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# THE CONFORMATION OF THE POLAR GROUP OF LYSOPHOSPHATIDYLCHOLINE IN $H_2O$; CONFORMATIONAL CHANGES INDUCED BY POLYVALENT CATIONS

H. HAUSER [a], W. GUYER [a], B. LEVINE [c], P. SKRABAL [b] and R.J.P. WILLIAMS [c]

*Eidgenössische Technische Hochschule, [a] Laboratorium für Biochemie and [b] Technisch-Chemisches Laboratorium, ETH-Zentrum, CH-8092 Zürich (Switzerland) and [c] Inorganic Chemistry Laboratory, Oxford University, South Parks Road, Oxford (U.K.)*

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## Summary

The conformation of the polar group of egg lysophosphatidylcholine and 1-myristoyl-*sn*-glycero-3-phosphorylcholine present as micelles in aqueous solution has been studied using NMR methods. In the absence of polyvalent cations the preferred conformation derived from spin-spin coupling constants is similar, but not identical, to that of phosphatidylethanolamine in the crystal structure (cf. Hitchcock, P.B., Mason, R., Thomas, K.M. and Shipley, G.G. (1974) Proc. Natl. Acad. Sci. U.S. 71, 3036—3040). The presence of lanthanides induces a conformational change involving primarily the phosphorylcholine group, e.g. torsion angle $\alpha_5$ changes from an all *gauche* to an approximate *trans* disposition. The *gauche* → *trans* transitions observed with torsion angles $\alpha_3$ and $\alpha_5$ produce a more extended orientation of the polar group (relative to the hydrocarbon chain axis). In the presence of lanthanides the conformation of lysophosphatidylcholine is very similar to that of the diacyl phosphatidylcholines observed in fully hydrated bilayers (cf. Hauser, H., Phillips, M.C., Levine, B.A. and Williams, R.J.P. (1976) Nature 261, 390—394) with the P-N vector at an angle of about 45° to the bilayer.

## Introduction

Knowledge of the structure and packing of the hydrocarbon chains in lipid aggregates, the predominant one being the lipid bilayer, is well advanced (for a review see refs. 1 and 2). In contrast, the conformation and molecular motion of the polar group region are less well understood. Here we report the polar group conformation of lysophosphatidylcholine dissolved in $^2H_2O$ in the presence and absence of polyvalent cations.

Lysophosphatidylcholine forms small micelles in aqueous solution and the resolution of the $^1H$-, $^{13}C$- and $^{31}P$-NMR spectra from those micelles (diameter

$<80$ Å) is such that spin coupling constants can be measured. This is the basis of our conformational analysis in the absence of cations. Lanthanides are then used as an isomorphous replacement for $Ca^{2+}$ [3]. The principle of the conformational analysis in the presence of lanthanides has been described before [4].

## Experimental Methods

*Materials*

Egg lysophosphatidylcholine (Lipid Products, South Nutfield, Surrey, U.K.) and 1-myristoyl-*sn*-glycero-3-phosphorylcholine (Applied Science Lab., State College, Pa., U.S.A.) were pure by thin layer chromatography standards. The gas-liquid chromatographic analysis showed that palmitic acid was the major fatty acid of egg lysophosphatidylcholine. The lipids retained methanol tenaciously which was removed by drying the samples in vacuo in the presence of solid KOH. Typical lipid concentrations used were 1—5% (w/v). The Stokes radius of the lysophosphatidylcholine micelles was determined by gel filtration on Sepharose 4B and the average value in aqueous solvent was $34 \pm 3$ Å [5]. Lanthanide nitrate (E. Merck AG, Darmstadt, G.F.R.) solutions in $^2H_2O$ of a nominal pH of 5.0—6.0 were prepared as described in ref. 7. Lanthanide concentrations were determined according to ref. 8 (Arsenazo III method).

*NMR methods*

$^1$H-NMR Fourier transform spectra were recorded on a Bruker HXS-360 MHz instrument with a digital resolution of 0.18 Hz/point. $^{13}$C- and $^{31}$P-NMR spectra were obtained at 22.63 (Bruker WH-90) and 36.43 MHz (Bruker HXE-90), respectively. Unless otherwise stated all experiments were carried out at $25 \pm 2$°C. The chemical shifts and coupling constants (Table I) were derived from computer simulations of the $^1$H-NMR spectra using the Nicolet ITRCAL version of the LAOCN3 programme on a Nicolet B-NC 12 computer equipment with a NIC-294 disk memory.

## Results

*Conformational analysis in the absence of polyvalent cations*

Previous $^1$H-NMR studies of lysophosphatidylcholine at 270 MHz [4—6] did not allow the complete assignment of the glycerol protons. The CH-OH (glycerol) signal unresolved at 270 MHz can be assigned unequivocally at 360 MHz on the basis of intensity measurements, titration with $Pr^{3+}$ (Fig. 2) and homonuclear double resonance experiments. Fig. 1a shows the $^1$H-NMR spectrum of lysophosphatidylcholine and Table I summarizes the chemical shifts and coupling constants of the lipid polar group derived from the computer simulation (Fig. 1c) of the expanded spectrum (Fig. 1b). The basic sets of spectral parameters used for the computation were obtained from decoupling experiments described below. The $CH_2O$-CO glycerol group gives a multiplet corresponding to the AB part of an ABC system indicating that the two protons are both chemically and magnetically non-equivalent. The $CH_2O$-CO signal does not show any $^{31}$P spin coupling and gives a four-line AB-

