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Some physico-chemical properties of phospholipids with choline and ethanolamine functional groups. Comparative characteristics and possible biological consequences of distinctions

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Comparative physico-chemical studies were carried out on glycero- and sphingophospholipids with choline and ethanolamine functional groups (phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, and keramideaminoethylphosphonate) at the *n*-heptane/water interface and in bilayer lipid membranes. Experimental results were analysed to determine the possible cause and effect of alterations in phospholipid composition of membranes of marine invertebrates in the course of their evolution.

Introduction

Phospholipids belong to the fundamental structure-forming substances of the bimolecular lipid leaflet of biological membranes, the principal of which are choline- and ethanolamine-containing phospholipids. They amount to 70–90% of total phospholipids (with the exception of many bacterial and plant membranes). In the membranes they are represented by phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin and keramideaminoethylphosphonate (KAEP). The latter lipid belongs to the phosphonolipids. It is found in considerable quantities in some marine invertebrates [1]. The difference between phospholipids and phosphonolipid lies in the type of bond between phosphorus and carbon atoms: in KAEP they are linked directly and in phospholipids through oxygen.

It has been established in [2] that in the course of evolutionary development of marine invertebrates changes in phospholipid composition of the membrane were in the direction of increase in choline-containing phospholipid. This was due to PC increasing while PE remained relatively constant, and also to the substitution of KAEP for sphingomyelin. As a result of these changes in phospholipid composition, the lipid matrix of the membranes in more advanced groups of marine invertebrates were enriched with the choline-containing phospholipid on the one hand and, on the other, with glycerophospholipids.

Enrichment of marine invertebrate membranes with choline-containing phospholipids was explained in Ref. 2 by the considerable differences in temperatures of phase transition of structures of synthetic PC and PE having identical saturated hydrocarbon chains, while the driving force in the evolutionary reconstruction in phospholipid composition was considered to be temperature change in the sea water. Nevertheless, differences in phase transformations do not explain the causes and significance of preferential accumulation in marine invertebrate membranes of glycerophospholipids over sphingophospholipids.

The present work has investigated choline- and

ethanolamine-containing phospholipids in identical conditions and on various models of biological membrane, with the aim of obtaining lacking information on the physico-chemical properties of phospholipids, and an understanding of the causes and significance of evolutionary reconstruction in phospholipid composition of marine invertebrates.

Materials and Methods

Isolation and identification of phospholipids. PC and PE from egg yolk were obtained chromatographically on aluminium oxide and silica gel (L40/100 μ, Czechoslovakia). Sphingomyelin from bull brain and KAEP from the mussel *Mythilus grayanus* were extracted after alkaline saponification of total lipid extracts on silica gel by the method proposed in Ref. 3. Analysis of methyl esters of fatty acids and sphingosine bases was accomplished as described in Ref. 3. In chromatographically pure phospholipids the following fatty-acid residues were found (in wt.%): PC, 16 : 0 (35.8), 16 : 1 (1.5), 18 : 0 (18.3), 18 : 1 (37.4), 18 : 2 (6.8); PE, 16 : 0 (21.1), 18 : 0 (39.1), 18 : 1 (35.1), 18 : 2 (4.3); sphingomyelin, 16 : 0 (10.6), 16 : 1 (3.4), 17 : 0 (4.5), 18 : 0 (30.3), 18 : 1 (9.1), 19 : 0 (1.7), 20 : 0 (1.1), 22 : 0 (1.1), 22 : 1 (3.2), 24 : 0 (3.7), 24 : 1 (15.6), 25 : 1 (2.2), 26 : 1 (6.4); KAEP, 16 : 0 (79.0), 16 : 1 (12.5), 17 : 0 (1.3), 18 : 0 (3.7). Average molecular weights of phospholipids were calculated for mentioned fatty acid composition of lipids.

The n-heptane/water interface. Interfacial tension was measured by the drop-volume method. Experimental setup was described earlier in Ref. 4.

Lipids were stored as solutions in benzene or in mixture of chloroform and methanol at -7°C to -10°C. Immediately before experiments, initial phospholipid solutions were evaporated to dryness at room temperature, blown with argon. Dried phospholipids were weighed and dissolved in the necessary volume of *n*-heptane. *n*-Heptane (chromatographically pure) was twice distilled and saturated with water.

