Conformational Analysis of the Polar Head Group in Phosphatidylcholine Bilayers: A Structural Change Induced by Cations†

Hideo Akutsu* and Toshiaki Nagamori

Department of Physical Chemistry, Faculty of Engineering, Yokohama National University, Hodogaya-ku, Yokohama 240, Japan

Received October 15, 1990; Revised Manuscript Received February 6, 1991

ABSTRACT: The conformation of the polar head group of phosphatidylcholine in a bilayer in the liquid-crystalline state was deduced by analyzing the deuterium quadrupole splittings of the choline group and the phosphorus chemical shift anisotropy of the phosphate group in combination with the restriction of the choline conformation determined in laser Raman studies. The latter efficiently reduced the number of candidates for the actual conformation. A family of conformations was obtained for both the dynamic-structure and rigid-structure models, respectively. The polar head group is oriented roughly parallel to the membrane surface in both models. Furthermore, they are close to conformation A of the crystal structure of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine. The dynamic-structure model was concluded to be more reasonable in view of the fact that the polar head-group structures in most crystals comprise two conformations, which are nearly mirror images of each other. Conformational analysis was also carried out for the polar head group in the presence of multivalent cations. A possible conformational change of the polar head group induced by cations is discussed in the light of the present results.

Phosphatidylcholine is one of the major phospholipids in membranes. The physicochemical properties of its bilayers have been extensively investigated. The crystal structure of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) has also been determined (Pearson & Pascher, 1979). However, it was difficult to determine the conformation of the molecule in the liquid-crystalline state, with which biomembranes can exert their functions. Conformational analysis of the polar head group was carried out with paramagnetic shift reagents in the early stages (Hauser et al., 1976). It turned out, however, that lanthanide ions induce conformational changes in the polar head group (Brown & Seelig, 1977; Akutsu & Seelig, 1981). Consequently, the results of the analysis were questionable from the methodological point of view. Then a model for the polar head-group structure was proposed on the basis of a simulation of the residual deuterium quadrupole splittings and phosphorus chemical shift anisotropy (Seelig et al., 1977). Although it was consistent with the observed data, there is great ambiguity when choosing a model from among the possible conformations (Skarjune & Oldfield, 1979). In the meantime, conformational analysis of relatively rigid groups such as sterols (Oldfield et al., 1978; Murari et al., 1986) or the head groups of glycolipids (Jarrell et al., 1986; Renou et al., 1989) was carried out by using ^{2}H NMR.

Since the polar head groups are located in the interzone between the hydrophobic region of membrane and hydrophilic aqueous solution, their conformation under physiological conditions is of great importance. It is particularly interesting to elucidate the mechanism underlying the interaction with molecules in the aqueous phase, such as metal ions. Extensive work has been carried out on the interaction between the polar head groups and ions for the last few decades, a wide variety of methods being used [cf. McLaughlin (1977) and Seelig et al. (1987)]. It was clearly shown that such an interaction induces a conformational change in the phosphocholine group, by means of ^{2}H NMR (Akutsu & Seelig, 1981). The mechanism underlying the interaction was further studied by means of ^{2}H NMR using a variety of cations (Akutsu & Seelig, 1981; Boulanger et al., 1981; Browning & Akutsu, 1982; Altenbach & Seelig, 1984, 1985; Macdonald & Seelig, 1987a,b; Seelig et al., 1988), anions (Macdonald & Seelig, 1988), cationic lipids (Seelig et al., 1987), and cationic membrane-bound peptides (Roux et al., 1989). It was also proposed that phosphatidylcholine head groups function as sensors of electric charge in membranes (Seelig et al., 1987).

We present a new approach for the conformational analysis of the polar head group of phosphatidylcholine in this paper. It involves analysis of the quadrupole splittings of the deuterons in the choline group in combination with analysis of the phosphorus chemical shift anisotropy in the phosphate group, with the restriction determined in laser Raman studies.

Materials and Methods

The chemical structure of phosphatidylcholine and the nomenclature for the choline group are given in Figure 1. The analysis was carried out by using the results for 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) bilayers at 59 °C (Akutsu & Seelig, 1981). The analysis was performed in the following way. For a molecule undergoing an axially symmetric motion such as a phospholipid molecule in a liquid-crystalline bilayer, the residual quadrupole splitting, $\Delta\nu_Q$, is related to the order parameter of the C–^{2}H bond, S_{CD}^{obs}, according to

$$\Delta\nu_Q = (3/4)(e^2qQ/h)S_{CD}^{obs} \quad (1)$$

where (e^2qQ/h) is the static quadrupole splitting constant, which was assumed to be 170 kHz as in earlier reports (Seelig et al., 1977). The molecular axis of phosphatidylcholine is considered to be parallel to the director axis on average. If the motions of the molecular axis and the intramolecular segment are independent,

$$S_{CD}^{obs} = S_{mol} \times S_{CD} \quad (2)$$

where S_{mol} and S_{CD} are the order parameter of the molecular axis with respect to the director and the orientational order parameter of the C–^{2}H bond with respect to the molecular axis,

† This work was partly supported by Special Coordination Funds of the Science and Technology Agency.