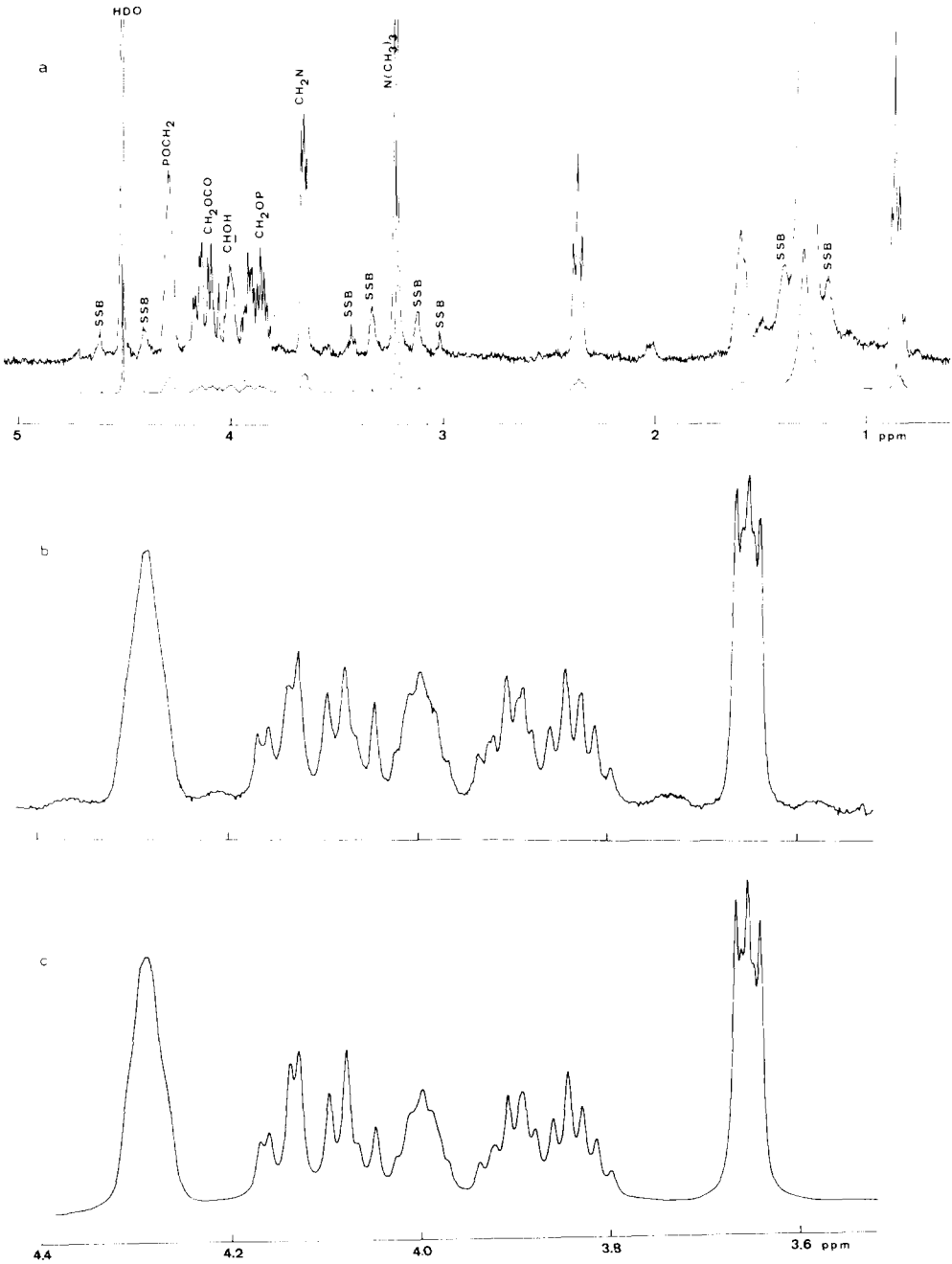


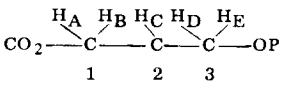

Fig. 1. (a) 360 MHz $^{1}$H-NMR spectrum of egg lysophosphatidylcholine in $^{2}H_2O$ (20 mg/ml = 0.04 M) at a nominal pH of 5.5. SSB, spinning side band. (b) Expanded spectrum of the lipid polar group ($N(CH_3)_3$ resonance not shown) and (c) its computer simulation.

TABLE I

$^1H$ AND $^{31}P$ CHEMICAL SHIFTS AND COUPLING CONSTANTS OF LYSOPHOSPHATIDYLCHOLINE IN $^2H_2O$

| Signal | δ (ppm) ** | Coupling constants (Hz) |
|---|---|---|
| Glycerol $CH_2$-O-CO * | $H_A$ 4.15; $H_B$ 4.08; | $^2J_{AB}$ = 1.14; $^3J_{AC}$ = 3.4; $^3J_{BC}$ = 6.5 |
| Glycerol CH-OH | $H_C$ 4.00 | |
| Glycerol $CH_2$-OP | $H_D$ 3.91; $H_E$ 3.84; | $^2J_{DE}$ = 10.8; $^3J_{CE}$ = 6.0; $^3J_{CD}$ = 4.1; $^3J_{P\text{-}H_D}$ = 6.0; $^3J_{P\text{-}H_E}$ = 5.8 |
| Choline $POCH_2$ | $H_{XX'}$ 4.29 *** | $^3J_{P\text{-}H_X} = {}^3J_{P\text{-}H_{X'}}$ = 6.0; $^3J_{N\text{-}H_X} = {}^3J_{N\text{-}H_{X'}}$ = 2.5 |
| Choline $CH_2N$ | $H_{MM'}$ 3.66 *** | $^3J_{MX} = {}^3J_{M'X'}$ = 2.5; $^3J_{M'X} = {}^3J_{MX'}$ = 6.9 |
| Choline $\overset{+}{N}(CH_3)_3$ | 3.21 | |
| $^{31}P$ | 3.46 | |

* The numbering of the C-atoms and lettering of the glycerol protons is as follows:



** Downfield from 3-(trimethylsilyl)propane sulphonate ($^1H$) and trimethyl phosphate ($^{31}P$) as internal standards.

*** The geminal coupling constants could not be extracted from the spectrum. The accuracy of the calculated chemical shifts and coupling constants is ±0.4 Hz and ±0.2 Hz, respectively, except for the $^3J_{N\text{-}H}$ coupling which has a larger error of ±1 Hz. $^3J_{N\text{-}H}$ is the only vicinal coupling constant for which no estimate could be obtained from decoupling experiments. The chemical shifts are given to the nearest 0.01 ppm because the accuracy of the experimental determination relative to an internal standard is ±0.01 ppm.

spectrum when the C<u>H</u>-OH proton is decoupled. The $CH_2OP$ glycerol protons are also non-equivalent characterized by vicinal $^{31}P$-$^1H$ spin coupling constants of 6.0 and 5.8 Hz. This coupling is evident from the ABX type spectrum to which the $CH_2OP$ signal is reduced when the <u>H</u>COH proton is decoupled. The quintet at 3.66 ppm (Fig. 1b) is assigned to the $CH_2N$ (choline) protons which are the MM′ part of an MM′XX′ system. Irradiating the broad resonance at 4.29 ppm caused the $CH_2N$ multiplet to collapse to a singlet indicating that there is no $^{14}N$-$^1H$ spin coupling. The vicinal coupling constants derived from the simulated spectrum (Fig. 1c) are 6.9 and 2.5 Hz (Table I). The broad resonance at 4.29 ppm is the asymmetric XX′ counterpart to the MM′ spin system. The asymmetry arises from coupling of the $POCH_2$ protons to both $^{31}P$ and $^{14}N$, which is evident from double resonance experiments irradiating the $CH_2N$ protons ($^3J_{P\text{-}H}$ = 6 Hz and $^3J_{N\text{-}H}$ = 2.5 Hz).

*Conformational analysis in the presence of paramagnetic lanthanides*

Fig. 2 shows the observed changes in chemical shift of all six $^1H$ resonances from the polar group of lysophosphatidylcholine as a function of lanthanide concentration. To check whether there was any diamagnetic contribution to the observed shift changes $La(NO_3)_3$ was added to lysophosphatidylcholine over the same concentration range. The diamagnetic shifts were negligible.

Fig. 3 gives the concentration dependence of the five $^1H$-shift ratios, $R_{ij}$, (cf. Table II) derived from the shift changes shown in Fig. 2. With the exception of $R_{ij}$ involving the glycerol signals $CH_2OCO$ and C<u>H</u>-OH, shift ratios are

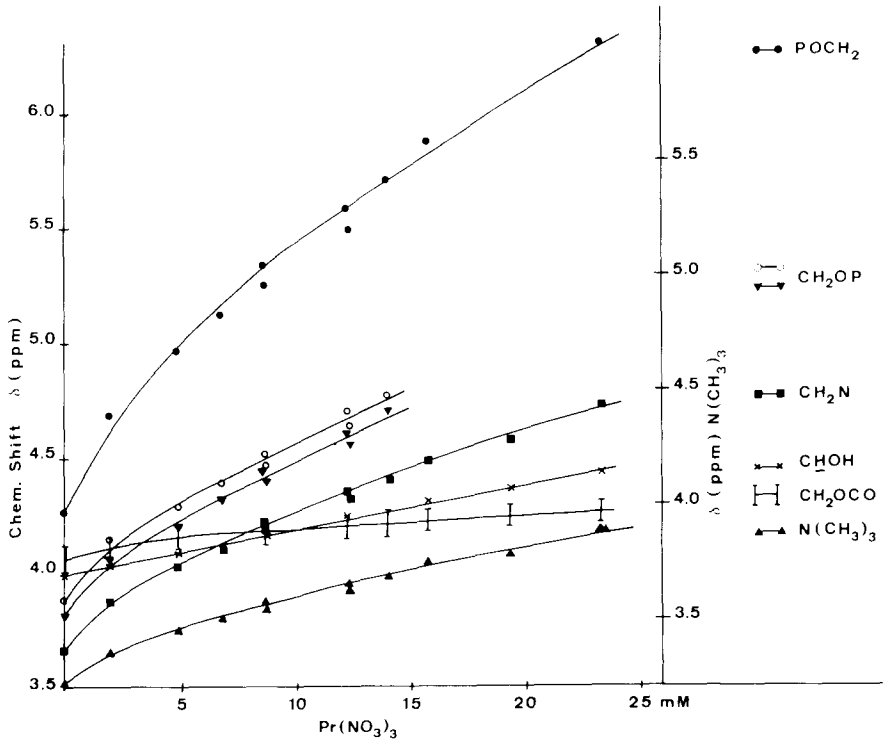



Fig. 2. Typical titration curves of observed chemical shifts $\delta$ (ppm) at 360 MHz of the polar group of lysophosphatidylcholine as a function of the total $Pr(NO_3)_3$ concentration. The concentration of lysophosphatidylcholine was 35.4 mM. With the $CH_2OP$ group giving two chemically-shifted $^1H$-signals (cf. Table I) it was possible to monitor the concentration dependence of $\delta$ of the two signals separately.

