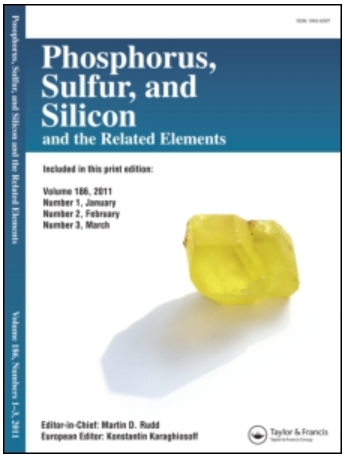

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Phospholipid-Alcohol Interactions: Effects of Chain-length and Headgroup Variations

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Introduction: Solvated phospholipids exhibit a rich polymorphism which depends not only on the lipid chemical structure but also on the characteristics of the bathing solution [1]. This is due to the strong and influential interactions between the lipid headgroups and the solvent or solute molecules. Particularly efficient in this respect are various cationic molecules or molecules with a high propensity to hydrogen bond formation which all bind avidly to the lipid phosphate groups. The di- and polyvalent cations, for example, are electrostatically attracted by the negative electronic cloud around the (typically ionized) PO_4^--group; protons donating or accepting molecules, such as the amino-compounds or the molecules with the readily accessible OH-residues, bind directly to the OH-groups on the phosphates via H-bonds. Water and various alcohols are the most prominent examples for this latter type of interaction.

In all practical phospholipid applications such interactions must be kept in mind and should also be well understood. We have thus attempted to highlight the molecular mechanisms of the solute- or solvent-glycerophosphate interactions but also their effects on the colloidal and phase properties of several common phospholipids. To this end we have studied systematically the outcome of alcohol interactions with the fully hydrated diacylphosphatidylcholines (PC-s) and related compounds [2, 3, 4, 5]. Specifically, the shifts of the lipid chain-melting (order-disorder = gel-to-fluid = $P'_\beta \rightarrow L_\alpha$) phase transition, pretransition ($L'_\beta \rightarrow P'_\beta$) and subtransition ($L'_c \rightarrow L'_\beta$) temperature as well as the changes in lipid vesicle morphology were determined as a function of the bulk alcohol concentration. The scanning differential calorimetry, X-ray diffractometry, the dynamic light scattering as well as fluorescent marker leakage studies afforded a fairly clear and general picture of the processes that are involved in the binding of solutes (and solvents) to the phosphate groups on the lipid molecules.

Materials and Methods: 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, purity > 99 %) was from Boehringer-Mannheim (D) or Sygena (Liesthal, CH). Ethanol (EtOH, p.a.) was obtained from Merck (Darmstadt, D). Water (18 MΩ cm) was doubly distilled in an all glass apparatus and was reprocessed by a water purification unit (Elgastat HQ). Differential scanning calorimetry was done with the multi-lamellar suspensions (usually 5 mM and aged, if necessary, for several weeks) in a MC2 scanning calorimeter (MicroCal, Inc., Amherst, MA) with the original data acquisition and analysis software (ORIGIN). X-ray diffractogrammes were measured with a Guinier x-ray diffraction camera (Huber Diffraktionstechnik, Rimsting, D) using a copper $L_{\alpha 1}$ line and approx. 150 mM multi-bilayer samples in the glass capillary positioned in a brass holder thermostated to ±0.1°C; by lifting the film holder and simultaneously changing the sample temperature continuous temperature scans were recorded.

To prepare the samples for the optical measurements, suspensions of multilamellar lipid vesicles (75 mM DPPC) in water were sonicated (Heat Systems W 380, USA) at 42°C) or extruded (LiposoFast, Avestin, Ottawa, Can) through a microporous (most often 100 nm (Poretics)) filter until the average vesicle diameter was approximately 50 to 60 nm. Vesicle size in the resulting (sterile) preparation was controlled prior to each experiment; it remained stable for several weeks, at least.

Results and Discussion: It is well known that the solvation of phospholipids in any protic solvent depends on the lipid affinity for the solvent as well as on the range of the inter-solvent interactions [7]. Phospholipid hydration first involves the water binding to the anionic phosphate group oxygens and then phosphodiester bridging by the water molecules [6]. (The avidity of water association with the other polar groups, such as amino-, choline, or glycerol-group, is lower than for the phosphate.)