Water solutions of sodium salt of tetraphenylborate (TPB-Na) (analytical grade, G.D.R.) and KCl (chemically pure) were prepared in twice-distilled water, previously saturated with alkane. Inorganic salt was twice recrystallized from water solution and roasted at 600°C for 8 h. HCl (chemically pure, twice distilled) or NaOH (chemically pure) were used to change the pH of water solutions.

Bilayer lipid membrane. Lipid membranes were formed by the method of Muller et al. in an aperture (diameter 1 mm) located in the partition of a two-chamber cell of Teflon. The cell was thermostated. All experiments were run at 25.0 ± 0.5°C. The experimental technique is described in greater detail in Ref. 5.

Solutions for preparation of membranes from PC and PE were made on *n*-decane (chromatographically pure); membranes from sphingomyelin and KAEP were prepared on mixtures of chloroform/methanol/*n*-decane (1 : 2 : 1, v/v) and *n*-dodecane/dioxane (2 : 1, v/v), respectively. The solvents used were chemically pure. Concentrations of PC, PE and KAEP in solution were 20 mg/ml; sphingomyelin, 10 mg/ml. In the latter case cholesterol (Serva) was added to give a 1 mg/ml concentration.

Water buffer solution contained 5 mmol/dm^3 Tris-HCl (Reanal) and 5 mmol/dm^3 citric acid (chemically pure). Solution pH was changed by addition of HCl (chemically pure) and NaOH (chemically pure).

Membrane capacitance was measured at 310 Hz by means of a.c. bridge R-5021. Kinetics of tetraphenylborate anions (TPB^-) passage through lipid bilayers were studied by current relaxation technique. The method of measurement was described in Ref. 6. Single voltage pulses (50 mV) were applied to the membrane by pulse generator G5-46, and membrane current variations were recorded by storage oscilloscope C8-13. Platinum grid electrodes were used for measuring.

Evaluation of the experimental parameters

(A) Areas (S) occupied by phospholipid molecules in the adsorption layer were computed from the Gibbs' equation

$$S = \frac{RT}{\partial\pi/\partial \ln C} \qquad (1)$$

from diagrams presented in Figs. 1 and 2. In the equation, π is the interfacial pressure equal to the difference in interfacial tensions at the *n*-heptane/

water interface in the absence and presence of phospholipid in non-aqueous phase; C is the concentration, R the gas constant, T the absolute temperature.

(B) Energy adsorption (ΔE) of phospholipids from the bulk non-aqueous liquid to the *n*-heptane/water interface was calculated using the equation:

$$-\Delta E = RT \ln \partial\pi/\partial C \tag{2}$$

from dependence diagrams π-C (not presented). The calculations were made, using as standard state for the interface $\pi = 1$ mN/m and for the bulk of *n*-alkane a hypothetical state in which the product of mole fraction and activity coefficient of phospholipid is unity [7].

(C) The concentration at which phospholipids begin to form micelle (critical micelle concentration) is found in Figs. 1–3 from the break in the curves. The number of molecules, n (aggregation number), forming micelles was calculated using the Freundlich adsorption isotherm [8]:

$$\pi = \pi_a + KC^{1/n} \tag{3}$$

where K is the constant, π_a the interfacial pressure, corresponding to the beginning of aggregate formation. To determine n graphs of dependence of interfacial pressure on the concentration in bilogarithmic coordinates were plotted (not presented). The aggregation number was found from the tangent to the linear parts of the curves at $\pi \gg \pi_a$ [8].

(D) The rate constant of TPB^- anions transport through the interior of lipid bilayer at zero transmembrane potential (k_i) and the partition coefficient of anion between the membrane and the bulk water solution (β) were calculated using equations [6,9]:

$$I_0 = zFA\beta C(k_i' - k_i'') \tag{4}$$

$$\tau = 1/(k_i' - k_i'') \tag{5}$$

$$k_i' = k_i \exp(zU/2) \tag{6}$$

$$k_i'' = k_i \exp(-zU/2) \tag{7}$$

where z is the ion charge, F the Faraday constant, C the concentration of salt in bulk water solution, A the lipid bilayer area, I_0 the initial current observed at the moment of imposition of voltage pulse on the lipid bilayer, τ the relaxation time, k_i', k_i'' the rate constants of ion translocation through the membrane from right to left and back when lipid bilayer is under electric field, $U = F\Delta\zeta/RT$, $\Delta\zeta$ is the transmembrane potential.

Relaxation time τ was found from the tangent to the linear part of the curve plotted in coordinates $\log I - t$ (presented in Ref. 10). Then, using in sequence Eqns. 5, 6, 7 and 4, k_i and β were calculated.

