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Biochimica et Biophysica Acta 1372 (1998) 389–393

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Rapid report

L_α-phase separation in phosphatidylcholine–water systems induced by alkali chlorides

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Received 8 April 1998; revised 23 April 1998; accepted 24 April 1998

Abstract

The effects of alkali chlorides on phosphatidylcholine–water bilayer systems in the L_α-phase were investigated by using small- and wide-angle X-ray scattering. The ternary system LiCl–POPC–H_2O under isothermal conditions has shown that above Li^+/POPC molar ratios of 0.1 and a lipid concentration above 5% (w/w), a splitting of the lamellar Bragg diffraction peaks into discrete components indicates a phase separation into different lamellar liquid crystalline (smectic A) phases. It is also shown that in saturated distearoyl phosphatidylcholine and in egg phosphatidylcholine, alkali chlorides induce L_α-phase separation. The number and repeat distance of the coexisting lamellar phases depend on the nature and concentration of the alkali chloride, the concentration of the phosphatidylcholine, and the degree of the acyl chain unsaturation.

Keywords: Phosphatidylcholine; Alkali-chloride (salt); Phase separation; Fluid crystalline phase; X-ray diffraction

Alkali ions are the most abundant inorganic cations in living systems and have therefore been extensively studied in their interaction with cellular and subcellular systems. Particular attention has been attracted by the lightest alkali metal, lithium, because of its pharmacological potential in the treatment of manic-depressive disease [1,2].

Alkali ions have considerable effects on the structural and conformational behavior of phospholipid bilayers. Phosphatidylcholine head groups behave as 'sensors' of the electrostatic charge at the membrane surface [3] such that, e.g., ions induce conformational changes in the polar head group region in the liquid crystalline L_α-phase [4–6], reorder the packing geometry of the saturated acyl chains in the ripple phase, and enhance the appearance of the submain transition [7]. The interaction between Li^+ and the negatively charged phosphatidylserine leads to dehydration of the phosphatidylserine-head groups, and to the formation of high-melting ion–lipid complexes [8,9], whereas Na^+ and K^+ only produce minor changes by interacting with the negatively charged bilayer structure of phosphatidylserine [10,11].

Of crucial interest, because of its possible influence on cell membrane fusion, is the formation of ion-induced phase separations [12,13]. So far only Ca^{2+}-induced phase separations in mixed systems containing zwitterionic and negatively charged phos-

Abbreviations: DSPC, distearoyl phosphatidylcholine; POPC, palmitoyl-oleoyl phosphatidylcholine; EYPC, egg phosphatidylcholine; PC, phosphatidylcholine; SAXS, small-angle X-ray scattering; WAXS, wide-angle X-ray scattering; SWAX, simultaneous small- and wide-angle X-ray scattering

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0005-2736/98/$19.00
PII: S0005-2736(98)00079-0

pholipids are known, in which the preferential association of Ca^{2+} with the negatively charged lipids leads to a rigid phase which excludes zwitterionic phospholipids [14–16]. Recently, a fluid–fluid phase separation was demonstrated in liquid crystalline phosphatidylserine–phosphatidylcholine mixtures [17]. Hitherto, however, phase separation was only known to occur in phospholipid mixtures, and we are not aware of any reports on phase separation occurring in one-component phospholipid bilayers systems.

In our present study, we focus mainly on LiCl-induced L_α-phase separation in the liquid crystalline phosphatidylcholine–H_2O bilayer systems by simultaneous small- and wide-angle X-ray scattering (SWAXS) studies. We also report on analogous experiments with NaCl and KCl.

1,2-Distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and egg-yolk phosphatidylcholine (EYPC) were purchased from Avanti Polar Lipids, Birmingham Alabama, and used without further purification. Multilamellar liposomes were prepared by dispersing weighted amounts of dry lipids (5–20%, w/w) in aqueous LiCl, NaCl and KCl solutions, covering the range of salt-to-lipid molar ratios between 0 and 1 (in one case, with EYPC, the Na^+/lipid molar ratio has been chosen as 3.1; see Table 1). Quartz-bidistilled, deionized water was used throughout. To ensure complete hydration, the lipid dispersions were left for about 4 h at room temperature, and during this period the lipid dispersions were vigorously vortexed under an N_2 atmosphere to prevent oxidation.

