Acyl and Phosphoryl Migration in Lysophospholipids: Importance in Phospholipid Synthesis and Phospholipase Specificity†

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ABSTRACT: Three isomeric lysophosphatidylcholines (1-palmitoyl-sn-glycero-3-phosphorylcholine, 2-palmitoyl-sn-glycero-3-phosphorylcholine, and 3-palmitoyl-sn-glycero-2-phosphorylcholine) have been prepared by the action of phospholipase A2 or lipase on 1,2-dipalmitoyl-sn-glycero-3-phosphorylcholine or phospholipase A2 on 1,3-dipalmitoyl-sn-glycero-2-phosphorylcholine. The structures of the lysocompounds have been confirmed by a complete assignment of the polar head groups using 1H NMR spectroscopy. The product of phospholipase A2 action on phosphatidylcholine is 1-acyl-sn-glycero-3-phosphorylcholine. Acyl migration between this compound and the 2-acyl isomer and phosphoryl migration between this compound and the 2-phosphoryl isomer were followed by 31P NMR. The acyl migration was found to be first order in both lysophospholipid and acid or base with a base-catalyzed second-order rate constant of about 4 × 10−4 M−1 s−1. At alkaline pHs, the equilibrium mixture contains about 90% of the 1-acyl and about 10% of the 2-acyl isomer. A slow acyl migration also occurs in organic solvents, most notably in the presence of basic catalysts used in common acylation procedures for the synthesis of phospholipids from lysophospholipids. At alkaline pHs, no phosphoryl migration was detected in the time scale of acyl migration and hydrolysis. 31P NMR could also directly demonstrate the positional specificity of phospholipase A2 and lipase, which acts as a phospholipase A1, by the direct observation of the products formed under conditions where migration was slow. While it is well-known that phospholipase A2 is specific for the sn-2 position of phospholipids in micelles and bilayer membranes, it was demonstrated by this technique that this specificity also holds for the monomeric phospholipid dibutyrylphosphatidylcholine.

The rearrangement of lysophospholipids via acyl migration has long been recognized as a serious problem that has to be taken into account in the determination of phospholipase specificity (de Haas et al., 1960; de Haas & van Deenen, 1961; van Deenen et al., 1963), synthesis of mixed acyl phospholipids (Gupta et al., 1977; Warner & Benson, 1977), and the elucidation of biosynthetic pathways that lead to mixed acyl phospholipids (van Golde & van den Bergh, 1977) involving lyso phospholipid intermediates (Homma et al., 1981). The importance of phosphoryl migration is illustrated by the recent observation that trace amounts of 1,3-diacetyl-sn-glycero-2-phosphorylcholine produced by phosphoryl migration during the synthesis of 1,2-diacetyl-sn-glycero-3-phosphorylcholine (Lammers et al., 1978; Ponpipom & Bugianesi, 1980) can increase the stability of sonicated vesicles of the latter by 100-fold (Larrabee, 1979).

There are six different lysophosphatidylcholines consisting of three enantiomeric pairs of positional isomers as shown in Figure 1. The possible acyl and phosphoryl migrations are indicated. The lack of a suitable analytical tool has previously prevented a detailed investigation of the reaction rates and equilibria involved in migration. We have now used 31P NMR to follow the kinetics and determine the equilibria of these reactions.

The phenomenon of acyl migration was first discovered by Fischer (1920) and has subsequently been found to occur frequently in chemical and biochemical systems [reviews: Akahori, 1965; Pavlova & Rachinskii, 1968]. It has been characterized quite extensively in the rearrangement between 1- and 2-monoglycerides, because the vicinal diol in 1-monoglycerides can be easily quantitated by using a periodate determination (Martin, 1953; van Lohuizen & Verkade, 1960; Wolfenden et al., 1964; Verkade, 1966; Serdarevich, 1967). However, such a reaction is not possible in lysophospholipids. A number of techniques have been used for studies on the rearrangement of diglycerides, such as a thin-layer chromatographic separation (Freeman & Morton, 1966), enzymatic breakdown (Mattson & Volpenhein, 1962), and 1H NMR spectroscopy (Serdarevich, 1967).

In the case of lysophospholipids, however, it was found to be impossible to separate compounds Ia and IIa by any thin-layer or paper chromatography technique (Slotboom et al., 1963; Eibl & Lands, 1970). Column chromatography on silicic acid or alumina causes rapid equilibration of lysophospholipids (Lands & Merkel, 1963; de Haas & van Deenen, 1965; Eibl & Lands, 1970; Gupta et al., 1977). No chemical methods have appeared which can quantitate mixtures of isomers of lysophospholipids without themselves causing isomerization (Hanahan et al., 1960). The difference in activity of phospholipase A2 toward Ia and IIa was used in an attempt to qualitatively assess the stability of the isomers (Slotboom et al., 1963; Albright et al., 1973), but this depends on the correct assignment by another, independent method. Another enzymatic technique, which has been employed to distinguish Ia and IIa, used the degradation of lysophospholipids to monoglycerides by phospholipase C (van den Bosch & van Deenen, 1965; Eibl & Lands, 1970). Although the resulting 1- and 2-monoglycerides can be analyzed by a variety of techniques (Hofmann, 1962; Wolfenden et al., 1964; Johnson & Holman, 1966; Satouchi & Saito, 1977; Sugatani et al., 1980; Matsuzawa & Hostetler, 1980), this is rather cumbersome and involves the problem of further acyl migration during the workup procedure. Spectroscopic methods such as IR (Slotboom et al., 1963, 1967; de Haas & van Deenen, 1965), 13C NMR (Schmidt et al., 1977), or 1H NMR (discussed below) can all detect spectral differences between the