0006-2960/91/0430-4510$02.50/0

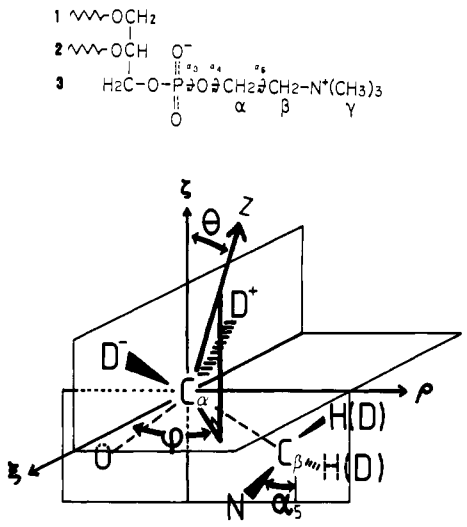

FIGURE 1: Nomenclature for and definition of the relevant atoms and angles. The labels of the carbons of the glycerol backbone are given on the left side. The Cartesian coordinate (ξ, ρ, ζ) was fixed at the α position in such a way that the ζ axis became a bisector of the angle DCD and the ρ axis was perpendicular to the plane DCD. Deuterons are denoted by D. The z axis represents the molecular axis of the phosphatidylcholine, which is parallel to the bilayer normal on average.

respectively (Petersen & Chan, 1977; Seelig, 1977; Oldfield et al., 1978). The simplest expressions of these order parameters are

$$S_{mol} = \langle 3 \cos^2 \eta - 1 \rangle / 2 \quad (3)$$

$$S_{CD} = \langle 3 \cos^2 \theta' - 1 \rangle / 2 \quad (4)$$

where η and θ' are the instantaneous angles between the director and the molecular axis and between the molecular axis and the C–^{2}H bond, respectively. The brackets $\langle\ \rangle$ denote the time average. S_{mol} represents the fluctuation of the molecular axis around the director. S_{CD} provides us with information of the molecular conformation in addition to the intramolecular fluctuation. This can be used for conformational analysis provided that S_{mol} is known and the mode of intramolecular motion is assumed. Seelig et al. (1977) estimated S_{mol} from the order parameter of the C_2–C_3 bond of the glycerol backbone. Following them, we estimated S_{mol} at 59 °C as 0.64 and used it in this paper. For conformational analysis, S_{CD} at different sites should be described as a function of common structural parameters. The parameter θ' is not adequate for this purpose. When a coordinate system (ξ, ρ, ζ) was introduced into the α position as shown in Figure 1, S_{CD} of any C–^{2}H bond in the choline group can be described as a function of at most three structural parameters (angles), θ, ϕ, and α_5. Here, the molecular axis is represented by the z axis. Definitions of three angles are also given in Figure 1. S_{CD} at the α position can be written as a function of θ and ϕ. Namely,

$$S_{CD}^{\pm} = \pm 0.707 \langle \sin 2\theta \cos \phi \rangle + 0.5 \langle \sin^2 \theta \cos 2\phi \rangle \quad (5)$$

where S_{CD}^{+} and S_{CD}^{-} are the orientational order parameters for the C–^{2}H(D)$^+$ and C–^{2}H(D)$^-$ bonds, respectively, and the bond angle of ^{2}H–C–^{2}H was assumed to be 109.4° (Seelig, 1977). S_{CD} at the β and γ positions can be obtained by transforming the electric field gradient tensor from the principal coordinate system at deuterium of a C–^{2}H bond into the molecule-fixed frame (x,y,z), where the molecular axis was taken as the z axis. This can be accomplished by using three angles, θ, ϕ, and α_5. In this transformation, the average bond angles of two structures in the glycerophosphocholine crystal, namely, 111.74° for O–C_α–C_β and 115.19° for C_α–C_β–N (Abrahamsson & Pascher, 1966), were used. The procedure of the transformation was similar to that used by Seelig et al. (1977). Since we have three observations (the quadrupole splittings at the α, β, and γ positions) for three parameters, we can basically deal with this problem in an analytical manner. In actual analysis, the quadrupole splitting was calculated as a function of θ, ϕ, and α_5, by using the relationships mentioned above, and compared with the observed splitting in the way shown later.

The phosphorus chemical shift anisotropy was calculated by transforming the chemical shift tensor from the principal coordinate system of phosphorus to the molecule-fixed coordinate system. The transformation was carried out through the ester bonds to the coordinate system at C_α by using α_3 and α_4 and then to the molecule-fixed frame using θ and ϕ. The principal values (−81, −25, and 110 ppm) (Herzfeld et al., 1978) were used for the phosphorus chemical shift tensor in the calculation. Finally, the obtained anisotropy was multiplied by the value of S_{mol}.

Results

For conformational analysis, we have to assume a motional model. Axially symmetric motions around the director axis were assumed in all cases, as mentioned in the previous section. As to the intramolecular motion of the polar head group, the analysis was carried out for the dynamic- and rigid-structure models. In the former, rapid exchange between the two conformations that were mirror images of each other was assumed, as in the case of Seelig et al. (1977), because they were found in most crystal structures of phospholipids. The NMR data used in this analysis were obtained for 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) bilayers and have already been published (Akutsu & Seelig, 1981), unless otherwise indicated. In the absence of ions, the quadrupole splittings for the α, β, and γ positions were 5.95, 4.22, and 0.96 kHz, respectively, at 59 °C. The phosphorus chemical shift anisotropy was −44 ppm. However, in the analysis based on the rigid-structure model, 6.0 and 6.3 kHz were used for the two deuterons at the α position because doublets were observed under proton-decoupling conditions and the two quadrupole splittings mentioned above were obtained from the spectrum (Akutsu and Seelig, unpublished data). On changing either the cation species or the ion concentration, the quadrupole splittings changed, the linear relationships being kept between the quadrupole splittings at the α and β, and β and γ positions, respectively (Akutsu & Seelig, 1981; Altenbach & Seelig, 1984). It should be noted that we cannot determine the sign of a quadrupole splitting from NMR measurements. Therefore, the analysis was carried out for both positive and negative values. Other important information we used was the results of laser Raman experiments on DPPC bilayers. It was shown that a Raman band due to the totally symmetric stretching vibration of the C–N bonds of the choline group is sensitive to the dihedral angle of the O–C–C–N^+ backbone, α_5 (Akutsu, 1981). It appeared at about 720 and 770 cm^{-1} for the gauche and trans conformations, respectively. In the Raman spectra of DPPC bilayers, the band appeared at 717 cm^{-1} in the liquid-crystalline state. No clear peak was observed at about 770 cm^{-1}. Thus, it was concluded that most of the choline groups in the DPPC bilayers take the gauche conformation in the liquid-crystalline state (Akutsu, 1981). It means that the dihedral angle, α_5, should stay in the range of the gauche conformation. The intensity of the band at 717 cm^{-1} did not change on addition of multivalent metal ions and local anesthetics (Akutsu et al., 1986). Therefore, the same restriction as mentioned above can be imposed on α_5 in the analysis of the DPPC bilayers in the presence of metal ions.