Simultaneous small- and wide-angle X-ray scattering (SAXS and WAXS) experiments were carried out at the SAXS-beamline at the Synchroton ELETTRA in Triest [18,19]. The scattering patterns were recorded with two one-dimensional position sensitive detectors (Gabriel type, Grenoble, France) monitoring the s-ranges ($s = 2\sin\theta/\lambda$, 2θ = scattering angle, $\lambda = 0.154$ nm) between $(75.0 \pm 4.0)^{-1}$ nm^{-1} and $(0.90 \pm 0.35)^{-1}$ nm^{-1}, respectively, with typical exposure times of 10 s. The position calibration of the detectors was performed by using the diffraction patterns of dry rat tail tendon collagen (d-spacing = 65 nm) and p-Br-benzoic acid (wide-angle region, reflections at $s_1 = (0.467\ nm)^{-1}$, $s_2 = (0.380\ nm)^{-1}$ and $s_3 = (0.370\ nm)^{-1}$) as a reference.

Further simultaneous small- and wide-angle X-ray scattering control measurements were carried out at a laboratory X-ray source by using a SWAX camera (Hecus MBraun Graz X-Ray Systems, Graz, Austria) [20], which employs two coupled linear position sensitive detectors as described before [7]. The lipid dispersions were measured in a thin-walled 1 mm diameter Mark capillary held in a steel cuvette which provides good thermal contact to the Peltier heating unit.

The raw scattering data were normalized against the incident beam intensity using the signal of the ionization chamber. The background was subtracted by fitted polynomials. To obtain the d-spacing of the Bragg reflections, the normalized data were fitted by a least square method based on the Levenberg–Marquardt algorithm [21]. The model function was given by a sum of Lorentzians

$$I_s = \sum_i \frac{2A_i}{\pi} \frac{\mathrm{FWHM_i}}{4(s-c_i)^2 + \mathrm{FWHM}^2},$$

where I is the intensity of the peak as a function of the scattering vector s, A is the total area under the curve, c is the center of the peak, and FWHM is the full width of the peak at half maximum. The significance of the number of Lorentzians used in the model was verified by statistical variance analyses (F-test). The fitting procedures were carried out with the software package Origin 4.1 (Microcal Software, Northampton, USA).

Table 1
Overview of prepared lipid dispersions: the weight concentrations of lipids, the alkali chlorides used and the salt-to-lipid molar ratios

PC	Lipid content (% w/w)	Alkali-chloride	Alkali-chloride/PC (mol/mol)
DSPC	20	KCl	0.6
POPC	5, 10, 25, 40, 50, 60, 70	LiCl	1
	20	LiCl	0.1, 0.2, 1
EYPC	20	NaCl	3.1