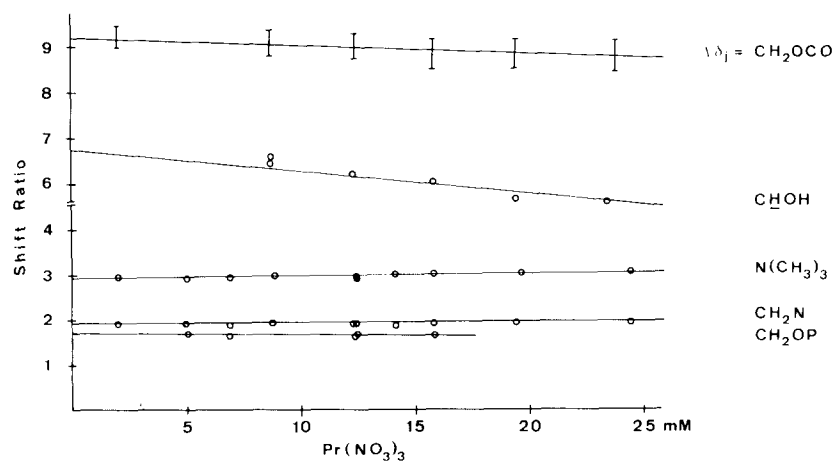



Fig. 3. Ratios of proton pseudo-contact shifts $R_{ij}$ (cf. ref. 4) as a function of total added $Pr(NO_3)_3$ concentration. Changes in chemical shifts $\Delta\delta$ (ppm) induced in the presence of $Pr(NO_3)_3$ were measured relative to the chemical shifts $\delta$ observed in the absence of lanthanides. The changes in chemical shift $\Delta\delta$ derived from Fig. 2 were corrected for any diamagnetic contribution and used to form the shift ratios $R_{ij} = \Delta\delta_i/\Delta\delta_j$, where $\Delta\delta_i$ is the change in chemical shift of the $POCH_2$ (choline) signal and $\Delta\delta_j$ designates the shift changes of the other polar group protons. The accuracy of the determination of ratio $R_{ij}$ (top curve) was less than that of other ratios, and the bars represent the experimental scatter.

TABLE II

AVERAGE PSEUDO-CONTACT SHIFTS $\Delta\delta$ INDUCED IN THE $^1$H-, $^{13}$C- AND $^{31}$P-NMR SPECTRA OF LYSOPHOSPHATIDYLCHOLINE BY PARAMAGNETIC LANTHANIDES

| Signal | $\Delta\delta$ (ppm) * | Shift ratio $R_{ij}$ ** | | $^{13}$C Signal | $\Delta\delta$ (ppm) *** |
|---|---|---|---|---|---|
| | | Average value | Value extrapolated to $[Ln^{3+}] = 0$ | | |
| Glycerol $CH_2$-OCO | 0.10 ± 0.06 | 10.0 | 8.9 | $CH_2$-OCO | 0.03 ± 0.15 |
| Glycerol CH-OH | 0.20 ± 0.05 | 5.0 | 6.5 | CH-OH | 0.22 ± 0.15 |
| Glycerol $CH_2$-OP | 0.61 ± 0.05 | 1.7 | 1.6 | $CH_2$-OP | 0.50 ± 0.20 |
| Choline PO-$CH_2$ | 1 | | | PO-$CH_2$ | 0.90 ± 0.15 |
| Choline $CH_2N$ | 0.53 ± 0.04 | 1.9 | 1.9 | $CH_2N$ | 0.48 ± 0.15 |
| Choline $N(CH_3)_3$ | 0.34 ± 0.04 | 3.1 | 3.0 | $N(CH_3)_3$ | 0.30 ± 0.10 |
| $^{31}$P | 3.30 ± 0.06 | 0.3 | | | |

* Average pseudo-contact shifts for various nuclei obtained when the induced shift for the $POCH_2$ (choline) protons equals 1 ppm. The average is taken over the lanthanide concentration range $[Ln^{3+}] = 0$—25 mM.

** Shift ratios $R_{ij}$; $R_{ij} = \Delta\delta_i/\Delta\delta_j$ where $\Delta\delta_i$ is the change in chemical shift of the $POCH_2$ (choline) group and $\Delta\delta_j$ is the change in chemical shift of any of the other protons or $^{31}$P (cf. Fig. 3); the average shift ratios $R_{ij}$ are related to $\Delta\delta$ (second column) by $R_{ij} = 1/\Delta\delta$.

*** $^{13}$C Pseudo-contact shift changes standardized to the $^1$H shift change $\Delta\delta_i$ of the $POCH_2$ (choline) signal. The $^{13}$C pseudo-contact shift changes were derived from the observed values after correcting for diamagnetic contributions using $La^{3+}$ and for contact contributions using the treatment described in ref. 7. The $^{13}$C shift changes induced by $Pr^{3+}$, $Dy^{3+}$, $Ho^{3+}$, $Tm^{3+}$ and $Yb^{3+}$ (as chlorides) were determined at a single lanthanide concentration (8 mM) and hence bear larger tolerances. The $^{13}$C pseudo-contact shifts quoted were obtained as follows: $\Delta\delta_X{}^{13}C/\Delta\delta^1H \times 0.34$ where $\Delta\delta_X{}^{13}C/\Delta\delta^1H$ is the derived pseudo-contact shift ratio of carbon signal X relative to the $N(CH_3)_3$ proton shift. The value 0.34 is the $N(CH_3)_3$ $^1$H-shift relative to the $POCH_2$ (choline) proton signal.

invariant with lanthanide concentration (cf. Table II). All shift ratios are, however, invariant upon titration with $Pr^{3+}$ in the presence of $La^{3+}$ ($\geqslant$10 mM). The average shift ratios were also invariant for $Nd^{3+}$, $Eu^{3+}$, $Tb^{3+}$, $Dy^{3+}$, $Ho^{3+}$ and $Tm^{3+}$.