The rate constants of ion desorption k_d were determined as in Ref. 9 * from the deviation of membrane current from diffusion kinetics, comparing experimental dependencies $I - 1/\sqrt{t}$ with theoretical ones, calculated by equation:

$$I(t) = I_0 \frac{2\sqrt{w}}{\pi} \int_0^\infty \frac{\exp[-U^2 kt]\,dU}{wU^2\left[g(U^2-1)-1\right]^2 + (U^2-1)^2} \tag{8}$$

where $w = D/k\beta^2$; $g = k/k_d$; $k = k_i' + k_i''$; D is the diffusion coefficient, t the time.

Rate constants of ion adsorption were found from equation [6]:

$$k_a = \beta k_d. \tag{9}$$

Results and Discussion

Because of significant differences in the methods used and in the analysis and interpretation of the experimental data, obtained with various models of biological membranes, for convenience, the experimental results will be examined separately.

n-Heptane/water interface

The adsorption isotherms of PC and PE at the *n*-heptane/water interface are shown in Figs. 1 and 2. The areas occupied by PC and PE molecules in the adsorption layer at concentrations near the critical micelle concentration, as calculated (Eqn. 1) from the graphs, were 120 and 85 Å^2, respectively. In the monolayers at the air/water interface at identical pressures, the PE molecule

* Our variant of the method has been described in greater detail in Shchipunov, Yu.A. and Drachev, G.Yu. (1985) Elektrokhimiaya, in the press.

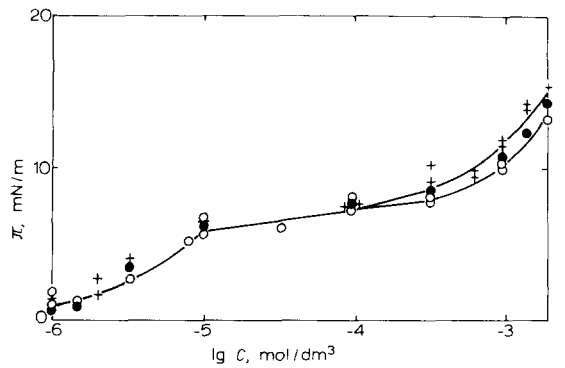

Fig. 1. Dependence of interfacial pressure at the *n*-heptane/water interface on the logarithm of PC concentration in non-aqueous phase at various pH of water solution: ●, 2.0; ○, 7.0; +, 12.0.

also occupied less area than the PC molecule [11,12].

Variation of the pH of water solutions from 2 to 12 influenced only PE adsorption. Shifting pH from 10 to 12 in the presence of lipid in nonaqueous phase (Fig. 2) led to increase of interfacial pressure at the *n*-heptane/water interface. Similar results were obtained on PE monolayers at the air/water interface and are explained by the charging of the lipid molecule by phosphate group due to deprotonization of the amino group [12,13].

At neutral pH (5–7) of water solution and identical concentrations of PC and PE in nonaqueous phase, interfacial pressure at the *n*-heptane/water interface differ insignificantly (Figs. 1 and 2). Lipid adsorption energies (Eqn. 2) from *n*-heptane at the interface of two immiscible liquids are equal to −42.5 and −43.6 kJ/mol, respectively.

Breaks in the curves (Figs. 1 and 2) are caused by micelle formation of phospholipids in nonaqueous solutions [8]. PE began to form aggregates at concentration $(5–7)\cdot 10^{-6}$ mol/dm^3 and PC at 10^{-5} mol/dm^3. The aggregation numbers (Eqn. 3) found on the graphs of interfacial pressure dependence on phospholipid concentrations plotted in bilogarithmic coordinates [8], are equal to 5 for both lipids.

Experimental data on adsorption of sphingomyelin and KAEP at the *n*-heptane/water interface are presented in Fig. 3. As calculated from the graphs, characteristics of lipid adsorption and aggregation, that is, the energy of adsorption (Eqn. 2), critical micelle concentration, and aggregation numbers (Eqn. 3) are identical for both lipids and equal to −36.6 kJ/mol, $3\cdot 10^{-5}$ mol/dm^3, and 2, respectively.