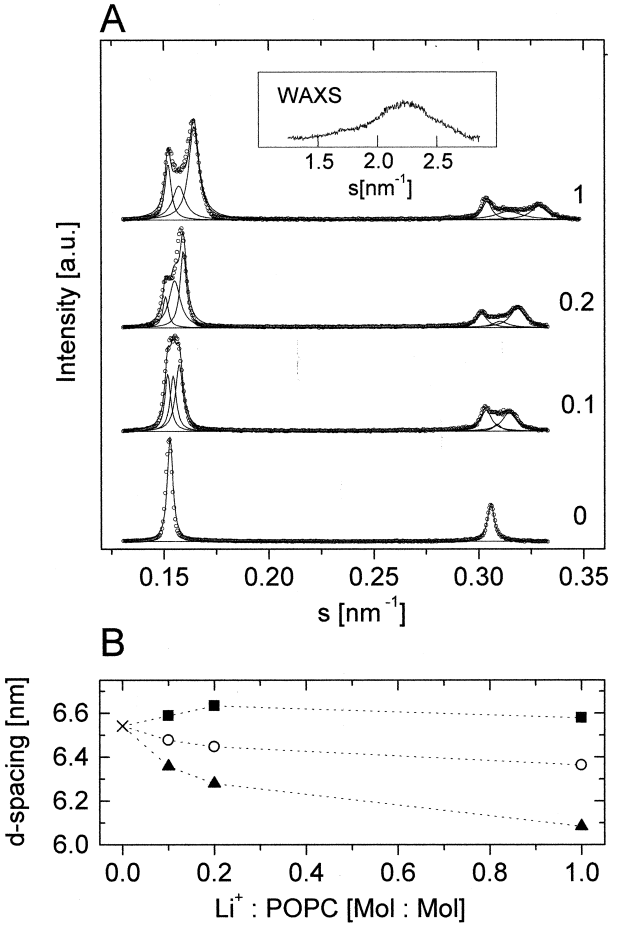

Fig. 1. (A) SAXS patterns (first and second order reflections) of POPC at a constant lipid concentration of 20% w/w and variable Li^+/POPC molar ratios (0, 0.1, 0.2 and 1), at 10°C. The patterns were fitted by the sums of 2 (in the case of no salt) and 6 Lorentzians, respectively. The insert shows the WAXS pattern of the Li^+–POPC–H_2O at an Li^+/POPC molar ratio of 1. Only one broad band centered at around $(0.45\ nm)^{-1}$ is present. (B) Dependence of the *d*-spacings on Li^+/POPC molar ratio obtained upon fitting. The *d*-spacings of the salt-induced lattices (closed squares, open circles, closed triangles) differ from that of the pure POPC–H_2O system (×).

Effects of Li^+ concentration were studied in the range of molar ratios Li^+/POPC of $R=0$ to $R=1$, at constant lipid contents of 20% (w/w), at 10°C, where the hydrocarbon chains of POPC are molten. Fig. 1A shows representative SAXS patterns, with the first and the second-order peaks of multilamellar bilayer systems; higher orders were also resolved, but are not shown here. The insert shows a wide-angle (WAXS) pattern of the system with $R=1$, confirming the liquid crystalline L_α-state of the hydrocarbon chains through the appearance of only a broad band centered around $(0.45\ nm)^{-1}$.

With increasing Li^+ contents, the SAXS peaks were found to broaden and to split into two major peaks. For the first order peaks, this appears initially as a broadening to larger *s*-values, and above $R=0.1$, this develops into a separate peak. Particularly visible with the second order reflections, the intensities do not reach the base-line between the dominating peaks, suggesting more than two components. Modelling by a sum of Lorentzians leads to a satisfactory fit with a set of three independent lamellar lattices, which all differ in their repeats from that of the salt-

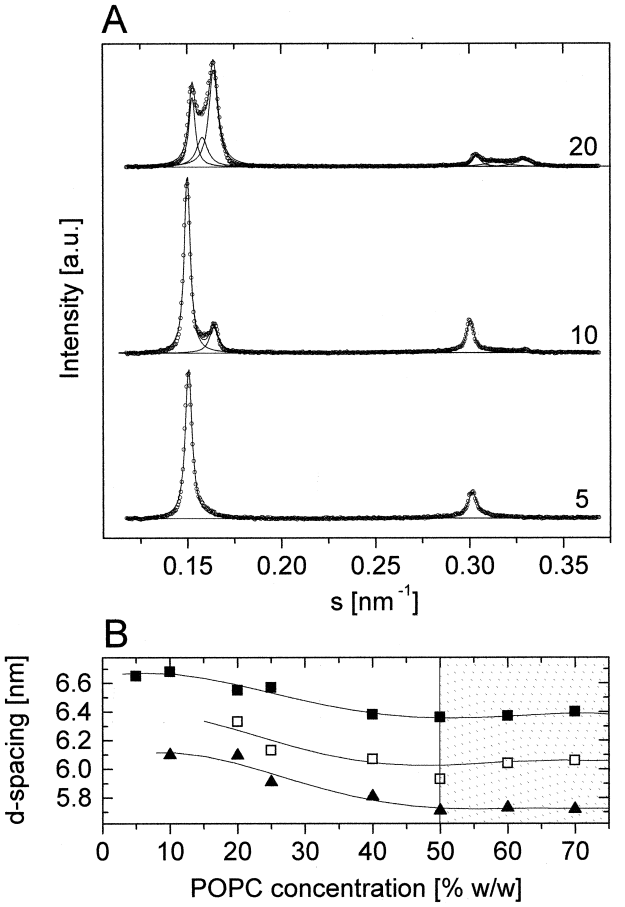

Fig. 2. (A) SAXS patterns (first and second order reflections) of POPC at a constant Li^+/POPC molar ratio of 1 and variable lipid concentration (5, 10 and 20% w/w), at 10°C. The patterns were fitted by the sums of 2, 4 and 6 Lorentzians, respectively. (B) Dependence of the *d*-spacings on POPC concentration obtained upon fitting. Above a POPC concentration of 50% (i.e. less than 42 molecules water per lipid molecule) the *d*-spacings of the three peaks remain constant, and the difference in the *d*-spacing is constant at about 0.3 nm.