From $^1$H (Fig. 2) and $^{31}$P (not shown) "titration" curves, $^{31}P/^1H$ shift ratios were calculated at given lanthanide concentrations; contrary to the $^1$H shift ratios (Table II and Fig. 3) the $^{31}P/^1H$ shift ratios were found to strongly depend on the nature of the lanthanide ion. It is noted that the ratio $^{31}P/^1H$ varied with lanthanide concentration below approximately 2 mM but was constant at higher lanthanide concentrations. The $^1$H and $^{31}$P titration curves were then used to separate the contact contribution from the total $^{31}$P shift observed. To this end the $^1$H and $^{31}$P shift changes were plotted according to ref. 7 and from the linear relationship thus obtained the pseudo-contact shift ratio $\Delta\delta^{31}P/\Delta\delta$ $(^1H_3C)_3N$ was derived as 9.0 ± 0.2. If the same treatment was carried out using the $^1$H signal of the $POCH_2$ (choline) group the pseudo-contact shift ratio was $\Delta\delta^{31}P/\Delta\delta^1H$ $(POCH_2) = 3.3 \pm 0.6$.

Table II also contains the $^{13}$C pseudo-contact shift changes of the polar group signals of lysophosphatidylcholine. The signals from both $^{13}$C atoms next to the phosphate group were affected by contact contribution while the shift changes of all other signals were pseudo-contact in origin, as shown by invariance of shift ratios with different lanthanides. With the two $CH_2OP$ and

$POCH_2$ $^{13}C$ resonances the contact contributions to the observed shift changes were separated to give the pseudo-contact shift changes included in Table II. Within the error of the measurement there is good agreement between the $^1H$ and $^{13}C$ shift ratios of Table II.

## Discussion

*The conformation of the polar group in the absence of cation*

Conformations A to C (Fig. 4) are the most likely ones of the $RCO_2CH_2$-CHOH bond assuming that the staggered conformations represent minimum energy conformations. The experimental values of the vicinal coupling constants $J_{AC}$ and $J_{BC}$ (Table I) represent averages of the so-called component coupling constants in rotamers A to C (see Fig. 4 and legend) weighted by the fractional populations $a$, $b$ and $c$:

$$J_{AC} = aJ_t^g + bJ_g^t + cJ_g^g \quad (1)$$

$$J_{BC} = aJ_t^t + bJ_g^{g'} + cJ_g^{g'} \quad (2)$$

$$1 = a + b + c \quad (3)$$

The fractional populations $a$ to $c$ can be calculated if the component vicinal coupling constants are known. These constants were derived according to Abraham and Gatti [9] using the electronegativity values of ref. 10 and are included in Fig. 4. The fractional populations calculated from Eqns. 1—3 are summarized in Table III. Since $H_A$ and $H_B$ (Fig. 4, A to C) cannot be assigned, two possible solutions are obtained: (1) with $J_{AC} > J_{BC}$ and (2) with $J_{AC} < J_{BC}$ (Table III). With $J_{AC} > J_{BC}$ the populations $b$ and $c$ are dominant with $\theta_3$ = *trans* and *gauche* and $\theta_4 = \pm$ *gauche*, respectively. With $J_{AC} < J_{BC}$ populations $a$ and $c$ are dominant. With $J_{AC} > J_{BC}$ conformation B makes up half of the total

TABLE III

| Fragment * | Rotamer * | Fractional population ** | | Torsion angle *** | Conformation |
|---|---|---|---|---|---|
| | | $J_{AC} > J_{BC}$ | $J_{AC} > J_{BC}$ | | |
| $RCO_2CH_2$-CHOH | A | 0.11 (0.08) | 0.42 | | − *gauche* (*trans*) |
| | B | 0.48 (0.50) | 0.05 | $\theta_3$ ($\theta_4$) | *trans* (+ *gauche*) |
| | C | 0.41 (0.42) | 0.53 | | + *gauche* (− *gauche*) |
| | | $J_{CD} > J_{CE}$ | $J_{CD} < J_{CE}$ | | |
| HOHC-$CH_2$OP | D | 0.18 (0.15) | 0.38 | $\theta_1$ ($\theta_2$) | + *gauche* (*trans*) |
| | E | 0.39 (0.42) | 0.14 | | *trans* (− *gauche*) |
| | F | 0.43 (0.43) | 0.48 | | − *gauche* (*gauche*) |
| $POCH_2$-$CH_2$N | G | 0 | | | *trans* |
| | H | 0.50 | | $\alpha_5$ | + *gauche* |
| | I | 0.50 | | | − *gauche* |
| $CH_2$O-PO | K | 1.0 | | $\alpha_1$ | *trans* |
| OP-$OCH_2$ | K | 1.0 | | $\alpha_4$ | *trans* |

* cf. Fig. 4.

** The populations of rotamers A, B . . . K are $a$, $b$ . . . $k$, respectively. Values in brackets are those from the component coupling constants of *trans*-2,3-dimethyl-1,4-dioxane.

