343

BBA Report

Comparative structural aspects of cation binding to phosphatidylserine bilayers

Helmut Hauser and G. Graham Shipley

Biophysics Division, Departments of Medicine and Biochemistry, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118 (U.S.A.)

X-ray diffraction data recorded for monovalent and divalent cation complexes of a series of phosphatidylserines (PS) varying in chain length reveal a simple structural pattern. Only two bilayer structural types differing in hydrocarbon chain tilt but with similar polar group conformations are observed for (i) anhydrous acidic PS, (ii) anhydrous K^+-PS, and (iii) Li^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, and Pr^{3+} complexes of 'hydrated' PS. The X-ray diffraction data suggest that PS becomes dehydrated on complexing with Li^+, Mg^{2+}, Ca^{2+}, and other divalent cations and adopts either the chain untilted (form I) or tilted (form II) bilayer structure.

The physico-chemical properties of uncharged zwitterionic phospholipids, notably phosphatidylcholine and phosphatidylethanolamine, have been the subject of extensive studies. This approach has provided detailed information on their phase behavior, their conformation and molecular packing, and the dynamics of the phospholipid molecules in these phases. This knowledge in turn has led to a better understanding of the molecular parameters that determine their phase behavior. For phosphatidylcholine and phosphatidylethanolamine, it is now possible to assign differences in chemical structure, more precisely differences in the conformation and packing of the head group [1,2], to macroscopically observable differences in the phase behavior [3].

In contrast, anionic membrane phospholipids such as phosphatidylserine (PS), phosphatidylglycerol and cardiolipin have received much less attention and precise details concerning the molecular packing, conformation and motion of these lipids are lacking. With respect to PS, physico-chemical studies of both natural PS, with mixed fatty acyl chains, and synthetic PS, with controlled fatty acyl chains, have been concerned primarily with the phase behavior of these lipids. For example, bovine brain PS forms a lamellar, bilayer phase exhibiting continuous hydration or swelling [4,5]. The structure and hydration of this bilayer phase has been shown to be sensitive to the presence of monovalent and divalent cations [6–16]. In particular, Ca^{2+} produces high melting 'crystalline' Ca^{2+}-PS complexes [17–20].

More recently, the structure and properties of chemically defined PS and details of the cation induced changes have been studied using differential scanning calorimetry [20–25], ESR spin labeling [24,26] and ^{31}P- and ^2H-NMR [23]. Our own studies have focused on the bilayer structure and properties of a homologous series (di $C_{10:0}$–di $C_{18:0}$) of synthetic diacyl-PS and the changes induced by monovalent and divalent cations [25,27–29]. Using primarily DSC and X-ray diffraction, we have shown that synthetic PS forms swelling, continuously hydrated bilayers exhibiting chain length dependent gel → liquid-crystal transitions [25]. Monovalent cations such as Na^+ and K^+ have little influence on PS bilayer structure, stability and chain packing; however, these ions do shield the negatively charged bilayer surface

resulting in an ionic strength-dependent reduction in the aqueous separation of adjacent bilayers [27]. In contrast, the monovalent cation Li^+ [27,28] and the divalent cations Mg^{2+} and Ca^{2+} [29], all with relatively small ionic radii, form strong, high melting, dehydrated metal ion-PS complexes and induce bilayer hydrocarbon chain crystallization. The larger divalent cations Sr^{2+} and Ba^{2+}, while exhibiting surface charge shielding and bilayer dehydration, apparently do not form these strong complexes with PS [29].

During the course of the X-ray diffraction studies of synthetic PS in the absence and presence of cations, we have noticed some simplifying features in terms of the bilayer structural characteristics. Consideration of the chain length-dependent X-ray diffraction data allows the influence of factors such as hydration and cation binding on PS bilayer structure to be evaluated. Notably, there appear to be two major ordered bilayer forms of PS-cation complexes differing primarily in hydrocarbon chain tilt.

Dihexanoyl (di $C_{6:0}$-PS, DHPS), didecanoyl (di $C_{10:0}$-PS, DDPS), dilauroyl (di $C_{12:0}$-PS, DLPS), dimyristoyl (di $C_{14:0}$-PS, DMPS), dipalmitoyl (di $C_{16:0}$-PS, DPPS), and distearoyl (di $C_{18:0}$-PS, DSPS) phosphatidylserines were synthesized according to the methods described in Refs. 30 and 31. The preparation of anhydrous acidic, NH_4^+, and K^+ salts of PS was described in Ref. 25. The preparation of Li^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, and Pr^{3+} salts from hydrated dispersions of PS was as described previously [27–29]. Samples were transferred to thin-walled glass capillaries (internal diameter = 1 mm) and X-ray diffraction data recorded at $20 \pm 1°C$. Nickel-filtered CuK_α X-radiation from an Elliott GX-6 rotating anode generator (Ellott Automation, Borehamwood, U.K.) was focused using a low angle X-ray camera with toroidal optics [32]. For more details, see original Refs. 25, 27–29.

