Hypothesis

THE FUNCTION OF PHOSPHOLIPID POLAR GROUPS IN MEMBRANES

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1. Introduction

In 1968, Pagano and Thompson [1] reported that an electrically silent chloride-flux across a tetradecane and egg phosphatidylcholine bilayer can be very much faster than the Cl^--current calculated from the electrical parameters of the system. Similar results were obtained by several workers and, in 1972, Bangham [2] suggested that the flux of $^{36}Cl^-$ across bilayers is due to the permeation of $H^{36}Cl$. However, arguments used by Toyoshima and Thompson [3] and Singer [4] implied that the permeation of $H^{36}Cl$ can make only a minor contribution to the observed $^{36}Cl^-$-flux. Toyoshima and Thompson [5] using a semispherical bilayer membrane of area as high as 0.3 cm^2, showed that an electrically silent $^{36}Cl^-$-flux is about 10^3-fold larger than the Cl^--current calculated from the electrical parameters Using the 'flip–flop' rates for phospholipids calculated from Kornberg and McConnell [6], Toyoshima and Thompson [7] suggested that the ^{36}Cl-flux might be due to a flip–flop of phosphatidylcholine, acting, in effect, as an HCl carrier. There is now considerable agreement that the flip–flop rates suggested by Kornberg and McConnell on the basis of ESR data are far too high for normal membranes since there is considerable permeation of the ESR-label in the membrane. This paper suggests a modification of the Toyoshima and Thompson hypothesis to explain the high rate of electrically silent chloride-flux, without depending on the improbable flip–flop of the phospholipid. It is suggested that this phenomenon may be an important clue to the functions of other phospholipid polar groups.

2. Previous experiments summarised

Toyoshima and Thompson [5,7] carried out experiments on semispherical bilayers formed from a decane solution of synthetic diphytanoylphosphatidylcholine and on vesicles formed from phosphatidylcholine (hen egg-yolk). Their results may be summarised as follows:

(i) In the semispherical bilayers the electrically silent ^{36}Cl flux, about 10^3-fold larger than the chloride-current, was independent of the applied electrical field.

(ii) The chloride-penetration and proton-penetration are interdependent with a stoichiometry of 1:1 in both the semispherical bilayers and the vesicles, suggesting coupling of H^+ and Cl^-.

(iii) Nitrate in the solution on the outside of either the semispherical bilayer, or the vesicles does not reduce the inward flux of $^{36}Cl^-$; however nitrate on the inside of the membranes of either almost completely prevents inward ^{36}Cl-flux. Thus inward and outward Cl^--fluxes are coupled and nitrate cannot substitute for Cl^-, suggesting a degree of specifity of the phosphatidylcholine.

(iv) Iodide, which is known to bind more strongly to phosphatidylcholine than Cl^- (Jendrasiak [8]) reduces the flux of $^{36}Cl^-$ by more than an order of

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magnitude, presumably by preventing the combination of chloride and phosphatidylcholine.

(v) The $^{36}Cl^-$-flux is markedly affected by the pH external to the bilayers. Over the range from pH 5–8, the exchange-flux decreases by more than one order of magnitude. The permeability of a neutral compound, glucose, is unaffected over the same pH-range, suggesting that the permeability properties of the vesicle bilayer per se do not change with pH. Because of the very low pK of HCl, it would be impossible for pH-effect over this range to be due to an increase in the species-HCl in the aqueous solution. Further, the slopes of the line for the logarithms of both net- and exchange-fluxes, plotted against pH, are –0.5.

(vi) Vesicle dispersions containing 0.5 M KCl show a titration in the pH-range 3.0–7.0, not shown by vesicles in the absence of KCl (e.g., in water or in the present of NaF). Thus, in the low pH-range, proton-uptake appears to be specifically induced by chloride. The slope of the line for proton-binding plotted against the pH is –0.5. Thus the pH-dependence of chloride-dependent proton-binding is the same as the pH-dependence of both the exchange and net chloride-fluxes. These results taken together suggest that H^+ and Cl^--binding by the phosphatidylcholine must precede the transbilayer movement of these species.