Table I shows experimental results at the *n*-heptane/water interface. Clearly, differentiation

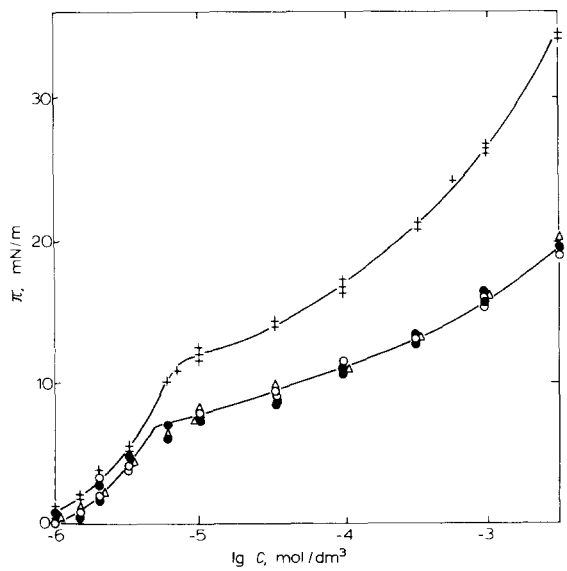

Fig. 2. Dependence of interfacial pressure at the *n*-heptane/water interface on the logarithm of PE concentration in non-aqueous phase at various pH of water solution: ●, 2.0; ○, 7.0; △, 10.0; +, 12.0.

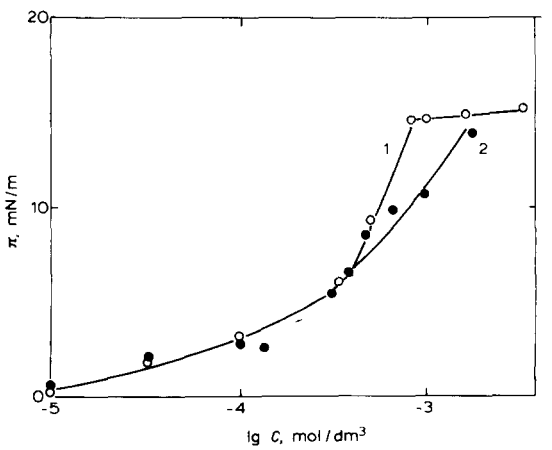

Fig. 3. Dependence of interfacial pressure at the *n*-heptane/water interface on the logarithm of sphingomyelin (1) and KAEP (2) concentration in non-aqueous phase. Water solution (pH 7.0) contained 10^{-3} mol/dm^3 KCl.

in surface-active properties of phospholipids within classes are insignificant. Thus, sphingomyelin and KAEP have identical surface-active properties, while PC and PE have similar parameters of adsorption. Certain differences in critical micelle concentration of PE and PC may be explained by the presence of intermolecular hydrogen bonds in the former lipid and their lack in the latter. The existence of these differences in intermolecular interaction of glycerophospholipids at the *n*-heptane/water interface has been shown by us [14]. Another proof of the existence of intermolecular hydrogen bonds between ethanolamine and the phosphate group in PE structures and their absence in PC ones is presented in Refs. 13, 15 and 16.

Noticeable differences in adsorption and aggregation characteristics are observed only between lipids of different classes. Concentrations at which sphingomyelin and KAEP begin to change interfacial pressure at the *n*-heptane/water interface are an order of magnitude higher than those of PC and PE. Accordingly, lower adsorption energy was found for sphingophospholipids (-36.6 kJ/mol) than for PC and PE (-42.5 and -43.6 kJ/mol, respectively), and less tendency to aggregate formation was also found. The data presented indicate that sphingophospholipids are less surface-active than glycerophospholipids.

The insignificant distinctions between the behaviour of sphingomyelin and KAEP and also between PC and PE at the *n*-heptane/water interface and, likewise, the marked distinctions in the adsorption characteristics of glycero- and sphingophospholipids point to the contribution in intermolecular interactions of some other functional groups besides phosphorylcholine and phosphorylethanolamine. We may assume it, in the case of sphingophospholipids to be the amide area of keramide, lying between phosphorylcholine or ethanolaminephosphonate and the hydrocarbon chains where intra- and intermolecular hydrogen bonds are formed [17,18]. In glycerophospholipids in the area of glycerine residue there are no such bonds. The observed distinctions in intermolecular interactions between sphingo- and glycerophospholipids may explain their differing behaviour at the boundary of two immiscible liquids.

TABLE I

BULK AND INTERFACIAL CHARACTERISTICS OF LIPIDS

PL, phospholipid, CMC, critical micelle concentration, SM, sphingomyelin.