Phosphate groups, consequently, are pushed apart by solvation which thence forces the phospholipid aggregates to swell in all directions [7]. In the low-temperature, ordered phase, characterized by the tight contacts and strong van der Waals attraction between the lipid chains, the resulting molecular area expansion, ΔA_L, is accompanied by the sliding of the parallel hydrocarbon chains along their long axes; simultaneously the lipid chain tilt, ϕ_{tilt}, increases [8]. The thickness of the interfacial region, d_p, which encompasses the lipid headgroups as well as the bound water molecules, also gets greater with increasing hydration [9]. The swelling-associated effects are limited, however, by the maximum number of the directly bound water molecules achievable and by the relatively short range of the strong inter-water interactions ($\Lambda_{eff} \sim \underline{0.1} \cdots 0.3$ nm) [7]. These maximum values were estimated to be $\Delta A_L \leq 25$ % [8], $d_p \geq 100$ %, and $\phi_{tilt} \leq 30°$ [10] which suffices for the induction of the isothermal lipid phase transitions in appropriate temperature and water-concentration ranges [11].

The short chain alcohols also bind to the phosphate groups like the first tightly bound water molecules. Alcohol binding thence involves the hydrogen bond formation between the anionic oxygens on the PO_4^--groups and the alcohol OH-residue. Some of the phospholipid's water of hydration, consequently, is replaced (or displaced) by the bound alcohol, as concluding from the FT-IR spectra of the phosphate and carbonyl-group regions (N. Nagel and G. Cevc, to be published). The interaction of alcohol's aliphatic chains with the outer membrane region is also important, the longer the alcohol the more so. The effects of alcohol partitioning into or binding to the bilayer are precisely the opposite, however, in the gel- and fluid-lipid phases. While the lipid chain packing in the former, ordered phase is improved by the presence of alcohols [3], the alcohol binding to the fluid-phase bilayers promotes the chain disorder [12]. Chain ordering is mirrored in the much sharper Bragg-peaks in the small-angle X-ray diffraction patterns of the multilamellar stack for the lipids with bound alcohol [4]; enhanced chain disorder is reflected in the more rapidly decaying order parameter profiles measured for the per-deuterated phospholipid chains by the ^{2}H-nuclear magnetic resonance (L. Löbbecke and G. Cevc, to be published).

In spite of such apparent discrepancy both effects have the same roots: any short-chain alcohol-lipid association always promotes the lateral as well as the transverse repulsion between the phospholipid molecules but the resulting stress may be dissipated differently in different phases.

In the ordered (L'_β-phase), the alcohol-induced lateral expansion has to enlarge the tilt of the ordered, fully extended, parallel and strongly interacting hydrocarbon chains (by up to 23°) [4]. The area of phosphatidylcholine molecules in the L'_β-phase, moreover, is increased by $\leq$ 40 % to the maximum possible value of $A_L \simeq 0.7$ nm^2 in the presence of alcohols. Alcohol-phospholipid association, furthermore, broadens the lipid-water interface and promotes the membrane solvation (inter-membrane separation). Gel-phase PC bilayers, consequently, accommodate up to 130 % more alcohol-water mixture than pure water [4].

When the solvation-induced hydrocarbon tilt reaches its sterically permissible maximum value of 53°, any further area increase must involve a different type of membrane adaptation. Chain-interdigitation is the most obvious and common solution to this problem as it doubles the area per lipid headgroup and yet maintains the close contacts between the chains.

Extensive alcohol binding therefore provokes the successive interdigitation of the ordered hydrocarbon chains. This occurs in molecular clusters due to the packing incompatibility between the interdigitated and normal bilayer regions. It seems that the interdigitated domains are distributed uniformly over the whole lipid membrane which forces the surface of all lipid vesicles in the presence of alcohols to get fragmented into the co-existing, but not parallel domains. Throughout the phase-coexistence region the highly permeable and perturbed boundaries are therefore formed in any gel-phase diacylphosphatidylcholine layer. Such domain borders are most abundant when the lipid interdigitation exceeds just about 50 % and are easily identifiable by an 'anomalous' decrease in the average vesicle diameter [3] or in the concomitant lipid (bi)layer permeability increase in the coexistence region [12].

This point is reached at some characteristic stoichiometric lipid/alcohol ratio (which decreases from 7 for the short chain alcohols to 2 for the longer chains) and corresponds to the mono-domain lipid membranes with un-tilted chains. Further rising the amount of the bound alcohol—and thus increasing the lateral repulsion—is again compensated by the chain tilting. The maximum chain tilt in the interdigitated seems to be the same as in the normal lipid bilayer, 53°.