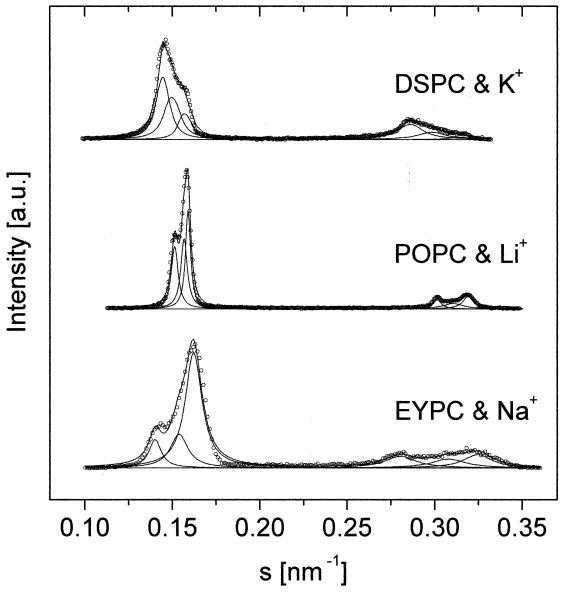

Fig. 3. SAXS patterns (first and second order reflections) of the K^+–DSPC–H_2O system, the Li^+–POPC–H_2O system and the Na^+–EYPC–H_2O system with molar ratios of 0.6, 0.2 and 3.1, respectively. The patterns were fitted by the sums of 6 Lorentzians.

free POPC–H_2O system (Fig. 1B). While the lattice with the largest *d*-spacing slightly increases at first, but remains within 6.6 ± 0.05 nm, the second dominant peak strongly shifts to smaller spacings approaching 6.1 nm at $R = 1$. The middle component peak also shifts to smaller spacings remaining symmetrically between the two major components and reaching about 6.4 nm at $R = 1$.

The salt-induced splitting of the diffraction peaks was found to depend strongly on the lipid-to-water ratio. This is shown in Fig. 2A, where representative SAXS patterns at 5, 10, and 20% POPC, at a constant Li^+/POPC molar ratio of $R = 1$, are plotted.

At low lipid concentration, at 5%, the salt effects are barely detectable; at 10% and above, a splitting into two peaks is clearly seen. Curve fitting suggests that the system, above 20%, is best approximated by a linear combination of three components. This behavior has been studied over POPC concentrations between 5 and 70%, and the resulting *d*-values are plotted in Fig. 2B. The *d*-spacings of the individual peaks decrease by about 0.3 nm with increasing lipid concentration up to approximately 50%, to stay constant above this level. It is noteworthy that this decrease runs practically parallel for the three peaks and that their differences in spacings remains constant at about 0.3 nm.

We have observed similar splittings of the SAXS diffraction peaks also with DSPC (above its main transition temperature) and EYPC, and also with KCl and NaCl; however, with NaCl at higher concentrations than with LiCl. A selected set of diffractograms is shown in Fig. 3. This serves as a demonstration that alkali chlorides in general, and not just LiCl, lead to a splitting of the multilamellar lattice into discrete components, and that this splitting is also affected by the hydrocarbon chain composition of the PCs.

The present results show unambiguously that alkali chlorides (other alkali salts are presently being studied) can induce the formation of discretely different multilamellar lattices in PC—water systems. Commonly, such behavior is referred to as phase separation, and we adhere to this notion, because the discreteness of the components in the diffractograms verifies that the underlying structures are physically and chemically homogeneous, i.e. with discrete compositions. To our knowledge so far, such phase separations have not been observed in pure phosphatidylcholine systems in the liquid crystalline state, with molten hydrocarbon chains.

The nature and underlying causes for this phase separation require further investigations. A conspicuous feature throughout the present results is the ubiquitous quantization of the splitting by about 0.3 nm, which is close to the dimensions of one water molecule, indicating that discrete hydration steps might be one cause, but others will also have to be considered.

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