PL	$-\Delta E$ (kJ/mol)	S (Å^2)	CMC (mol/dm^3)	n
PC	42.5	120	10^{-5}	5
PE	43.6	85	$(5–7)\cdot10^{-6}$	5
SM	36.6	–	$3\cdot10^{-5}$	2
KAEP	36.6	–	$3\cdot10^{-5}$	2

Bilayer lipid membranes

Glycerophospholipids, PC and PE, are widely used for lipid bilayer preparation. Conditions of formation and properties of membranes from them have been well described in detail (see, for example, Refs. 19 and 20). Less well studied are sphingomyelin membranes. Data on lipid bilayer formation from KAEP are not available in the literature.

We were not successful in forming stable lipid bilayers from sphingomyelin. The lifetime of membranes from this lipid was at best a few seconds. Only addition of cholesterol gave us stable film. A similar situation was observed in the case of phosphatidylcholine: lipid bilayer in the absence of cholesterol was formed with great difficulty and was not stable [20].

In this connection, the dependence existing in the cell membranes between sphingomyelin and cholesterol content may be noted. It manifests itself in the fact that on membranes with relatively high concentration of sphingomyelin, e.g., plasmatic membranes, the highest ratios of cholesterol to total phospholipid content are observed, while on membranes where there is little or no sphingomyelin, e.g., on mitochondrial membranes, this ratio is minimal [13,17,21,22]. Experimental data obtained on model systems enable us to suggest that the observed dependence is caused by the necessity for stabilization of the lipid matrix of the cell membranes containing sphingomyelin.

Both ethanolamine-containing phospholipids form equally stable membranes which are much more stable than lipid bilayers from sphingomyelin. Apparently, as film is formed from KAEP, additional stabilizing bonds occur which are ab-

sent in the formation of bilayer structure from sphingomyelin. In the case of PE, membranes are stabilized by intermolecular hydrogen bonds formed by phosphate and ethanolamine [13]. Probably, similar intermolecular interactions take place in KAEP structures. In sphingomyelin and PC the choline group is not capable of forming intermolecular hydrogen bonds [13,15,16]; therefore, other conditions being equal, interactions between molecules will be weaker than on phospholipids with an ethanolamine group.

The formation of bilayer membranes on a level with dependence on the nature of the membrane-forming phospholipid depends on temperature and on pH of the water solution. Experimental data are presented in Table II.

Acidification and alkalization of water solutions did not influence lipid bilayer formation from PC and PE (they were stable at pH range 2–12) but noticeably affected lipid bilayer formation from sphingophospholipids. Membranes from KAEP and a mixture of sphingomyelin and cholesterol are formed in acid and neutral aqueous media, while in alkali solutions (pH $\geqslant$ 8) they do not form.

This fact may be explained as follows. In sphingophospholipids, as mentioned above, intermolecular hydrogen bonds are formed between hydroxyl and amide groups. The destabilizing action of alkali may be the cause of their rupture as a result of dissociation of the corresponding functional groups. This yet again confirms our conclusion on the significant contribution of the amide area of keramide in the intermolecular interaction of sphingophospholipids, made above from data on lipid adsorption at the *n*-heptane/water interface.

Figs. 4 and 5 show temperature dependencies of capacitances of lipid bilayers from sphingomyelin and KAEP, Breaks on curves may be due to phase transition of the membrane from liquid-crystalline to gel-like state [23]. In the case of sphingomyelin (Fig. 4) several breaks were observed in a wide interval of temperatures, which may be caused by heterogeneity of the fatty acid content of the natural lipid. Such a situation has been observed [17,24]. On lipid bilayer made from KAEP, containing mainly palmitic acid residue (79%), the breaks are more clearly expressed (Fig. 5). At temperatures lower than that of the break (the temperature phase transition, T_p), lipid bilayer 'solidifies', loses stability and ceases to form. A similar picture is observed on lipid bilayers from glycerophospholipid with saturated fatty acid residues in phase transition from liquid-crystalline to gel-like state [19,20]. Evidently, the decline in temperature caused this effect on lipid bilayer from KAEP. At least, the phase transformation of membranes from natural KAEP takes place near T_p dipalmitoylglyceroethanolaminephosphonate (61.4°C [25]) and dipalmitoylPE (63°C [26]).