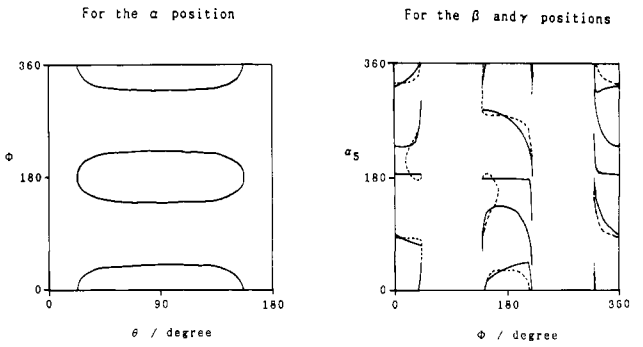

FIGURE 2: Schematic representation of the analysis of the quadrupole splittings of the choline group. Contour curves for the observed quadrupole splittings (5.95, 4.22, and 0.96 kHz for the α, β, and γ positions, respectively) were drawn on the $\theta - \phi$ and $\phi(\theta) - \alpha_5$ maps. Left: the solid line gives the relationship between θ and ϕ, which satisfies the observed quadrupole splitting at α. Right: the solid and broken lines represent the quadrupole splittings at β and γ, respectively. The quadrupole splittings on this map were calculated by using one set of the $\theta - \phi$ relationship obtained on the left map. The cross points of the solid and broken lines are candidates for the actual orientation.

Table I: Orientational Angle Sets Obtained from ^{2}H NMR Data in the Absence of Cations under the Restriction of $40° \leq \alpha_5 \leq 90°$ (Dynamic-Structure Model)[a]

$\Delta\nu_Q(\alpha)$	α_5	ϕ	θ
5.95 kHz	63.5	139.2	84.3
	73.7	40.4	72.8
	82.7	40.8	86.2
	83.2	6.3	22.7
	89.5	161.6	154.7
-5.95 kHz	55.0	130.8	88.5
	61.4	50.1	65.6
	65.2	130.8	89.2
	69.3	49.4	78.3
	76.5	130.9	76.7

[a] Angles are given in degrees.

This restriction reduced the number of possible conformations very efficiently.

A Dynamic-Structure Model. In this model, the electric field gradient tensors should be averaged for two deuterons at the α and β positions because of the rapid exchange of two conformations that were mirror images of each other. The orientational order parameter of the C–^{2}H bond at the α position was obtained by averaging the two S_{CD} of eq 5, which led to

$$S_{CD}(\alpha) = 0.5(\sin^2\theta \cos 2\phi) \quad (6)$$

By use of eqs 1, 2, and 6, contour plots for $\Delta\nu_Q(\alpha) = \pm 5.95$ kHz on the $\theta - \phi$ map were obtained, as shown on the left panel of Figure 2. These plots gave the relationship between the angles θ and ϕ that satisfies the observed quadrupole splitting. Taking this relationship into account, similar contour curves for the β and γ positions were obtained on the ϕ (or θ) $- \alpha_5$ map as shown on the right panel of Figure 2. The cross points of the contour curves for the β and γ positions represent candidates for the actual orientation. Those angles are summarized in Table I. Here, the absolute value of α_5 was assumed to be in the range of 40°–90°, because the O–C–C–N^+ backbone takes on the gauche form in the liquid-crystalline state (Akutsu, 1981). For symmetrical reasons, the angles are only given for the range of 0°–180°. The obtained candidates numbered ten in total.

As the next step, the chemical shift anisotropy of phosphorus was calculated as a function of the dihedral angles, α_3 and α_4, by taking each orientation in Table I into account. The contour curves for the observed chemical shift anisotropy were obtained on the $\alpha_3 - \alpha_4$ map. Then, whether or not the contour curve runs through the ranges $40° (270°) \leq \alpha_3 \leq 90° (320°)$ and $120° \leq \alpha_4 \leq 240°$ was examined, because the dihedral angles in the crystal structures of glycerophospholipids are generally in this range (Pearson & Pascher, 1979; Hauser et al., 1980; Pascher et al., 1981, 1987; Pascher & Sundell, 1986), except for in the case of 1,2-dilauroyl-*rac*-glycero-3-phosphoethanolamine, in which $\alpha_3 = 66°$ and $\alpha_4 = 106°$ (Elder et al., 1977). Seven orientations in Table I could be accommodated in the region defined above. However, one of them could not generate the orientation of the C_2–C_3 bond roughly parallel to the director axis in the glycerol backbone. This can be disregarded as a candidate. The other candidates are summarized in Table II. The dihedral angles, α_3 and α_4, in Table II are the typical ones in each region. These six conformations can be classified into two families, namely, of conformations 1a, 1b, and 2, and those of 3, 4, and 5, respectively. Conformations 1a and 3 are depicted in Figure

