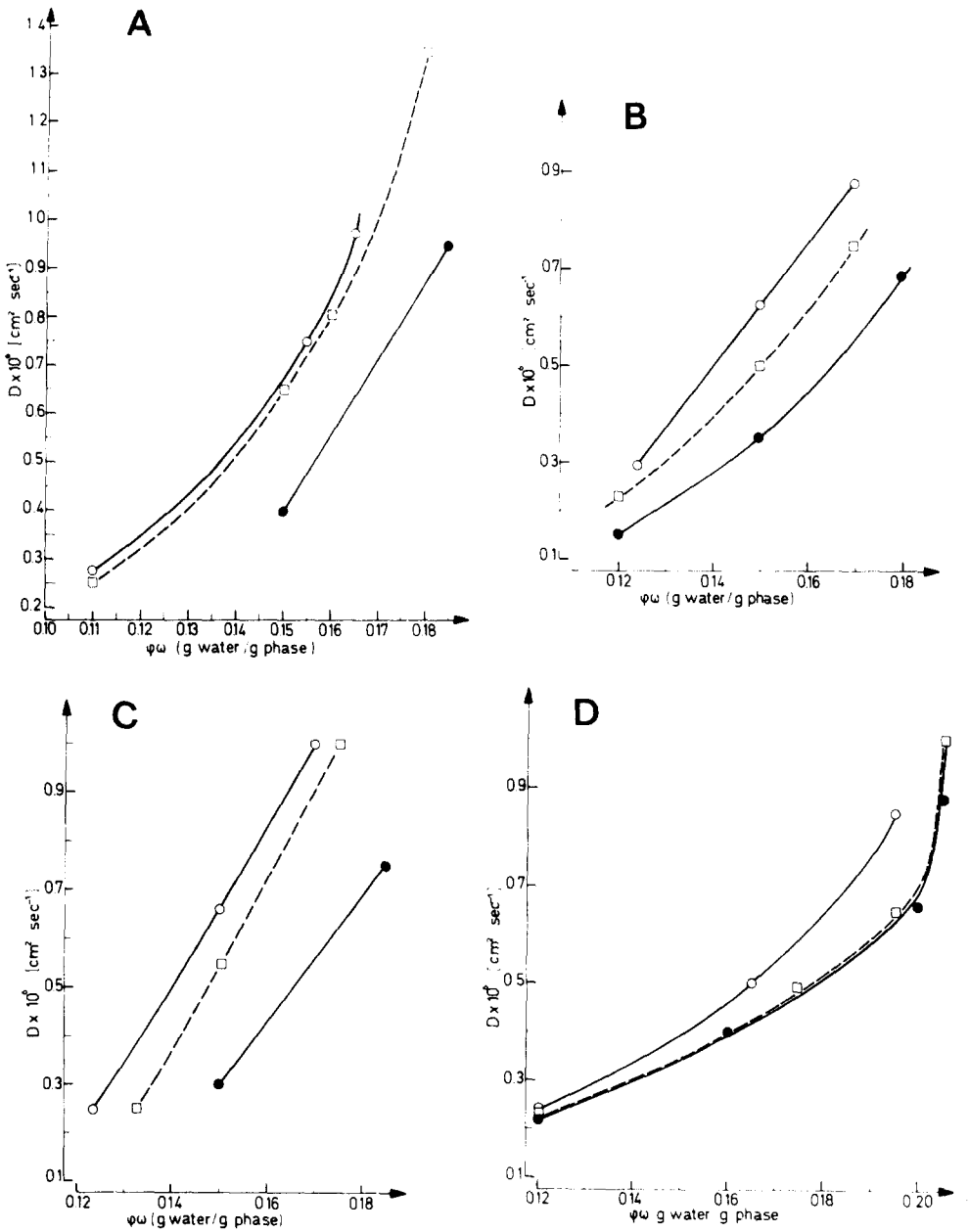

Fig. 3. Effects of Ca^{2+} and Mg^{2+} on diffusion coefficients of Cl^- (A), Na^+ (B), Rb^+ (C) and ethylene glycol (D) in phosphatidylcholine/water lamellar phases in the low water range ($\varphi_w \leqslant 0.20$). Phases were made in the presence of 10^{-2} M of $CaCl_2$ (●), $MgCl_2$ (○) or NaCl (□).

expanding the ordered structure in which ions diffuse more and more easily. From this point of view, pH effects on the ionic diffusion as well as on non-electrolyte diffusion had shown [8,9], that at low phase water content the presence of H_3O^+ is necessary for the formation of the complete ordered hydra-

ted organization of the hydrophilic path (the diffusion rates are systematically lower for all solutes at pH 9.0 than at pH 4.6).