*** For the notation of the torsion angles see ref. 19.

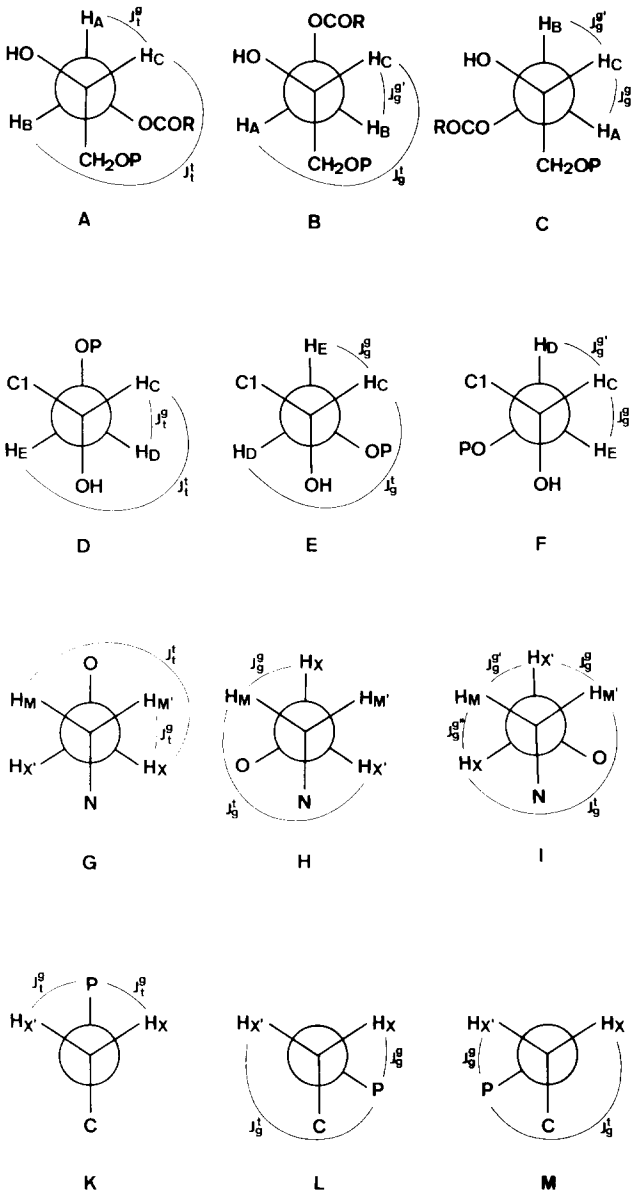



Fig. 4. Staggered conformations of minimum free energy for the polar group of lysophosphatidylcholine. The component vicinal coupling constants $J$ were taken from refs. 9, 11 and 18. The subscript of $J$ denotes the isomer, the superscript the orientation of the coupled protons. A to C, Rotamers about the $RCO_2CH_2$-CHOH bond of the glycerol group; component vicinal coupling constants: $J_t^t$, 11.9 Hz; $J_t^g$, 5.8 Hz; $J_g^t$, 12.3 (11.5) Hz; $J_g^{g'}$, 2.4 (2.7) Hz; $J_g^g$, 0.45 (0.60) Hz; the numbers in parenthesis taken for comparison are from the component coupling constants of *trans*-2,3-dimethyl-1,4-dioxane [11,12]. D to F, Rotamers about the HOCH-$CH_2$OP bond of the glycerol group; component vicinal coupling constants as in A to C. G to I. Rotamers about the POC$H_2$-$CH_2$N bond of the choline group; component vicinal coupling constants from ref. 18. $J_t^t$, 12.31 Hz; $J_t^g$, 5.48 Hz; $J_g^t + J_g^{g'}$, 13.62 Hz; $J_g^g + J_g^{g''}$, 4.84 Hz. K to M, Rotamers about the $CH_2$-OP bond ($\alpha_1$) or the PO-$CH_2$ bond ($\alpha_4$); $J_g^t$, 18—28 Hz; $J_g^g \approx J_t^g$, 1.5—6 Hz.

population with the torsion angles $\theta_3$ = *trans* and $\theta_4$ = *gauche* being consistent with the conformation of 1,2-dilauroyl-DL-phosphatidylethanolamine determined by X-ray crystallography [13,14]. With $J_{AC} < J_{BC}$ rotamer A becomes significant; in the case of diacyl phosphatidylcholine a large proportion of A is unlikely because the torsion angle $\theta_4$ = *trans* would not allow for the well known parallel alignment of the hydrocarbon chains observed in bilayers.

The above treatment applied to the second C-C bond of the glycerol fragment (Fig. 4, D—F) gives the following results; regardless of the assignment of the observed vicinal coupling constants $J_{CD}$ and $J_{CE}$ a major proportion of the population is made up by rotamer F. Conformation F is observed in the crystal structure of phosphatidylethanolamine indicating that that conformation is a preferred one.

The quintet observed for the $CH_2N$ (choline) group indicates that rotamers G, H and I (Fig. 4) are not equally populated. This is true for lysophosphatidylcholine in water as well as in organic solvents ($CHCl_3$/MeOH = 2 : 1). The quintet observed for the $CH_2N$ resonance (Fig. 1) was analyzed according to the methods described in refs. 15—18. The observed vicinal coupling constants $J_{MX}$ and $J_{MX'}$ are expressed in terms of the component vicinal coupling constants:

$$J_{MX} = 2.5 = gJ_t^t + hJ_g^g + iJ_g^{g''} \quad (4)$$

$$J_{MX'} = 6.9 = gJ_t^g + hJ_g^t + iJ_g^{g'} \quad (5)$$

Since $h = i$ we have:

$$g + 2h = 1 \, . \quad (6)$$

From this analysis it is clear that the conformation of the N-C-C-O choline group is preferred *gauche*. This is consistent with the crystal structures of phosphatidylethanolamine [13] and of lipid constituents such as glycerylphosphorylcholine and glycerylphosphorylethanolamine [19]. That the *gauche* conformation about the N-C-C-O bond is preferred has also been shown for dipalmitoyl phosphatidylcholine [12,20] and phosphatidylethanolamine [21] in organic solvents, for acetylcholine and many related compounds in aqueous solution [18,20,22] as well as for many 1,2-disubstituted ethanes [23]. It is noteworthy that there is particularly good agreement between the spectral parameters of the N-C-C-O group in lysophosphatidylcholine and acetylcholine [18].

An alternative method of obtaining an estimate of the fractional populations of the N-C-C-O rotamers is to use the observed $J(^{14}N\text{-C-C-H})$ coupling constants. The angular dependence of that vicinal coupling constant has been given in ref. 24. The value $^3J_{NH}$ = 2.5 Hz (Table I) derived from the simulated spectrum (Fig. 1c) is in good agreement with a predominantly *gauche* conformation (cf. ref. 18).

Of the remaining torsion angles of the lysophosphatidylcholine polar group ($\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha_4$) information about $\alpha_1$ and $\alpha_4$ may be deduced from the vicinal $^3J_{PH}$ coupling constants (Table I). However, no information concerning the torsion angles $\alpha_2$ and $\alpha_3$ and, thus, the conformation of the phosphodiester can be derived.