Anhydrous PS, monovalent cation salts of PS (M^+-PS), and divalent cation salts of PS (M^{2+}-PS) all exhibited lamellar diffraction patterns at 20°C. The diffraction patterns showed a series of sharp low angle diffraction lines with Bragg spacings in the ratios $1:1/2:1/3:1/4\ldots$ etc., from which the bilayer periodicity is derived. In contrast, the diffraction pattern observed in the wide angle

differed markedly for the different forms of PS (see Refs. 25, 27–29 for details). This indicates different lateral packing modes, with different (and so far undefined) hydrocarbon chain packing modes. In spite of significant variations in the crystal form, when the bilayer periodicity, d, is plotted as a function of PS-chain length, a relatively simple structural pattern is observed (Fig. 1). The bilayer periodicities fall on (or close to) two straight lines with different slopes and slightly different intercepts on the ordinate axis.

As discussed previously [25], anhydrous acidic PS can exist in two crystal forms. Form 1 shows a linear dependence of d with chain length; data from a linear regression analysis are summarized in Table I. The value for the slope, representing the increment per CH_2 group, is 2.8 Å per CH_2 group. Although this is somewhat greater than the theoretical value of 2.52 Å calculated for an idealized all-*trans* chain configuration, we believe that the inclusion of data from the lower ($C_{6:0}$ and $C_{10:0}$) chain length PS (see Fig. 1) in the regression analysis of the acidic PS and Li^+-PS (see below) series accounts for the anomolously high value of the calculated increment in both cases (see Table I). The two shorter chain PS and their metal ion complexes may not exhibit strictly homologous behavior to the longer chain compounds. In any case, this increment demonstrates clearly that the orientation of the fatty acyl chains is normal to the plane of the bilayer in this type of PS bilayer. Furthermore, the extrapolation to zero chain length gives an intercept value of 11.3 Å, corresponding to the projected thickness of two glycerophosphorylserine groups. The d-values of the corresponding anhydrous NH_4^+-, and K^+-salts of PS all

TABLE I

SUMMARY OF THE LINEAR REGRESSION ANALYSIS FOR DIFFERENT DATA SETS SHOWN IN Fig. 1

	Intercept	Slope	$r^{2\ a}$
Acidic PS (form I)	11.3	2.8	0.99
Acidic PS (form II)	13.6	2.1	1.00
Li^+-PS complexes	13.2	2.7	0.99
Ca^{2+}-PS complexes	12.9	2.2	0.99

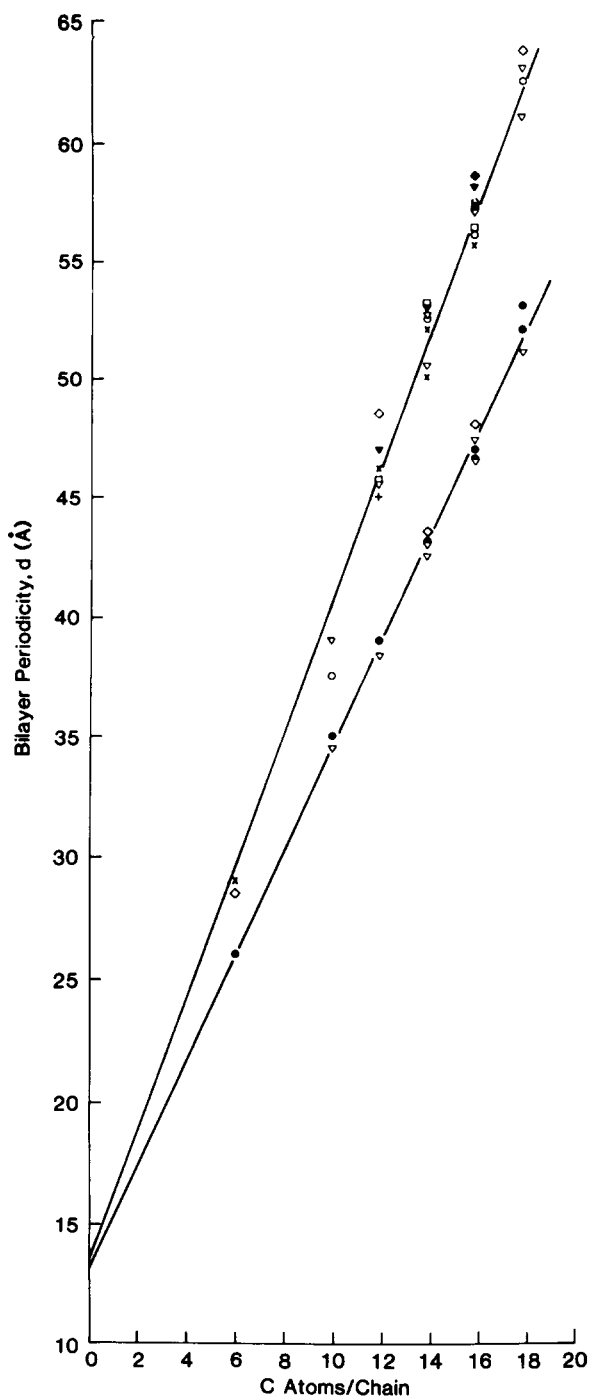[a] r^2, coefficient of determination, a value of 1 representing a perfect fit.