The effects of Mg^{2+} can be interpreted along this line. It is well known that this alkaline earth cation is a water-structure ordering ion [22] and by imposing upon the water a different structure which is more highly ordered than was the intrinsic structure, Mg^{2+} could favour the formation of the first hydration shell around the choline phosphate group, leading to an increase of all the diffusion coefficients measured (anions as well as cations or non-electrolytes).

This type of interpretation is not applicable to the effects of the other alkaline earth cation, Ca^{2+}. The lack of effect on non-electrolyte diffusion indicates that the presence of $CaCl_2$ does not promote a general breakdown of the hydrophilic pathway and that Ca^{2+} affects only the electric field in the vicinity of the dipole constituted by the choline phosphate group; however, this change on the electric field is not specific in regard to the anionic or the cationic diffusion, as observed in the high water range. The Ca^{2+}, by binding or screening the negative PO_4 sites, can modify, as observed on monolayers [23], the area occupied per phosphatidylcholine molecule and if, for any reason, head group spacing is critical, small changes could have a deep influence on the structure of the choline-phosphate dipole.

This has to be related to the fact (1) that in phosphatidylcholine-water lamellar phases, the most important increase in the area per molecule occurs between 10 and 20% water in the phase. The lack of discrimination between anions and cations is probably due to the fact that what we observe in our diffusion measurements is the mean general change in the electric field of the dipole.

(b) The results obtained in the high water range ($\varphi_w > 0.30$), are shown in Fig. 4. The presence of Mg^{2+} has no influence on diffusion rates. On the contrary, the presence of 10^{-2} M $CaCl_2$ in the phase decreases the diffusion rates of Cl^- and increases Na^+ and Rb^+ diffusion. No action on non-electrolyte diffusion (ethylene-glycol) is observed.

Whether one considers that Ca^{2+} screens or binds to the negative sites of choline phosphate groups, the modification of diffusion rates may be interpreted as the result of a decrease in the monovalent cation-site interaction, and an increase of the anion-site interaction.

This effect of calcium is quantitatively small *. Neither at low nor at high water content in the phase, are the cation selectivity sequences modified. This may be considered an indication that equilibrium binding affinity is not the predominant factor in determining diffusion rates. On the other hand, it may be seen that in the range of high water content, for instance, the effect of Ca^{2+} on the diffusion rates of Na^+ and Rb^+ is significantly different; the ratios of the diffusion coefficients measured in the presence and absence of Ca^{2+} are at 35%

* In the present study an electrolyte concentration of 100 mM was used. For the sake of comparison the concentration of the choline phosphate group may be calculated and is approx. 3 M, i.e. more then 300 times the concentration of any of the free cations studied. This overwhelming concentration of choline phosphate groups with respect to mobile cations seems to indicate that if Ca^{2+} promotes a local change in the conformation of the dipoles, this change has to be cooperative in the lamellar phase to explain the small, but significative, differences observed by our diffusion measurements.

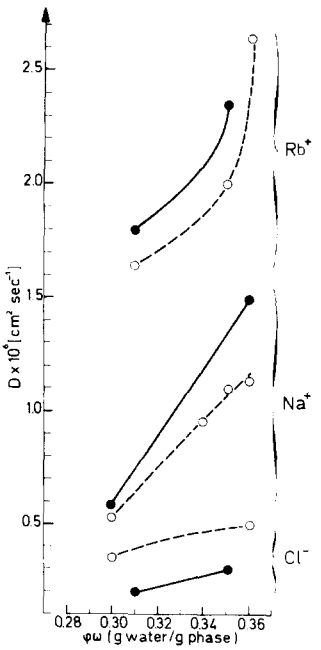

Fig. 4. Effects of Ca^{2+} on diffusion coefficients of monovalent ions (Na^+, Rb^+, Cl^-), in phosphatidylcholine/water lamellar phases in the high water range ($\varphi_w \geq 0.30$). Phases were made in the presence of 10^{-2} M of $CaCl_2$ (•) or NaCl (○).

water, 1.20 ± 0.1 for Rb^+, 1.40 ± 0.1 for Na^+. Following the line of the above reasoning, if Ca^{2+} competes with alkali monovalent cations for the same sites, there will be an inverse relationship between the equilibrium binding constant of each alkali cation for the site and the magnitude of increase in the diffusion rate; the more strongly bound a cation is to the site, the less it will be displaced by Ca^{2+} and the increase in the diffusion rate will be less. From our results, it seems that Na^+ diffusion is influenced more by Ca^{2+} than is Rb^+ diffusion; this indicates a greater affinity of Rb^+ for the negative sites comparatively to Na^+.