Table II: The Conformations Obtained from ^{2}H and ^{31}P NMR Data in the Absence of Cations (Dynamic-Structure Model)[a]

	α_3	α_4	α_5	ϕ	θ	$\alpha\beta\gamma$[b]
I						
1a	-59	-150	83.2	6.3	22.7	+++
b	51	120	83.2	6.3	22.7	+++
2	56	130	89.5	161.6	154.7	+-+
II						
3	-57	130	82.7	40.8	86.2	+++
4	-47	120	73.7	40.4	72.8	+++
5	-41	120	69.3	49.4	78.3	-+-

[a] Angles are given in degrees. [b] The signs of the quadrupole splittings at the α, β, and γ positions.

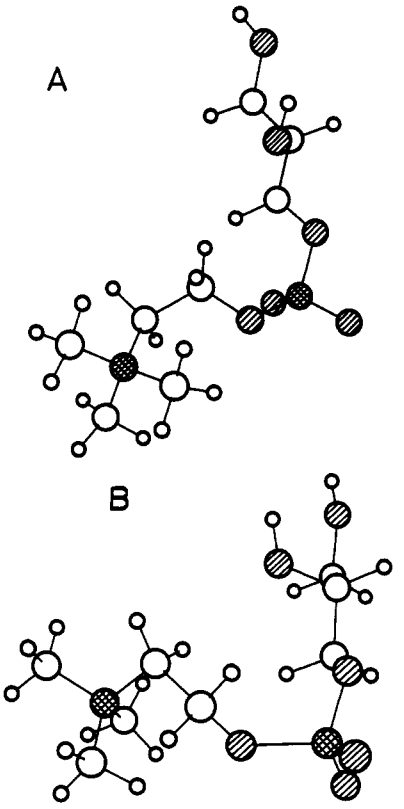

FIGURE 3: The conformations of the polar head group of DPPC in the absence of cations obtained with the dynamic-structure model. The vertical direction is the membrane normal. The dihedral angles other than those shown in Table II were taken from the crystal structure of L-α-glycerophosphocholine (Abrahamsson & Pascher, 1966). Shown are (A) number 1a in Table II and (B) number 3 in Table II. Symbols: small open circles, H; large open circles, C; circles with lines, O; cross-hatched circles, N and P.

Table III: The Conformations Obtained from ^2H and ^{31}P NMR Data in the Absence of Cations under the Restriction of $40° \leq \alpha_5 \leq 90°$ (Rigid-Structure Model)[a]

	α_3	α_4	α_5	ϕ	θ	$\Delta\nu_Q(\alpha)$/kHz +,	$\Delta\nu_Q(\alpha)$/kHz −[b]
1	x[c]	x	56.2	135.1	94.3	6.0,	−6.3
2a	52	127	54.2	123.5	5.6	6.0,	−6.3
b	−52	−118	54.2	123.5	5.6	6.0,	−6.3
3a	61	140	55.1	56.5	174.4	6.0,	−6.3
b	−58	−140	55.1	56.5	174.4	6.0,	−6.3
4a	52	120	61.3	56.5	5.6	−6.3,	6.0
b	−52	−127	61.3	56.5	5.6	−6.3,	6.0
5a	60	134	62.9	123.5	174.4	−6.3,	6.0
b	−64	−130	62.9	123.5	174.4	−6.3,	6.0

[a] Angles are given in degrees. [b] The signs of the deuterons shown in Figure 1. [c] x, no adequate angles.

3, A and B, respectively. The conformation was determined only for the phosphocholine group. The dihedral angles of glycerol through phosphate in Figure 3 were taken from the crystal structure of L-α-glycerophosphocholine (Abrahamsson & Pascher, 1966). This is also the case with the conformational models shown later, unless otherwise mentioned. The common feature of the structures presented in Figure 3 is that the orientation of the polar head group is roughly parallel to the membrane surface. In the case of family II, however, the P–N vector is inclined into the membrane, as can be seen in Figure 3B. This is incompatible with the better packing of the hydrocarbon chains because of the bulky size of the choline group. In conclusion, the conformations shown in Figure 3A (family I) are the most reasonable solutions obtained in this analysis.

A Rigid-Structure Model. In this model, the conformation at C_α was assumed to be rigid, and a rapid conversion around the C_α–C_β bond was assumed also. The latter assumption is based on the fact that although a doublet signal was observed for deuterons at C_α under proton-decoupling conditions, only a singlet was observed for those at C_β under any conditions. The electric field gradient tensors were averaged for two deuterons at C_β. The quadrupole splitting of each deuteron can be calculated as a function of θ and ϕ according to eq 5. This gives two sets of contour curves on the $\theta - \phi$ map. When these curves were overlapped, the candidates for the actual solution can be obtained as the cross points. Since we do not know the assignments or the signs of the quadrupole splittings, 24 candidates were obtained in total.

Then, the quadrupole splittings at the β and γ positions were calculated as a function of α_5, by using the set of θ and ϕ values mentioned above. The α_5 that satisfies the observed values at both the β and γ positions was searched for. In this analysis, an experimental error of ±0.5 kHz was taken into account. Five sets of θ, ϕ, and α_5 values were found to satisfy the observed quadrupole splittings at three positions. They are summarized in Table III. Phosphorus chemical shift anisotropy was calculated as a function of α_3 and α_4 by using the set of θ and ϕ values in Table III. Whether or not the dihedral angles that give the observed value fall in the reasonable range was examined. With conformations 2, 3, 4, and 5, we could find two sets of reasonable α_3 and α_4 regions, respectively. Representative values for α_3 and α_4 are included in Table III. These conformations are very similar to one another. As far as the choline group is concerned, all the conformations are symmetrically correlated to one another, as can be seen in the values of θ and ϕ. One of them is presented in Figure 4.