3. Hypothesis

Our hypothesis retains the idea suggested by Toyoshima and Thompson [7] that a phospholipid in which the charges of the polar group are neutralised, will have decreased hydrophilicity and may sink back into the hydrophobic region of the bilayer. This idea is exactly similar to the 'bobbing up and down' hypothesis used by Robertson and Boardman [9] to suggest that reduced ubiquinone in its unprotonated (hydrophilic) form moves its polar group into the aqueous region of other polar groups in the membrane but on becoming protonated and losing its hydrophilicity moves back into the lipophilic region.

In the passage of chloride across phosphatidylcholine bilayers, we believe that a proton is likely to associate with the phosphate group of the phosphatidylcholine, with resulting decrease in the zwitterionic properties and increased positive charge around the base to attract a chloride-ion; then the phosphatidylcholine hydrochloride will sink back into the hydrophobic region of the bilayer. In that environment the proton and the chloride (which, as can be seen by building a model, are very close together) will be more likely to form a covalent-bond to give hydrogen chloride than to stay attached to the charges on the polar group.
The completely undissociated hydrogen chloride in the hydrophobic environment will then be free to move by diffusion, while the phosphatidylcholine, having become a zwitterion again, will put its polar group out into the aqueous-phase. Our hypothesis therefore differs from that of Toyoshima and Thompson by eliminating the flip–flop of phospholipid; a diagrammatic scheme for the hypothesis is shown in fig.1.
This explanation fulfills the requirements of Toyoshima and Thompson's observations that the net chloride-flux and its coupled proton-flux must be due either to an HCl-carrier or to molecular HCl. This explanation does not depend on ordinary partitioning of a hydrogen chloride molecule from the fully dissociated H^+ and Cl^- in the water, an event which would occur only very infrequently. The phenomenon whereby the HCl is introduced into the lipophilic region by the polar group of phosphatidylcholine may be termed 'activated partitioning'.

Both HCl-carrier permeation, to get molecular HCl formed within the bilayer, and HCl movement in the lipophilic region, are necessary. On the inner side of the bilayer, in the presence of chloride, some molecules of phosphatidylcholine will also add hydrogen and chloride and, with their non-charged polar groups will therefore be in the lipophilic region. There $^{36}Cl^-$ would exchange for the Cl^- and $^{36}Cl^-$ attached to the polar group would pass to the inner side of the membrane. Thus the passage of Cl^- inward on one side and outward on the other is dependent on the polar groups. The absence of ^{36}Cl-flux when NO_3^- is on the inside of the semispherical bilayer or of the vesicles is probably because no effective nitrate–hydrogen compound can be formed with phosphatidylcholine. The more diffuse negative charge of the NO_3^--ion is probably insufficient to complete the formation of a compound which neutralises the zwitterionic polar group, so phosphatidylcholine remains with its polar group in the aqueous-phase. The HCl pictured as forming in the lipophilic region, presumably cannot escape readily to the inside of the bilayer, i.e., it does not come into contact with the water on the inner side

