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SOLUBILITY OF PHOSPHOLIPID POLAR GROUP MODEL COMPOUNDS IN WATER

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Summary

The aqueous solubilities of the Na^+ and Ca^{2+} salts and the free acid forms of phosphorylcholine, phosphorylethanolamine and D,L-phospho-serine, respectively, were found to be below the polar group concentrations calculated for bilayers of the corresponding phospholipids.

It has been stated that the concentration of lipid head groups in bilayer membranes corresponds to a bulk electrolyte concentration of 5 to 10 M [1, 2]. However, it has been unknown whether this concentration range is below or above the bulk solubility of phospholipid polar groups in water.

Table I contains the following data. The experimentally determined solubilities of the Na^+ and Ca^{2+} salts and the free acid forms of phosphorylcholine, phosphorylethanolamine and D,L-phospho-serine, respectively; the apparent concentrations of these polar group model compounds in the crystalline state, as calculated from reported crystal densities; the apparent polar group concentrations in liquid crystalline bilayers of the corresponding phospholipids, as calculated from published hydration data.

Polar group concentrations calculated for phospholipid bilayers were between 4.6 and 5 M, and those calculated for the crystalline state of the polar group model compounds were between 4.8 and 11 M. The aqueous solubilities of the polar group model compounds were lower (0.04 to 4 M). These latter values probably were still too high because these compounds, when compared with the corresponding phospholipids, contain one additional dissociating group on the phosphate moiety. Such charged groups are preferred sites of hydration [3].

TABLE I

CONCENTRATION VALUES OF PHOSPHOLIPID POLAR GROUP MODEL COMPOUNDS

Materials and methods were as follows.

Materials. Phosphorylcholine (Ca^{2+} salt), phosphorylethanolamine (acid form) and D,L-phospho-serine (acid form) were purchased from Sigma, Munich, Serva, Heidelberg, and Fluka, Neu-Ulm, respectively. The Na^+ salt and the free acid form of phosphorylcholine were prepared by addition of stoichiometric amounts of Na_2SO_4 and H_2SO_4, respectively, to aqueous solutions of Ca^{2+} salts, followed by precipitation with propanol-2. The Na^+ and Ca^{2+} salts of phosphorylethanolamine and D,L-phospho-serine were isolated in the same way after addition of stoichiometric amounts of NaOH or $CaCl_2$, respectively, to the free acid forms. All compounds were recrystallized from water/propanol-2 mixtures until homogeneity was achieved by two criteria: a single spot in paper chromatography and a free phosphate value of less than 1% of the value for total phosphate content (see below). The flame-photometric analysis of the various Na^+ and Ca^{2+} salts gave satisfactory values, with the exception of the Ca^{2+} salt of phosphorylethanolamine which, after preparation by the above procedure, contained only 0.2 mol Ca^{2+} per mol of total phosphate.

Determination of solubility. An excess of the solid compound to be tested was kept, with occasional shaking, in aqueous suspension until saturation was reached (4 days, at the desired temperature). Solid material was removed by centrifugation. Aliquots of the supernatant solutions were assayed for free and for total phosphate content, as indicated below. Decomposition was below 2% in all cases, when the content of free phosphate is taken as a measure of decomposition.

Analytical procedures. The solvent system for paper chromatography consisted of isobutyric acid/1 M ammonia, (5: 3, v/v). Detection was by the ninhydrin and/or the Hanes-Isherwood reagent [7]. Free phosphate was determined by a colorimetric assay [8]. The amount of total phosphate was determined by the same assay [8] after ashing with $Mg(NO_3)_2$ [9].

Calculations. Concentration values for the crystalline state of the model compounds were calculated by division of the reported crystal densities by (molecular weight $\times 10^{-3}$). In the case of phosphorylcholine, the crystal density has apparently so far not been reported, and the reported value for L-α-glyceryl-phosphorylcholine [10] has been used instead. Polar group concentrations of lipid bilayers were calculated by division of 55.5 by the number of water molecules in the inner and the main hydration shells [5]. The hydration values used had been determined by deuteron magnetic resonance spectroscopy. Similar values have been obtained by a variety of other methods [11].

Concentration of								
Saturated aqueous solutions (experimental, 20° C)*			Crystalline substances (calculated)			Liquid-crystalline lipid bilayers (calculated)		
Substance	Cation	Concn. (M)	Substance	Cation	Concn. (M)	Substance	Cation	Concn. (M)
Phosphoryl-choline	H^+ Na^+ Ca^{2+}	3.0 0.4 0.8	L-α-glyceryl-phosphoryl-choline [10]	H^+	4.8	Egg lecithin [5]	**	4.6
Phosphoryl-ethanolamine	H^+ Na^+	1.4 4.0	Phosphoryl-ethanolamine [12]	H^+	11	Egg phospha-tidylethanol-amine [5]	**	4.6
D,L-Phospho-serine	H^+ Na^+ Ca^{2+}	0.15 1.2 0.04	D,L-Phospho-serine·H_2O [13]	H^+	8.2	Bovine phos-phatidyl-serine [5]	Na^+	5.0

*Solubility values were 10 to 20% higher at 37° C and between 20 to 50% lower at 0° C.
**Not indicated in ref. 5.

From the data of Table I it is apparent that the lipid polar group concentrations of bilayer membranes are above the bulk saturation concentrations of the corresponding polar group model compounds. This near-crystalline solution state of the polar groups might conceivably restrain phospholipid bilayer fluidity. Indeed, some correlation between the present solubility data and known polar group effects on bilayer fluidity is possible. The addition of calcium ions to phosphatidylserine or phosphatidic acid bilayers soldifies the bilayer structure, including the hydrocarbon portion, and shifts T_c temperatures to higher values [4]. This might correspond to the 30-fold lower solubility of the Ca^{2+} salt of D,L-phospho-serine relative to the Na^+ salt (Table I). The very low solubility of the Ca^{2+} salt of phosphoric acid, which is a primitive model compound for the polar group of phosphatidic acid, is well known. The free acid form of phosphatidylserine fails to form fluid bilayers in excess water, whereas the Na^+ salt readily does [5]. It might be a possible analogy that the free acid form of D,L-phospho-serine was about 8-fold less soluble than the Na^+ salt (Table I).

More generally, the present results might be related to the principle of opposing forces by which micelle formation can be thermodynamically described [6]. The oversaturated bilayer polar groups will tend to dissolve in excess water, this repulsive force being more than compensated for by the attractive force originating from the positive entropy term of hydrophobic interactions between the lipid fatty acyl chains.

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