Various examples of the angular dependence of the vicinal coupling constants $^3J_{P\text{-}O\text{-}C\text{-}H}$ have been reported [25—28]. In lysophosphatidylcholine the $^{31}P$ atom is coupled to the $CH_2OP$ (glycerol) as well as to the $POCH_2$ (choline) protons, with all coupling constants rather close to 6 Hz (Table I) [29]. According to ref. 26 the $^3J_{P\text{-}O\text{-}C\text{-}H}$ coupling constants in Table I correspond to torsion angles P-O-C-H of either 55° (cf. Fig. 4, K) or 125°. The latter can be ruled out because such a conformation is energetically less favourable than conformation K because of the P-atom being eclipsed with the neighbouring C-atom. Rotamers L and M are expected to give $^3J_{P\text{-}O\text{-}C\text{-}H}$ values larger than 6 Hz because the observed values would be the average of a *gauche* ($J_g^g$) and a *trans* ($J_g^t$) coupling constant. Hence, rotamer K represents the preferred conformation about the $C_2$-$C_3$-$O_{31}$-P and the P-$O_{32}$-$C_{31}$-$C_{32}$ bonds with torsion angles $\alpha_1$ and $\alpha_4$ being approximately *trans* (Table III). Even though this conformational analysis permits us to differentiate only between three staggered conformations, the *trans* conformation of $\alpha_4$ is still considered to be significantly different from the crystal structure of phosphatidylethanolamine [13]; the value of $\alpha_4$ is, however, consistent with the crystal structures of phospholipid constituents [19,30].

Synthetic myristoyl-phosphatidylcholine gives a $^1H$-NMR spectrum identical to that shown in Fig. 1. Furthermore, the coupling constants of both myristoyl and egg lysophosphatidylcholine do hardly change between 25 and 70°C. This indicates that both lysophosphatidylcholines have the same conformation which does not change significantly over that temperature range. From the small changes with temperature in the $^1H$ signals of the choline group it can be calculated that at 70° the *trans* conformation of torsion angle $\alpha_5$ amounts to 3—4%.

*The conformation of the polar group in the presence of lanthanides*

The mode of lanthanide binding to the polar group of lysophosphatidylcholine closely resembles that of lanthanide binding to phosphatidylcholine [5,6]. This is evident from the following observations.

(a) With both lipids the observed $^1H$ shift ratios are independent of the nature of the lanthanide ion indicating that the observed shifts are pseudo-contact in origin and that the lanthanide · lipid complex has effective axial symmetry [4].

(b) Within the experimental error of the measurement, the $^1H$ and $^{13}C$ pseudo-contact shift ratios of phosphatidylcholine and its lysocompound are in good agreement [4].

(c) With both lipids almost identical values for the pseudo-contact shift ratios $\Delta\delta^{31}P/\Delta\delta^1H$ are obtained; using the $N(CH_3)_3$ $^1H$ signals these ratios are 9.1 ± 0.2 and 9.0 ± 0.2, and using the $POCH_2$ $^1H$ signals they are 3.1 ± 0.6 and 3.3 ± 0.6 for phosphatidylcholine [4] and its lysocompound, respectively.

The pseudo-contact shift ratios (Fig. 3 and Table II) used in the conformational analysis of lysophosphatidylcholine were the average values (Table II, 2nd column), rather than the values extrapolated to zero, of lanthanide concentration. The reason for this is that the $^1H$ shift ratios were found to be independent of $Pr(NO_3)_3$ when the titration was carried out in the presence of 20 mM $La(NO_3)_3$. The values of the shift ratios thus obtained were consistent

with the average values listed in Table II. This result suggests that the observed concentration dependence of the shift ratios involving the $^{31}P$ signal and the $CH_2OCO$ and CH-OH (glycerol) resonances (Fig. 3) may be due to a change in molecular packing/orientation at the micelle surface induced by lanthanide binding at concentrations $\lesssim$ 10 mM (cf. ref. 5) above which the lanthanide binding sites are nearly saturated (cf. ref. 31).

Since the pseudo-contact shift ratios observed with both phosphatidylcholine and its lysocompounds are similar, we can expect the conformations of the lanthanide · lipid complexes of the two lipids to be similar. The conformational analysis of the lysophosphatidylcholine · lanthanide complex was carried out in the same way as described for phosphatidylcholine [4], and the results are shown in Fig. 5. The two orthogonal views represent the central solution of a small family of closely related conformations all of which, individually and