Therefore, one would come to the conclusion that the greater the affinity for the site, the faster the diffusion rate.

Discussion

The present study shows clearly that ionic diffusion in an hydrophilic channel lined by polar sites, with no net charge such as choline-phosphate dipole, may be selective. A clear discrimination is observed not only between the diffusion of anions and cations, but between the series of monovalent cations Na^+, K^+, Rb^+ and Cs^+.

The most important feature of the results is that a series of diffusion rates sequences is obtained as the water content of the lamellar phase increases, all of them corresponding to the equilibrium affinity sequences VI, V, IV, III and II predicted by Eisenman's theory.

Ion transport through channels has been analysed in some detail by Laüger [24,25] on the basis of the Eyring's rate reaction theory. According to this

theory ion movements through a channel may be considered as a sequence of energy barriers over which ions have to jump. The ion specificity of the channel depends, on the one hand, upon the change in free energy associated with the transfer of the ion from a bulk aqueous solution into the pore medium. On the other hand, it depends upon the rate constant of ion movement, that is the frequency of jumps from site to site within the pore.

The present study is concerned only with the second kinetic parameter. As stressed before, ion diffusion in the lamellar system proceeds entirely within the hydrophilic path and ion partition between bulk and channel is not involved.

According to the theory of absolute reaction rate, Laüger's analysis comes to the following conclusion. The jump frequency over the energy barrier within the pore is related to the pore energy of interaction. As a consequence, if there is only little interaction between the ion and the binding site, transport rate may be high, but the selectivity of the channel will be poor [24,25].

The experimental results obtained in the Gramicidin A pore [5,26] seem consistent with this viewpoint: the permeability of this pore model is high. The activation energy of ion transport and the sequence observed are very similar to that measured in bulk water. Therefore, the selectivity of the Gramicidin pore is considered negligible [5,26].

The situation in the case of the phosphatidylcholine pore model studied in the present work appears different, although ion diffusion rates are also high, and the activation energy not much larger than in the case of Gramicidin A. By varying the water content a series of diffusion sequences is obtained, none of which is the bulk water diffusion sequence. The ratio of diffusion rates for two ions may vary by a factor of 3 with phase water content: for instance, D_{Na}/D_{Rb} varies from 1.16 (at $\varphi_w = 0.135$) to 0.43 (at $\varphi_w = 0.41$) D_{Na}/D_{Cs} varies from 1.36 (at $\varphi_w = 0.135$) to 0.53 (at $\varphi_w = 0.41$).

It is difficult to account for this significant selectivity by the above reasoning. Assuming that diffusion rates are inversely related to ion-site affinity constant, the diffusion sequences obtained should be inverse from the equilibrium affinity sequence predicted by Eisenman's theory, and not these affinity sequences themselves, as they are obtained experimentally.

To interpret the experimental results, it must be assumed that diffusion rates are the direct function of equilibrium affinity sequences, i.e. that the more strongly ions bind to the site, the faster they diffuse. This implies that within the channel the spatial organization of the site, at a given water content, favours the jumping process of a given ion from site to site, as is assumed to be the case in protonic diffusion.

In this case the diffusion sequences will not necessarily differ from equilibrium affinity sequences. In the strict organization of the hydrated choline-phosphate dipole in which there is no free water molecule, an ion, although hydrated, may jump from site to site without dragging the water with it. Such a mechanism would imply a specific geometrical pattern of the interaction between water, ions and sites and a definite relationship between the jump rate and the relaxation time of the sites.

Whatever the precise mechanism involved, the experimental data obtained on this model system clearly raise the possibility that selectivity can arise from the

dynamics of diffusion and not only from equilibrium affinity. This gives some support to the conception of ion selectivity developed in the recent years by Hille [27] and Armstrong [28] on the sodium and the potassium channel of the axon. These authors, although they ascribe the selectivity to the "mouth" of the channel, describe it not in terms of affinity, but of exclusion, the ion being selected as able to cross because of an adequate organization of interactions.

Obviously, the analogy must be restricted to the level of the physico-chemical general principle, since the phosphatidylcholine/water lamellar phase is in no way a model for the particular molecular mechanism underlying the properties of the axon's highly specific proteic channel. Nonetheless, it is clear that lipid/water systems, in allowing the experimental study of diffusion processes in organized hydrophilic structures in which the number and nature as well as the geometrical organization of the interactive site can be controlled, appears to be a useful tool for approaching the difficult study at the molecular level of local physico-chemical parameters which govern selectivity.

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