Variation in the pH of the aqueous solution from 2 to 8 did not affect the temperature of phase transformation (T_p) of lipid bilayers made from PC and PE [13]. Characteristics of sphingomyelin membranes (Fig. 4) were also unchanged. In the case of KAEP, acidification of water solution leading to the decrease of pH from 6.6 to 2.0 caused displacement of the T_p from 58° to 38°C (Fig. 5, Table II).

TABLE II

PHASE TRANSITION TEMPERATURES, CONDITIONS OF FORMATION, AND CHARACTERISTICS OF LIPID BILAYERS

T_p was at pH 7.0. The initial solution for sphingomyelin (SM) contained 1 mg/ml of cholesterol. PL, phospholipid.

PL	T_p (°C)	Formation range of pH	C (μF/cm^2)
PC	−10 [26]	2–12	0.34
PE	0 [24]	2–12	0.33
SM	33.5 [24]	2–8	0.33
KAEP	59	2–8	0.31
	38 [a]		

[a] at pH 2.0.

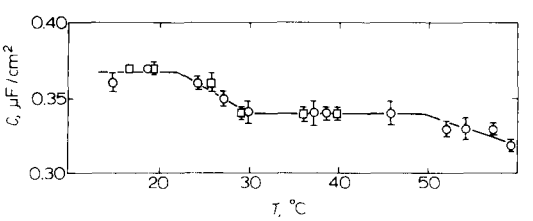

Fig. 4. Dependence of capacitance of lipid bilayer made from sphingomyelin on temperature at pH of water solution: ○, 6.5; □, 2.0.

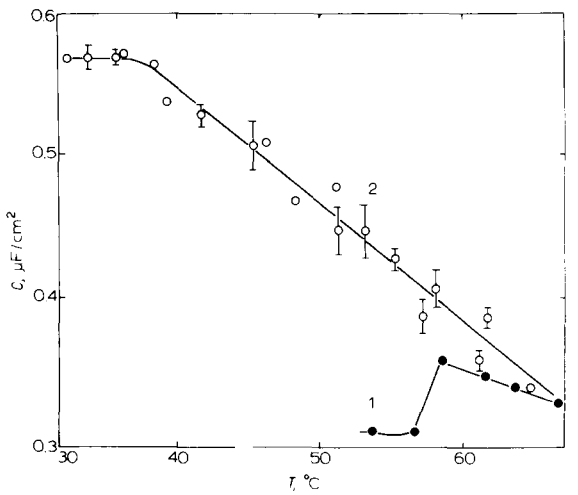

Fig. 5. Dependence of capacitance of lipid bilayer made from KAEP on temperature at pH of water solution 6.5 (1) and 2.0 (2).

The data presented in [25] on temperatures of phase transition of structures of dipalmitoylPE and its phosphonic derivative show that T_p of these lipids are practically identical. On this basis, a conclusion was drawn on the independence of lipid phase behaviour from the type of bond between the phosphorus atom and ethanolamine. However, our data indicate that in phospholipid molecules substitution of the ester bond between phosphoric acid and ethanolamine for phosphonic acid is reflected in the phase behaviour of the lipid. Experimental verification of this fact is shown by the decrease of T_p in lipid bilayer made from KAEP by 20°C when the water solution was acidified to pH 2.0 (Fig. 5). In phospholipids this was not observed (Fig. 4) [13].

We connect the distinctions found between phospho- and phosphonolipid with differences in the chemical bonds between phosphorus and carbon atoms. Apparently, the type of bond determines the conformations and intermolecular interactions in phospholipid polar regions. This is indicated by distinctions found in NMR spectra of PE and its phosphonic derivative [27].

Interaction of phospholipids with hydrophobic ions

Experiments were carried out at the *n*-heptane/water interface and on lipid bilayers. Anions of tetraphenylborate were used as hydrophobic ions.

Fig. 6 shows the dependence of the increment in interfacial pressure at the *n*-heptane/water interface when TPB-Na was added to water solution on the logarithm of concentration of PC (solid lines) and of PE (dashed line) in non-aqueous solution. Clearly, joint adsorption at the boundary of two immiscible liquids, PC and PE with TPB^- anions markedly differed: in the presence of PC and TPB-Na, interfacial pressure increased more than in the presence of PE. In the last case the growth of π was observed only at relatively large concentrations of TPB-Na and lipid in the system.