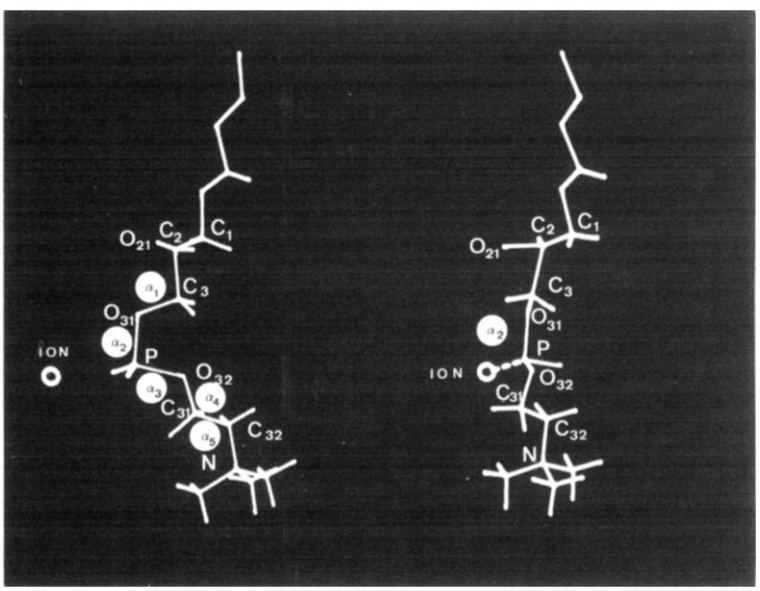



Fig. 5. Two orthogonal views of the conformation of the lysophosphatidylcholine polar group in the presence of lanthanides. The conformation on the right is related to the one on the left by a rotation of 90° about an axis approximately parallel to the hydrocarbon chains. The average torsion angles of the central solution of the small family of possible solutions are as follows: $\alpha_1$ ($C_2$-$C_3$-$O_{31}$-P) = 172°; $\alpha_2$ ($C_3$-$O_{31}$-P-$O_{32}$) = 0°; $\alpha_3$ ($O_{31}$-P-$O_{32}$-$C_{31}$) = 170°; $\alpha_4$ (P-$O_{32}$-$C_{31}$-$C_{32}$) = 155°; $\alpha_5$ ($O_{32}$-$C_{31}$-$C_{32}$-N) = 155°; For the notation of torsion angles see ref. 19. The numbering of atoms used to define the torsion angles is different from that suggested by Sundaralingam [19] and is chosen such that it is consistent with the stereospecific numbering [35]. The family of closely related solutions is defined by the variation of the torsion angles $\alpha_1$—$\alpha_5$ given to the nearest 5°.

| $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_4$ | $\alpha_5$ |
|---|---|---|---|---|
| 150—160° | 35 to —10° | 185—170° | 125—150° | 185—160° |
| 160—170° | 35 to —10° | 185—175° | 130—160° | 185—150° |
| 170—180° | 20 to —15° | 180—160° | 140—170° | 180—140° |
| 180—190° | 15 to —25° | 180—160° | 140—185° | 170—130° |
| 190—200° | 15 to —35° | 175—155° | 150—185° | 165—125° |

The range of variation in torsion angles $\alpha_1$ to $\alpha_5$ is determined by allowing freedom of rotation about C-C bonds so that computed shift and relaxation data are within 20% of the experimentally determined values.

therefore collectively, will fit the experimental data. The central solution is defined by the torsion angles summarized in the legend of Fig. 5. The family of closely related conformations is defined by the range of the possible torsion angles given in the legend of Fig. 5. A considerable population of conformations much outside this family is ruled out [4].

Comparison of Fig. 5 with Figs. 1 and 2 of ref. 4 shows that in the presence of lanthanides the conformation of the lysophosphatidylcholine polar group indeed closely resembles that of the diacyl compound described in ref. 4. The discussion therein on the conformation, stoichiometry and symmetry properties of the complex is equally valid for the lysophosphatidylcholine · lanthanide complex. In addition to the previous discussions [4,36] we would like to stress that even though the polar group as a whole is more extended than that of phosphatidylethanolamine [13], the angle of the P-N vector with respect to the bilayer plane is about 45°.

*Comparison of the two conformations of lysophosphatidylcholine in the absence and presence of polyvalent cations*

Torsion angle $\alpha_5$(N-C-C-O) merits a more detailed discussion. The addition of polyvalent cations causes the N-C-C-O group to undergo a *gauche* (folded) → *trans* (extended) conformational change. Sundaralingam [19,32] pointed out that the preferred conformation of $\alpha_5 = \pm$ *gauche* both in the solid state (crystal) and in solution is probably stabilized by electrostatic interaction between the positively charged nitrogen and the electronegative oxygen. In agreement with that proposal is the finding that the replacement of oxygen by sulfur or selenium, which have significantly larger atomic radii * and smaller electronegativities, leads to a reduction of the electrostatic interaction in the S(Se)-C-C-N segment and consequently to a *gauche* → *trans* conformational change [18,33,34]. In the light of these results the conformational change upon binding of polyvalent cations to the phosphate group of lysophosphatidylcholine is to be expected. The proximity of the lanthanide ion with 3 positive charges is likely to repel the positively charged nitrogen so that the O-C-C-N bond attains the more extended *trans*-conformation. The torsion angles $\alpha_1$ and $\alpha_4$ do not seem to differ when lanthanides are added. The analysis of coupling constants does not allow the determination of torsion angles $\alpha_2$ and $\alpha_3$ defining the conformation of the phosphodiester group. However, it was shown recently [37] that the phosphodiester group in dipalmitoyl phosphatidylcholine present in fully hydrated, liquid crystalline lamellar phases is characterized by a *gauche-gauche* conformation. From the good agreement between the conformation of the diacyl lipid and the conformation discussed above it is likely that the similarity extends to the torsion angles $\alpha_2$ and $\alpha_3$. This would imply that the addition of lanthanides also induces a conformational change of the phosphodiester group from a *gauche-gauche* to a *gauche-trans* disposition [38]. Recently Brown and Seelig ** [39], using an independent approach, have also

* The atomic radius of O is 0.66 Å while the atomic radii of S and Se are 1.04 Å and 1.17 Å, respectively.

** Dr. J. Seelig has written to us saying that he finds a conformational change on binding of ions to the surface of phosphatidylcholine bilayers. This independent conclusion was published [39] after this paper was submitted. We thank Dr. Seelig for this communication.

come to the conclusion that lanthanides induce a conformational change in the polar group of phosphatidylcholine.

In conclusion, the conformational changes induced by lanthanides involve primarily the phosphorylcholine group while the effects on the glycerol backbone are minor. The latter involve a change in distribution of the different populations (Table III). The predominant conformation of the glycerol group in the presence of lanthanides is characterized by $\theta_1$ ($\theta_2$) = *gauche* (*gauche*) and $\theta_3$ ($\theta_4$) = *trans* (*gauche*) consistent with the crystal structure of phosphatidylethanolamine [13].

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