The Structure in the Presence of Metal Ions. In order to visualize the effect of metal ions on the conformation of the polar head group, the analysis was carried out for the data in the presence of metal ions. There could be two models for interpretation of the linear relationship between the quadrupole splittings at different sites (Akutsu & Seelig, 1984). One is rapid exchange between the free and ion-bound forms (referred to as two-site exchange hereafter). The other is a continuous conformational change under the influence of the average electric field (continuous change). In the case of the two-site exchange associated with the dynamic-structure model, $\Delta\nu_Q(\alpha) = -3.00$ kHz, $\Delta\nu_Q(\beta) = \pm 7.92$ kHz, and $\Delta\nu_Q(\gamma) = 0.85$ kHz were used as the limiting values of the deuterium quadrupole splittings of the ion-bound form because the quadrupole splittings at the α and γ positions for family I in Table II were always positive and the sign of $\Delta\nu_Q(\alpha)$ changed in the presence of cations (Akutsu & Seelig, 1981). The values were obtained from the linear relationship between the quadrupole splittings. Four solutions were obtained for the angles ϕ, θ, and α_5, which are summarized in Table IV. No other solution was found, even when the limit of α_5 was extended to 120°. On checking of the consistency with the phosphorus chemical shift anisotropy of −52 ppm, they were reduced to only one (1a and 1b), as shown in Table IV. Here, we imposed the same restrictions for α_3 and α_4 as in the case of the free form, because the dihedral angle, α_3, remains in the gauche range in the crystal state of the L-α-glycerophosphocholine–cadmium chloride complex (Sundaralingam & Jensen, 1965). Conformation 1a is given in Figure 5. This structure cannot be achieved through a continuous change from a free conformation, satisfying the linear relationships among the quadrupole splittings. When we changed the conformations in Table II continuously, the structures shown in Table V were

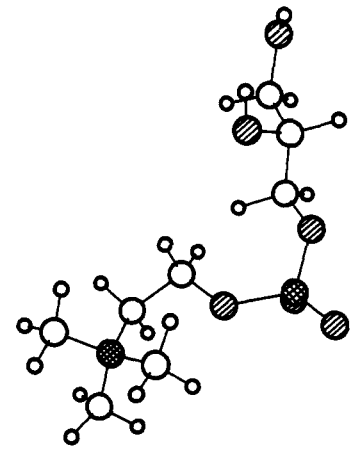

FIGURE 4: The conformation of the polar head group of DPPC in the absence of cations obtained with the rigid structure model (number 3a in Table III). The vertical direction is the membrane normal. The dihedral angles other than those shown in Table III are the same as those in Figure 3. Symbols: small open circles, H; large open circles, C; circles with lines, O; cross-hatched circles, N and P.

Table IV: The Conformations Obtained from ^2H and ^{31}P NMR Data in the presence of Cations under the Condition of $\Delta\nu_Q(\alpha) = -3.0$ kHz, Assuming Rapid Exchange between the Free and Ion-Bound Forms (Dynamic-Structure Model)[a]

	α_3	α_4	α_5	ϕ	θ	$\alpha\beta\gamma$[b]
1a	−67	170	54.7	132.9	97.5	−−+
b	−60	120	54.7	132.9	97.5	−−+
2	x[c]	x	58.1	132.9	84.5	−+−
3	x	x	68.9	47.7	62.4	−++
4	x	x	76.3	47.3	74.7	−++
5	x	x	77.9	132.7	73.0	−++

[a] Angles are given in degrees. [b] The signs of the quadrupole splittings at the α, β, and γ positions. [c] x, no adequate angles.

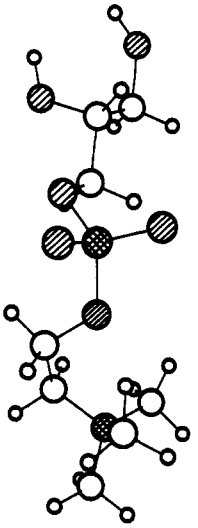

FIGURE 5: The conformation of the polar head group of DPPC in the presence of cations obtained with the dynamic-structure model assuming rapid exchange between the free and ion-bound forms (number 1a in Table IV). The vertical direction is the membrane normal. The dihedral angles other than those shown in Table IV are the same as those in Figure 3 with the modification of α_1 and α_2. Symbols: small open circles, H; large open circles, C; circles with lines, O; cross-hatched circles, N and P.

Table V: The Conformations Obtained by Changing the Structures in Table II Continuously to Those with $\Delta\nu_Q(\alpha) = 0$ kHz in the Presence of Cations (Dynamic-Structure Model)[a]

	α_3	α_4	α_5	ϕ	θ	conformation[b]
1a	−43	−142	65.5	45.0	10.1	1a
b	43	123	65.5	45.0	10.1	1b
2	60	129	69.5	135.0	169.0	2

[a] Angles are given in degrees. [b] The starting conformation in Table II.