Fig. 1. Plot of bilayer periodicity, d, as a function of chain length for (i) anhydrous phosphatidylserine bilayers: $\triangledown$, acidic PS (L, L); $\square$, acidic PS (DL, L); $\bigcirc$, NH_4^+-PS (L, L); +, NH_4^+-PS (DL, L); $\blacktriangledown$, K^+-PS (L, L), and (ii) complexes formed from hydrated phosphatidylserine (L, L), bilayers: $\times$, Li^+-PS; $\Diamond$, Mg^{2+}-PS; $\bullet$, Ca^{2+}-PS; $\blacksquare$, Sr^{2+}-PS; $\triangle$, Ba^{2+}-PS; $\blacklozenge$, Pr^{3+}-PS.

lie close to the regression line of form I of acidic PS (Fig. 1), suggesting that these anhydrous salts have a similar chain orientation (i.e. perpendicular to the bilayer surface) and polar group conformation. In addition, Li^+ forms crystalline bilayer complexes with hydrated PS of different chain length [27,28]. The bilayer periodicities of the Li^+-PS complexes are also similar to those of the acidic PS, form I (Fig. 1). From the linear regression analysis (Table I), values for the increment (2.70 Å per CH_2 group) and intercept (13.2 Å) are in good agreement with those of crystal form I, again suggesting structural similarity with respect to hydrocarbon chain tilt and polar group conformation.

Under certain conditions, acidic PS can crystallize into a different form II [25] with different bilayer periodicities (see Fig. 1). From the linear regression analysis of the chain length dependence, for form II of acidic PS the increment is 2.1 Å per CH_2 group (Table I) indicating that, in contrast to form 1, the hydrocarbon chains are tilted with respect to the bilayer normal. The intercept of form II (13.6 Å) is slightly (but probably not significantly) greater than that of form I. Ca^{2+} forms crystalline bilayer complexes starting with hydrated PS of different chain lengths [29]. The bilayer periodicities of the Ca^{2+}-PS are similar to those of the acidic PS, form II (Fig. 1). From the linear regression analysis (Table I) both the increment (2.2 Å per CH_2 group) and the intercept (12.9 Å) show good agreement with the corresponding values for the acidic PS, form II. These data strongly suggest that on Ca^{2+} complex formation, PS becomes dehydrated and a tilted chain bilayer structure is formed. The behavior of the Mg^{2+}-PS complexes is more complicated. Mg^{2+}-DMPS and Mg^{2+}-DPPS exhibit bilayer periodicities similar to those of the corresponding Ca^{2+} complexes (i.e. type II structures, see Fig. 1), whereas the bilayer periodicities of Mg^{2+}-DLPS and Mg^{2+}-DSPS would appear to form type I structures (see Fig. 1). Although the complex chain length dependence is difficult to rationalize, it is clear that only the two types of bilayer structure (forms I and II) are favored.

346

Finally, DPPS complexes with Sr^{2+}, Ba^{2+}, and Pr^{3+} exhibit bilayer periodicities of 57.0, 57.7 and 58.5 Å, respectively [29]. These values are indicative of the type I structure, rather than the type II bilayers formed by both Mg^{2+}- and Ca^{2+}-DPPS.