In the fluid L_α phase the phospholipid molecules are less constrained in their conformational freedom than in the L_β state. They are therefore not forced to adapt themselves to the ambient stress in cohorts, as in the gel phase. Lipids above their chain-melting phase transition temperature, consequently, increase their area simply by the progressive chain fluidization. This permits any initial structural changes originating from the alcohol binding to be gradual. (The propensity of the fluid, alcohol-doped membranes to fuse [4] provides a circumstantial evidence for the conclusion that domains of different alcohol/phospholipid stoichiometry may exist.)

Ultimately, the fluid phase phospholipids are solubilized by the bound alcohol molecules. This transition occurs in several steps and is complete when the PC/alcohol molar association ratio exceeds the value of 2-3 [12].

The chain-melting phase transition temperature of fully solvated phospholipids, T_m, depends on the chain-length of the added alcohol: short-chain alcohols (up to n-propanol) first decrease and then increase the T_m-value. For the alcohols with longer chains (up to n-heptanol), however, the T_m-value decreases over the whole investigated alcohol concentration range, however [5]. Both pretransition as well as subtransition

temperature of PC-s are first monotonously (but non-linearly) lowered by increasing the bulk alcohol concentration. The former transition disappears at some characteristic, chain-length dependent alcohol concentration. This point, K, in the solute-dependent phase diagram of PC marks the complete hydrocarbon interdigitation [5].

The K-value, and most other alcohol-dependent lipid layer characteristics, decreases with increasing alcohol chain-length and is largely determined by the free energy of transfer of alcohols from the aqueous sub-phase into the phospholipid layer. n-Alcohols as well as alcohols with OH-groups at the second or third position on aliphatic chain obey this trend, provided that their length does not exceed approximately half of the phospholipid chain length. This experimentally established functional dependence can be described quantitatively within the framework of an apparent chain-length concept allowing for the fact that the long-chain alcohols have a much higher probability to partition into the bilayer than the short-chain ones [5]; going from methanol to heptanol thus decreases the K-value from 3 M to 8 mM, for example, in the log-linear manner. Phosphatidylcholine solubilization follows the same trend.

Phospholipids sensitivity to the perturbation by the phosphate-bound alcohol increases with their lipid chain-length [12] and, in particular, with the headgroup polarity [13]. This confirms indirectly our view that the alcohol-induced hydrocarbon interdigitation is, indeed, chiefly a consequence of the solute-induced lateral repulsion (and of the resulting expansion) in the phospholipid headgroup region. When this effect is balanced by the corresponding repulsion in the lipid chain region no chain interdigitation is observed. This explains why the long-chain alcohols, which disorder the phospholipid chains, do not induce interdigitation. On the other hand, this is also the reason why the less polar phospholipids (such as phosphatidylethanolamine-N,N-dimethyl) interdigitate less readily than phosphatidylcholines.

References

1. G. Cevc, Ed. (1993) 'Phospholipids Handbook.' Marcel Dekker, New York.
2. Rowe, E. S. (1983) *Biochemistry* 22, 3299–3304; Rowe, E. S. (1987) *Biochemistry* 26, 46–51.
3. Nagel, N. E., Cevc, G. & Kirchner, S. (1992), *Biochim. Biophys. Acta* 1111,263–269.
4. Vierl, U., Nagel, N., Löbbecke, L. & Cevc, G. (1994) Biophys. J. 67, 1067-1079.
5. Löbbecke, L., Cevc, G. (1995) Biochim. Biophys. Acta, 1237, 59–69.
6. Cevc, G., Marsh, D. (1987) *Phospholipid Bilayers* (Wiley, New York).
7. Cevc, G. (1993) In: 'Hydration of Biological Macromolecules' (E. Westhof, ed.) Macmillan Press, New York, 338-390.
8. Janiak, M. J., Small, D. M. & Shipley, G. G. (1979) *J. Biol. Chem. 254*, 6068-6078.
9. Büldt, G., Gally, H. U., Seelig, A., Seelig, J. & Zaccai, G. (1978) *Nature 271*, 182-184.
10. Wiener, M. C., Suter, R. M. & Nagle, J. F. (1989) *Biophys. J.* 55, 315–325.
11. Cevc, G. (1991) *Chem. Phys. Lipids* 57, 293—307.
12. Löbbecke, L. (1995) Ph.D. Thesis, TU München.
13. Nagel, N. (1994) Ph.D. Thesis, TU München.