Table VI: The Conformations Obtained from ^2H and ^{31}P NMR Data in the Presence of Cations under the Condition of $\Delta\nu_Q(\alpha) = \pm 3.0$ kHz (Rigid-Structure Model)[a]

	α_3	α_4	α_5	ϕ	θ	conformation[b]
1	60	132	65.6	138.0	2.0	5a
2a	51	140	69.2	42.1	2.0	3a
b	−52	−130	69.2	42.1	2.0	3b

[a] Angles are given in degrees. [b] The starting conformation in Table III.

obtained for $\Delta\nu_Q(\alpha) = 0$ kHz. The overall orientations of these structures are quite similar to the original ones. However, no solutions were found for $\Delta\nu_Q(\alpha)$ smaller than −2.0 kHz, when the conformation was changed continuously.

In the case of the two-site exchange associated with the rigid-structure model, we searched for a structure for $\Delta\nu_Q(\alpha)^{\pm} = \pm 3.00$ (or ∓ 3.00), because two deuterons at the α position have different signs in the Table III. The analysis of deuterium quadrupole splittings gave only the two proper solutions shown in Table VI. The obtained conformations could also clear the restrictions of phosphorus chemical shift anisotropy. These conformations can be achieved by changing conformations 3 and 5 in Table III continuously, following the linear relationships among the quadrupole splittings. Therefore, these structures are compatible with not only the two-site exchange model but also the continuous change model. The conformational change induced by cations was also minor in this case.

Discussion

Conformational analysis of the polar head group of phosphatidylcholine was carried out at first by Seelig et al. (1977).

Table VII: The Displacements of the Averaged Proton Positions of the Polar Head Group from the α Position Along the Director Axis (Å) in the Absence of Metal Ions

position	neutron scattering[a]	Figure 3A[b]	Figure 3B[b]	Figure 4[b]	Figure 5 (cation)[b]
α	0.0 ± 1.0	0.00	0.00	0.00	(0.00)
β	0.2 ± 1.0	0.81	−0.56	1.04	(0.79)
γ	0.8 ± 0.6	1.84	−0.28	1.93	(1.49)
GC3[c]	−3.6 ± 1.5 (−2.0 ± 1.5)[d]	−0.87	−0.46	−0.86	(−2.08)

[a] DPPC with 25% (w/w) water at 50 °C in the L_α phase (Büldt et al., 1979). [b] The distance obtained from the coordinate of each atom was multiplied by S_{mol} (= 0.64). [c] The C3 position of the glycerol backbone. The values, except for neutron scattering, are tentative. They were calculated from the conformations given in the indicated figures. [d] The same sample at 28 °C in the $L_{\beta'}$ phase.

They fixed the coordinate of the molecular axis in the glycerol backbone. Quadrupole splittings were simulated by changing five dihedral angles (α_1–α_5). They calculated the phosphorus chemical shift anisotropy and the deuterium quadrupole splittings, searching for a conformation that satisfied the observations and that was close to the crystal structure of 1,2-dilauroyl-*rac*-glycero-3-phosphoethanolamine. Skarjune and Oldfield (1979) analyzed the same system by searching for a reasonable conformation with wider ranges of dihedral angles. They came to the conclusion that they could not reach a particular conformation, but found three or four families of conformations. The analysis carried out in the present work is different from the earlier ones in two points. In the first place, we defined the molecular axis in the coordinate system at the α position instead of the glycerol backbone. Thus, we could describe the quadrupole splitting of any deuteron in the choline group through the use of three parameters, θ, ϕ, and α_5. Since we have three independent observations at the α, β, and γ positions, we can obtain the solution in an analytical way. The solutions, however, were not unique. Therefore, the results of laser Raman spectroscopy and phosphorus chemical shift anisotropy were used in the second place to find the actual solution. These restrictions worked very efficiently. Although a unique solution did not emerge, even with the use of these restrictions, the obtained solutions belonged to one or two families.

The results of our analysis can be compared with those in the study of neutron scattering of the oriented bilayers of specifically deuteriated DPPC (Büldt et al., 1979). The displacement of the deuterons at the α, β, and γ positions along the bilayer normal determined for the DPPC bilayer with 25% water at 50 °C is summarized in Table VII. The corresponding distances in the structures obtained in our analysis are included in the table as well. Although the C3 position of the glycerol backbone has not yet been established, the value in the Table VII can give an idea of the relative distance to the α position. In the conformation of family I (Figure 3A) of the dynamic-structure model, the four positions are of the same order as in the case of the neutron-scattering results. The displacements are also consistent with the observations, taking into account experimental errors. In contrast, the four positions in family II (Figure 3B) are in a different order from the observations. Furthermore, the latter positions were within a 0.6-Å distance, although they spanned from 2 to 6 Å in the case of the neutron-scattering results. Thus, only the conformations of family I are consistent with the results of neutron scattering in the case of the dynamic-structure model. This supports the conclusion mentioned under Results. The conformations obtained for the rigid-structure model are also very similar to those of family I. However, this motional model

is less plausible, as mentioned above. The above leads us to the conclusion that the conformation of family I is the most reasonable structure of the polar head group of the DPPC bilayer in the liquid-crystalline state. These conformations are close to conformation A in the crystal structure of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (Pearson & Pascher, 1979). The P–N vector is, however, more parallel to the membrane surface in the conformation of family I than in the crystal structures.

In the presence of metal ions, the problem was more complicated. To analyze the data under the assumption of two-site exchange, we have to know the limiting values of the deuterium quadrupole splittings and phosphorus chemical shift anisotropy. We did not know the exact values. However, it turned out that the magnitude of the absolute value is not very important for obtaining a rough image of the ion-bound conformation. Even if the limiting shift of $\Delta\nu_Q(\alpha)$ was assumed to be −10 kHz, a similar conformation was obtained. The conformational feature seemed to be determined by the combination of the signs of three quadrupole splittings. Since the continuous conformational change in the dynamic-structure model could not cover the entire region of the observed quadrupole splittings, two-site exchante must be the most reasonable explanation for the changes induced by cations.