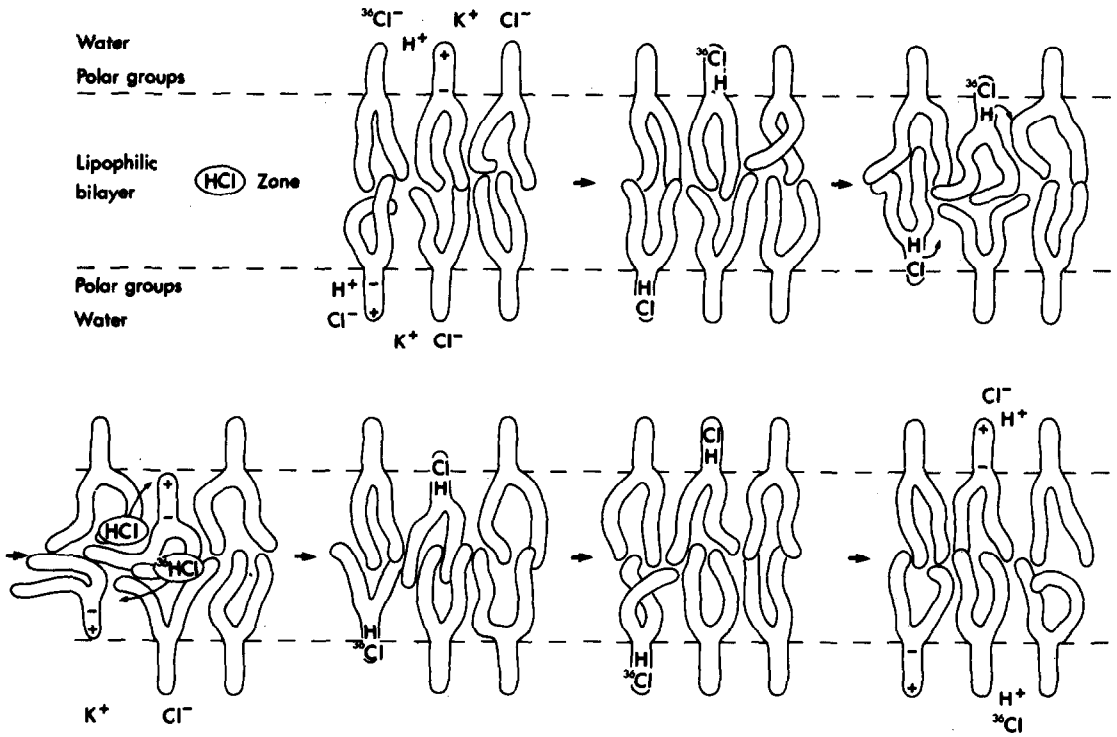

Fig.1. A diagram illustrating the hypothesis, showing the phosphatidylcholine molecules in the bilayer, the combination of H^+ and Cl^- with the polar groups and the formation of hydrogen chloride in the lipophilic zone of the bilayer.

where it would ionise immediately and diffuse in the water. Phosphatidylcholine can apparently act as an effective barrier to the diffusion of the small molecule HCl, presumably due to the packing of the hydrocarbon chains of adjacent lipid molecules near the polar groups. This packing is not destroyed by pH as evidenced by the lack of effect of pH on glucose permeability. Thus, not only the formation of HCl towards the outside of the bilayer is dependent on the combination with 'bobbing up and down' of phosphatidylcholine, but also the escape of hydrogen and chloride on the inside. The vertical movement of the phosphatidylcholine is more likely to be responsible for the Cl^- entry into and exit from the bilayer, than any difference in packing on the two sides. Some small asymmetry may be expected when the ions in the double-layer on one side of the membrane are nitrate and on the other side are chloride. Some difference in symmetry may be expected in vesicles due to the curvature, but not much difference would be expected in the semi-spherical bilayers with areas as large as 0.3 cm^2.

This hypothesis combines several important ideas about the properties of a phosphatidylcholine bilayer:

(i) The effective specificity for the Cl^--ion.

(ii) The tight packing of the hydrocarbon chains and the consequent low permeability to even small molecules like HCl and glucose.

(iii) The importance of the vertical movement of the phospholipids – the bobbing up and down – in taking substances into the lipophilic region of the bilayer.

4. A function for the polar groups of lipophilic substances?

The principle developed here for the phosphatidylcholine molecule, hydrogen and chloride might apply to other ionic compounds at the surface of bilayers. It has been difficult to suggest functions for the polar groups of the phospholipids with their different combinations of charge – the single negative charge of

phosphatidylinositol and phosphatidylglycerol, the negative and positive charges of phosphatidylethanolamine, phosphatidylcholine, phosphatidyl-3′*O*-alanylglycerol and sphingomyelin, the two negative charges and one positive charge of phosphatidylserine and the two negative charges of cardiolipin. It may be that these charges are related to the combining power of these polar groups for particular ions (including protons) and that a degree of specificity results. If decrease in hydrophilic properties follows, the phospholipid probably sinks into the lipophilic region, taking the ion or group with it. There the ion or group would be able to detach if it had some lipid-solubility (e.g., some of the acids of the Krebs cycle) or to interact in a lipophilic environment with lipophilic side-chains on a functional protein. Thus specificity, seen so commonly in membrane transmission, could be due either to the phospholipid specificity or to the protein specificity. This hypothesis for the functions of polar groups may explain also why phospholipids occur in different proportions in membranes of different functions.

The general principle, that lipophilic compounds can move up and down in the membrane with changing hydrophilicity of their polar groups, may be of considerable biological importance. Experiments to verify this suggestion will be carried out in both our laboratories.

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