In summary, (i) two bilayer structural forms differing in hydrocarbon chain tilt but probably with similar polar group conformation are favored by PS in the absence and presence of monovalent, divalent and trivalent cations; (ii) the contribution of approx. 6 Å per polar group to the bilayer thickness suggests that the polar group has the configuration with the phosphoserine group lying approximately parallel to the bilayer surface, similar to phosphatidylethanolamine and phosphatidylcholine [1,2], in both the absence and presence of different cations (However, it should be stated that other possible conformations and packing arrangements of the PS head group are consistent with this value of the intercept. Further crystallographic, diffraction and NMR methods will be necessary to define the interfacial packing arrangement.); (iii) starting with hydrated PS, Li^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} produce essentially dehydrated complexes of two main structural types (see above), albeit with different hydrocarbon chain packing modes [27–29]; and (iv) these effects on PS bilayer hydration and chain packing induced by different cations may help explain their mode of action at membrane interfaces.

References

1 Hitchcock, P.B., Mason, R., Thomas, K.M. and Shipley, G.G. (1974) Proc. Natl. Acad. Sci. USA 71, 3036–3040
2 Pearson, R.H. and Pascher, I. (1979) Nature 281, 499–501
3 Hauser, H., Pascher, I., Pearson, R.H. and Sundell, S. (1981) Biochim. Biophys. Acta 650, 21–51
4 Papahadjopoulos, D. and Miller, N. (1967) Biochim. Biophys. Acta 135, 624–638
5 Atkinson, D., Hauser, H., Shipley, G.G. and Stubbs, J.M. (1974) Biochim. Biophys. Acta 339, 10–29
6 Abramson, M.B., Katzman, R. and Gregor, H.P. (1964) J. Biol. Chem. 239, 70–76
7 Hauser, H. and Phillips, M.C. (1973) J. Biol. Chem. 248, 8585–8591
8 Hope, M.J. and Cullis, P.R. (1980) Biochem. Biophys. Res. Commun. 92, 846–852
9 Ohki, S. and Kurland, R. (1981) Biochim. Biophys. Acta 645, 170–176
10 Eisenberg, M., Gresalfi, T., Riccio, T. and McLaughlin, S. (1979) Biochemistry 18, 5213–5223
11 McLaughlin, S., Mulrine, N., Gresalfi, T., Vaio, G. and McLaughlin, A. (1981) J. Gen. Physiol. 77, 445–473
12 Puskin, J.S. (1977) J. Membrane Biol. 35, 39–55
13 Ohki, S., Düzgünes, N. and Leonards, K. (1982) Biochemistry 21, 2127–2133
14 McLaughlin, A.C. (1982) Biochemistry 21, 4879–4885
15 Loosley-Millman, M.E., Rand, R.P. and Parsegian, V.A. (1982) Biophys. J. 40, 221–232
16 Hauser, H., Finer, E.G. and Darke, A. (1977) Biochem. Biophys. Res. Commun. 76, 267–274
17 Papahadjopoulos, D., Vail, W.J., Newton, C., Nir, S., Jacobson, K., Poste, G. and Lazo, R. (1977) Biochim. Biophys. Acta 465, 579–598
18 Papahadjopoulos, D., Portis, A. and Pangborn, W. (1978) Ann. N.Y. Acad. Sci. 308, 50–63
19 Jacobson, K. and Papahadjopoulos, D. (1975) Biochemistry 14, 152–161
20 Newton, C., Pangborn, W., Nir, S. and Papahadjopoulos, D. (1978) Biochim. Biophys. Acta 506, 281–287
21 MacDonald, R.C., Simon, S.A. and Baer, E. (1976) Biochemistry 15, 885–891
22 van Dijck, P.W.M., de Kruijff, B., Verkleij, A.J., van Deenen, L.L.M. and de Gier, J. (1978) Biochim. Biophys. Acta 512, 84–96
23 Browning, J.L. and Seelig, J. (1980) Biochemistry 19, 1262–1270
24 Cevc, G., Watts, A. and Marsh, D. (1981) Biochemistry 20, 4955–4965
25 Hauser, H., Paltauf, F. and Shipley, G.G. (1982) Biochemistry 21, 1061–1067
26 Luna, E. and McConnell, H.M. (1977) Biochim. Biophys. Acta 470, 303–316
27 Hauser, H. and Shipley, G.G. (1983) Biochemistry 22, 2171–2178
28 Hauser, H. and Shipley, G.G. (1981) J. Biol. Chem. 256, 11377–11380
29 Hauser, H. and Shipley, G.G. (1984) Biochemistry 23, 34–41
30 Hermetter, A., Paltauf, F. and Hauser, H. (1982) Chem. Phys. Lipids 30, 35–45
31 Aneja, R., Chadha, J.S. and Davies, A.P. (1970) Biochim. Biophys. Acta 218, 102–111
32 Elliott, A.J. (1965) J. Sci. Instrum. 42, 312–316