In this case, the induced conformational change is rather significant. The P–N vector is inclined by 63° from the membrane surface in Figure 5, while it is inclined by 18 ° in the ion-free form (Figure 3A), neglecting the contribution of S_{mol}. The relative displacements of the averaged proton positions in Figure 5 are also included in Table VII. The ion-bound conformation in Figure 5 is consistent with many reported observations. Although pentalysine did not have any effect on the polar head group of phosphatidylcholine, an amphiphilic oligopeptide, Lys-Lys-Gly-Leu_{20}-Lys-Lys-Ala-$CONH_2$, which was incorporated into the membrane, showed significant effects on the deuterium quadrupole splittings of the polar head group (Roux et al., 1989). These facts suggest that the positive charge should be located within the polar group, and not at its surface, to affect the conformation of the polar head group. This idea is further supported by the observations as to the effects of monovalent cations. Although monovalent metal ions only slightly affect the quadrupole splittings of the choline group (Akutsu & Seelig, 1981), hydrophobic cations such as lipids (Seelig et al., 1987), local anesthetics (Boulanger et al., 1981; Browning & Akutsu, 1982; Seelig et al., 1988), and organic ions (Akutsu & Seelig, 1981; Seelig et al., 1987) changed them significantly. These hydrophobic cations are expected to penetrate into the polar head-group region. A neutron-scattering study also showed that calcium ions bound to DPPC bilayers are located within the polar head-group region (Herbette et al., 1984). The penetrated cations would stay in the vicinity of the phosphate group. They might induce a conformation such as that in Figure 5 because of the electrostatic repulsion between the cation and the quaternary ammonium ion. An X-ray diffraction study on the hydrocarbon chain tilt also suggested that multivalent cations induced a conformational change of the polar head group of DPPC so that the cross section of the polar region became smaller (McIntosh, 1980). This was explained by a more extended conformation of the polar head group with the choline group extending farther from the bilayer center than the phosphate group. Hauser et al. (1976) proposed a model for the polar head-group conformation of phosphatidylcholine on the basis of the results of paramagnetic probe analysis. This can be taken as the ion-bound conformation (Hauser et al., 1981). The P–N vector in this model was also oriented more parallel to the bilayer normal. Therefore, there is rough agreement between their model and ours in spite of the significant differences in the dihedral angles. As to cation-bound complex formation, 1:1 and 1:2 (cation/lipid) stoichiometries were reported (Altenbach & Seelig, 1984; Macdonald & Seelig, 1987b; Petersheim et al., 1989). Our model fits either stoichiometry.

It was indicated that the change in the quadrupole splitting is sensing the change in the electric field at the membrane surface (Seelig et al., 1987). A model explaining this was proposed by Roux et al. (1989). Namely, the electric field due to the surface charge produces a torque that rotates the polar head groups. Here, the surface charge is represented as a uniform charge distribution. If this is the case, the conformation of the polar head group should change continuously. This would only be compatible with the rigid-structure model, as mentioned above. Although the rigid-structure model seems to be less proper, we cannot eliminate this possibility at this stage. However, there would be another explanation for the sensing of the electric field. Namely, the polar head groups sense the relative number of bound charges rather than the average electric field. This model can also explain the linear relationship between the change in quadrupole splittings and the charge density at the surface. We do not need to assume a firm cation–polar head-group complex in this model. The localization of the charge in the vicinity of the phosphate group, which induces the conformational change, is enough for explanation of the observations. The importance of the location of the positive charge was also indicated by Roux et al. (1989). The effects of a negative charge and dipole moment should be taken as different processes, because they do not fall on the same linear relationship given by cations.

Registry No. DPPC, 2644-64-6.

References

Abrahamsson, S., & Pascher, I. (1966) *Acta Crystallogr. 21*, 79–87.

Akutsu, H. (1981) *Biochemistry 20*, 7359–7366.

Akutsu, H., & Seelig, J. (1981) *Biochemistry 20*, 7366–7373.

Akutsu, H., Suezaki, Y., Yoshikawa, W., & Kyogoku, Y. (1986) *Biochim. Biophys. Acta 854*, 213–218.

Altenbach, C., & Seelig, J. (1984) *Biochemistry 23*, 3913–3920.

Altenbach, C., & Seelig, J. (1985) *Biochim. Biophys. Acta 818*, 410–415.

Boulanger, Y., Schreier, S., & Smith, I. C. P. (1981) *Biochemistry 20*, 6824–6830.

Brown, M. F., & Seelig, J. (1977) *Nature 269*, 721–723.

Browning, J. L., & Akutsu, H. (1982) *Biochim. Biophys. Acta 684*, 172–178.

Büldt, G., Gally, H. U., Seelig, J., & Zaccai, G. (1979) *J. Mol. Biol. 134*, 673–691.

Elder, M., Hitchcock, P., Mason, R., & Shipley, G. G. (1977) *Proc. R. Soc. London, A 354*, 157–170.

Hauser, H., Phillips, M. C., Levine, B. A., & Williams, R. J. P. (1976) *Nature 261*, 390–394.

Hauser, H., Pascher, I., & Sundell, S. (1980) *J. Mol. Biol. 137*, 249–264.

Hauser, H., Guyer, W., & Paltauf, F. (1981) *Chem. Phys. Lipids 29*, 103–120.

Herbette, L., Napolitano, C. A., & McDaniel, R. V. (1984) *Biophys. J. 46*, 677–685.