In Table III rate constants of TPB^- anion passage through bilayer membranes made from PC and PE, found from current relaxation experiments (Eqns. 4–9) are presented. At neutral pH of water solution, significant differences were observed in the parameters of ion translocation through lipid bilayers from various phospholipids. We note the following regularity: lower rate constants of ion passage through lipid bilayer (k_i) and desorption (k_d) on PC membrane correspond to higher values of the partition coefficient (β) of anions between surface lipid bilayer and bulk water solution in comparison with similar parameters on PE membranes. The character of differences between rate constants and β indicates a stronger interaction of penetrating hydrophobic anions with the surface of membranes made from PC than from PE. Variation of interfacial potential at the PE membrane/water solution interface within

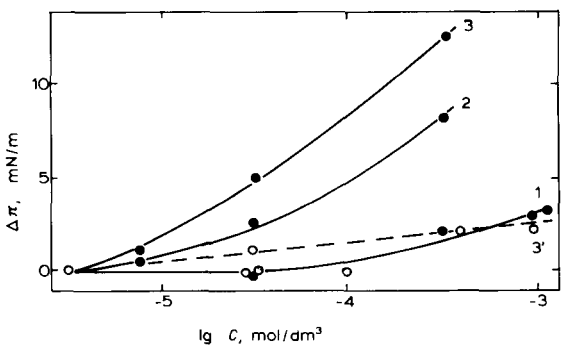

Fig. 6. Dependence of the increment of interfacial pressure at the *n*-heptane/water interface, obtained by addition of TPB-Na to the water solution, on the logarithm of PC (———) and PE (- - - - -) concentration in non-aqueous phase. The aqueous solution contained: 1, 10^{-4}; 2, 10^{-3}; 3 and 3', 10^{-2} mol/dm³ TPB-Na.

100–200 mV by changing the charge of the functional groups as a result of shifted pH of water solution led to corresponding changes in kinetic parameters of ion transport (Table III), but changes in rate constants and partition coefficient did not attain the differences in similar parameters on PC and PE membranes at neutral pH. Since differences in surface potentials between egg PC and PE monolayers attained only 20 mV [12], differing interaction of TPB^- anions with functional groups of phospholipids cannot be explained from an electrostatic position.

The regularity noted above in changing kinetic parameters of TPB^- ion passage through lipid bilayer by sign of charge of the PE molecule and by the substitute of phosphorylethanolamine for phosphorylcholine is illustrated in Fig. 7 by the relative changes in the profile of potential energy of TPB^- anion in the membrane. In the construction the arbitrary profile of potential energy of the TPB^- ion in the lipid bilayer made from PE at pH 6.6 was taken as a basis. Relative changes in the height of potential barriers were found from the ratio of rate constants by the equation:

$$k_1/k_2 = \exp\left[\frac{\Delta\phi_2}{\Delta\phi_1}\right] \qquad (10)$$

where $\Delta\phi$ is the activation energy of the process.

The diagram (Fig. 7) shows that on membranes made from PC, the potential minima at the interfacial boundaries were deeper than on membranes made from PE at various charges of the lipid molecule. The adsorbed ion is bound by choline [28] and just as it penetrates the lipid bilayer with difficulty, it finds difficulty in desorbing the water solution. The reason for differences in interaction

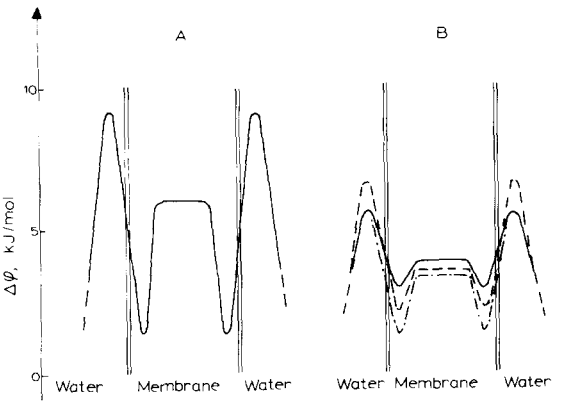

Fig. 7. Profiles of potential energy of TPB^- anion in bilayer membranes from PC (A) and PE (B). Positively charged (— — —), neutral (- - - - -), and negatively charged (– · – · –) membranes. See text for further details.

of PC and PE with TPB^- ions at the *n*-heptane/water interface (Fig. 6) and with the surface of the bilayer membrane (Fig. 7, Table III) becomes clear if we take into account the nature of anions and lipid functional groups. Choline and TPB^-, because their charged centres are surrounded by methyl and phenyl groups, are hydrophobic. In water solution there is a strong attraction of nonpolar groups for one another. Since methyl groups in ethanolamine are absent, TPB^- anions with $-\overset{+}{N}H_3$ should mainly interact electrostatically. The hydrophobic interactions as established in Ref. 29 are an order of magnitude stronger than electrostatic. This leads to the revealed differences between PC and PE in interaction with lipophylic ions at the membrane/water and *n*-heptane/water interfaces [10].