Herzfeld, J., Griffin, R. G., & Haberkorn, R. A. (1978) *Biochemistry 17*, 2711–2718.

Jarrell, H. C., Giziewicz, J. B., & Smith, I. C. P. (1986) *Biochemistry 25*, 3950-3957.
Macdonald, P. M., & Seelig, J. (1987a) *Biochemistry 26*, 1231-1240.
Macdonald, P. M., & Seelig, J. (1987b) *Biochemistry 26*, 6292-6298.
Macdonald, P. M., & Seelig, J. (1988) *Biochemistry 27*, 6769-6775.
McIntosh, T. J. (1980) *Biophys. J. 29*, 237-246.
McLaughlin, S. A. (1977) *Curr. Top. Membr. Transp. 9*, 71-144.
Murari, R., Murari, M. P., & Baumann, W. J. (1986) *Biochemistry 25*, 1062-1067.
Oldfield, E., Meadows, M., Rice, D., & Jacobs, R. (1978) *Biochemistry 17*, 2727-2739.
Pascher, I., & Sundell, S. (1986) *Biochim. Biophys. Acta 855*, 68-78.
Pascher, I., Sundell, S., & Hauser, H. (1981) *J. Mol. Biol. 153*, 807-824.
Pascher, I., Sundell, S., Harlos, K., & Eibl, H. (1987) *Biochim. Biophys. Acta 896*, 77-88.
Pearson, R. H., & Pascher, I. (1979) *Nature 281*, 499-501.
Petersen, N. O., & Chan, S. I. (1977) *Biochemistry 16*, 2657-2667.
Petersheim, M., Halladay, H. N., & Blodnieks J. (1989) *Biophys. J. 56*, 551-557.
Renou, J.-P., Giziewicz, J. B., Smith, I. C. P., & Bloom, M. (1989) *Biochemistry 28*, 1804-1814.
Roux, M., Newmann, J.-M., Hodges, R. S., Devaux, P. F., & Bloom, M. (1989) *Biochemistry 28*, 2313-2321.
Seelig, A., Allegrini, P. R., & Seelig, J. (1988) *Biochim. Biophys. Acta 939*, 267-276.
Seelig, J. (1977) *Q. Rev. Biophys. 10*, 353-418.
Seelig, J., Gally, H. U., & Wohlgemuth, R. (1977) *Biochim. Biophys. Acta 467*, 109-119.
Seelig, J., Macdonald, P. M., & Scherer, P. G. (1987) *Biochemistry 26*, 7535-7541.
Skarjune, R., & Oldfield, E. (1979) *Biochemistry 18*, 5903-5909.
Sundaralingam, M., & Jensen, L. H. (1965) *Science 150*, 1035-1036.

Bilayer Packing Stress and Defects in Mixed Dilinoleoylphosphatidylethanolamine and Palmitoyloleoylphosphatidylcholine and Their Susceptibility to Phospholipase A_2[†]

Arindam Sen,* Thomas V. Isac, and Sek-Wen Hui

Membrane Biophysics Laboratory, Department of Biophysics, Roswell Park Memorial Institute, Buffalo, New York 14263

Received May 25, 1990; Revised Manuscript Received January 3, 1991

ABSTRACT: The hydrolysis of mixed dilinoleoylphosphatidylethanolamine (DiLinPE) and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) dispersions by porcine phospholipase A_2, under conditions leading to the bilayer-to-nonbilayer phase transition, has been studied. Two structurally distinct forms of the dispersions were used, multilamellar vesicles (MLV) and supercritical large unilamellar vesicles (SCLUV). In MLV, maximum free fatty acid was produced in dispersions containing 85 mol % DiLinPE. The peak in the fatty acid release is found at the onset of appearance of the nonbilayer defects reported earlier. DiLinPE was found to be preferentially hydrolyzed as compared to POPC. When cholesterol was added to the mixed DiLinPE/POPC MLV, the onset of the observable appearance of nonbilayer defects, the positions of the peaks for total hydrolysis, and the preferential hydrolysis of DiLinPE were all shifted toward lower DiLinPE concentrations. In SCLUV, where the appearance of nonbilayer structures is prevented by constraining the lipids in bilayer configuration, the hydrolysis by PLA_2 increases with increasing DiLinPE as predicted from the increase in the calculated monolayer bending energy. The results are interpreted to be related to the pretransition molecular-packing stress and defects at the onset of the bilayer-to-nonbilayer transition. Results indicate that the porcine pancreatic phospholipase A_2 activity is controlled by bilayer-packing stress, which may cause structural defects of the substrate, among other factors. Results also indicate a preferential localization of PE at stress-related defect regions.

Phospholipases play an important role in membrane phospholipid metabolism. In recent years, the regulation of their activities has been the focus of many investigations because of their putative role in signal transduction processes (Berridge & Irvine, 1984). Recently, phospholipase A_2 (PLA_2) has also been suggested to play a pivotal role in the repair of damage by lipid peroxidation (van Kruijk et al., 1987). The study of the regulatory mechanism of this enzyme is thus of much current interest.

The hydrolytic function of PLA_2 is believed to be based on a sequence of events involving binding and activation steps (Waite, 1985). The activation step is very sensitive to the physical state of the substrate. For instance, bilayers are poorer substrates than micelles. For bilayer substrates, the activation requires structural defects or fluctuations that culminate at the gel-to-fluid phase transition (Romers et al.,

[†] This work was supported by USPHS Grant GM-28120 from the National Institutes of Health.

* To whom correspondence should be addressed.

0006-2960/91/0430-4516$02.50/0