Accordingly, we see from the experimental material that the physico-chemical properties of

TABLE III

KINETIC PARAMETERS OF TPB^- ANION TRANSPORT THROUGH LIPID BILAYERS

PL, phospholipid.

PL	pH	k_d (s^{-1})	k_a (m/s)	k_i (s^{-1})	β (m)
PC	6.6	0.05	$1.3\cdot10^{-6}$	0.65	$2.6\cdot10^{-5}$
PE	2.0	1.3	$1.4\cdot10^{-5}$	56	$1.1\cdot10^{-5}$
	6.6	6.5	$3.0\cdot10^{-5}$	40	$4.6\cdot10^{-6}$
	12.0	1.8	$3.0\cdot10^{-5}$	17	$1.7\cdot10^{-5}$

choline- and ethanolamine-containing phospholipids on the one hand, and glycero- and sphingophospholipids on the other, differ essentially. These differences allow us to take a new look at the causes and consequences of evolutionary reconstruction in the phospholipid composition of marine invertebrates [2].

Formation and stability of lipid membranes

The barrier properties of cell membranes and, consequently, the vital functioning of the whole cell are dependent on the ability of phospholipid to form stable bilayer structures capable of resisting external influences. Physico-chemical investigations, including the present work, show that properties of lipids are largely determined by the structure of polar regions. In the course of evolutionary development phospholipids with functional groups that form stable bilayer membranes will be selected. Indications of lipid choice may be found by analysis of composition and content of phospholipids in membranes of phylogenically associated groups of animals. Such a possibility is offered by the marine invertebrates [2].

In the phylogeny of marine invertebrates, among phospholipids, prevalent significance is acquired by PC and PE, whose relative contents grow to attain as much as 80% of the total phospholipid content. Compared with sphingophospholipids, the share of glycerophospholipids increases. Comparative studies, carried out in the present work, show the formation and stability of lipid bilayers from sphingophospholipids to be sensitive to the pH of water solution. This might serve as a basis for the restriction of widespread sphingophospholipids in biological membranes, and change in the pH of sea water may be the driving force of evolution. It is a common view [30,31] that the pH of sea water has changed in the course of evolution from 6.0 to the present level of 8.2–8.3. Experiments on model membranes show that sphingophospholipids form lipid bilayers at pH $\leqslant$ 8.0.

Unlike sphingophospholipids, the stability and phase state of membranes made from PC and PE in the same range were not sensitive to alteration of the pH of water solution. This is caused by the ability of the glycerophospholipid molecules to retain electroneutrality in the broad range of pH. Adsorption characteristics of PC, for instance, did not change when pH was varied between 2 and 12 (Fig. 1). A change appeared on PE molecules only at pH > 10 (Fig. 2). It is logical to suppose that evolutionary reconstruction in phospholipid composition of membrane of marine invertebrates toward principal enrichment of them with PC and PE was caused by less dependence of their bilayer structures on changes on environmental conditions.

The role of functional groups of phospholipid in the interactions with substances

For PC and PE an asymmetric distribution is characteristic in biological membranes, including the membranes of marine invertebrates [32]. It is expressed in enrichment of the inner (cytoplasmic) surface of the cellular wall by lipids with the ethanolamine functional groups and of the outer surface with the choline group [33]. As we have shown in the present work, at the interface of two immiscible liquids including lipid bilayers PC interacts more strongly with hydrophobic anions than PE. This may have great significance for adsorption and for binding biologically important substances at the surface of cell membranes. For example, the differences in hydrophobic interactions may promote the selective separation of substances and asymmetrical distribution of proteins in biological membranes, which is especially important for the functioning of the enzymatic systems. The contrary is not infeasible, that is, in the case of formation of frame of biological membranes by proteins, the differences in interaction of ethanolamine and choline with hydrophobic parts of proteins may lead to revelation in membranes of asymmetrical distribution of phospholipids. It is difficult to foresee all possible variants of the manifestation of peculiarities of PC and PE interactions with substances. But, without doubt, they must take place and must have considerable influence on many processes occurring in the living cell.

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