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is palmitic acid and X is choline, the structures correspond to single spots. 31P NMR showed single peaks for all phosphoglycer-3-phosphorylcholine (Slotboom et al., 1981) and purified by column chromatography on silicic acid. All phospholipids were checked for purity by thin-layer chromatography on silica gel (solvent CHCl3-CH3OH-H2O, 65:25:4 v/v/v) and were found to give single spots. 31P NMR showed single peaks for all phospholipids, except for 2-palmitoyl-sn-glycero-3-phosphorylcholine (IIa), which contained about 20% 1-palmitoyl-sn-glycero-3-phosphorylcholine (Ia) formed by migration during the lipase reaction and purification. The nonionic detergent Triton X-100 was obtained from Rohm and Haas. Deoxycholate was obtained from Sigma. All other chemicals were of the highest purity available and were used without further purification.

Enzymes. Phospholipase A2 from cobra venom (Naja naja naja), obtained from the Miami Serpentarium, lot no. NNP9STLZ, was isolated and purified as described previously (Deems & Dennis, 1975; Roberts et al., 1977). Lipase (Rhizopus arrhizus) was a gift of Dr. Hans Nilsson, Kockums Chemical AB, Malmö, Sweden, or obtained from Boehringer.

31P NMR Spectroscopy. 31P NMR spectra were obtained at 360 MHz on a modified Varian instrument equipped with quadrature phase detection and a 1080 FT Nicolet system. The spectra were run at ambient temperature (~22 °C). Samples were generally 30 mM in lysophospholipid in D2O at pH 5.0 and contained TSP2 as an internal chemical shift standard. Computer simulations of the 31P NMR spectra were made by using the itrcal program on the Nicolet computer.

Experimental Procedures

Phospholipids and Detergents. 1,2-Dipalmitoyl-sn-glycero-3-phosphorylcholine (dipalmitylophosphatidylcholine) and 1,3-dipalmitoyl-sn-glycero-2-phosphorylcholine were obtained from Calbiochem. 1-Palmitoyl-sn-glycero-3-phosphorylcholine (Ia), 2-palmitoyl-sn-glycero-3-phosphorylcholine (IIa), 1-palmitoyl-sn-glycero-2-phosphorylcholine (IIa), 3-palmitoyl-sn-glycero-1-phosphorylcholine (Ib), 2-palmitoyl-sn-glycero-1-phosphorylcholine (IIb), and 3-palmitoyl-sn-glycero-2-phosphorylcholine (IIIb).

Lysophospholipid isomers but are not particularly suitable for kinetic studies. We have found that 31P NMR provides a simple, sensitive, and rapid method to observe the kinetics of acyl and phosphoryl migration in lysophospholipids, and this has now made possible a detailed analysis of these processes which is reported here.

Migration Reactions. Buffer, containing 50 mM Tris-HCl or 50 mM citrate and 20% D2O, was brought to the pH indicated. The pH meter reading was not corrected for D2O. The lysophospholipid was dried from CHCl3 under a stream of N2, and the last traces of solvent were removed under high vacuum. The buffer was added to the dry phospholipid and the solution immediately transferred to the NMR tube. The final phospholipid concentration generally was 20 mM. Spectra were accumulated and stored on the computer disk in such a fashion that at least 10 time points per reaction half-time were obtained. Reactions were run either to completion or for 24 h. At pH 1.0, a slow precipitation during the reaction was observed with concomitant spectral broadening. Therefore, Triton X-100 was added in this case to give a detergent:lysophospholipid ratio of 8:1, resulting in a clear solution.

2 Abbreviations: TSP, sodium salt of 3-(trimethylsilyl)tetradecatetraenoic acid; NOE, nuclear Overhauser effect; Tris, tris(hydroxymethyl)aminomethane; dipalmitylophosphatidylcholine, 1,2-dipalmitoyl-sn-glycero-3-phosphorylcholine.
Enzymatic Reactions. Enzymatic reactions were run so that the time (indicated in the figures) for completion of the reaction was very short compared to the half-time of the migration reaction under those conditions.

Results

Structural Determination of the Isomeric Lysophosphatidylcholines. The $^1$H NMR spectra of 1-palmitoyl-$sn$-glycero-3-phosphorylcholine (I), 2-palmitoyl-$sn$-glycero-3-phosphorylcholine (II), and 3-palmitoyl-$sn$-glycero-2-phosphorylcholine (III) are shown in Figure 2. The assignment of the polar head group of 1-palmitoyl-$sn$-glycero-3-phosphorylcholine (I) is that of Hauser and co-workers (Hauser et al., 1978). When their values for chemical shifts and coupling constants for compound I were used, an excellent fit of the computer-simulated spectrum to the observed spectrum was obtained. We also used their values for the coupling constants of the choline methylene protons in the simulations of the other two isomers; however, the chemical shift of the choline methylene groups was found to be slightly different in all positional isomers, and the glycerol backbone of all these compounds gave distinctly different signals. All assignments are summarized in Table I. The $sn$-2-methine proton $H_2$ of the 2-palmitoyl-$sn$-glycero-3-phosphorylcholine (II) shows a first-order quintet at 5.03 ppm, whereas it is a second-order multiplet at 4.02 ppm for the 1-palmitoyl-$sn$-glycero-3-phosphorylcholine (I). This is consistent with it being attached to a $>\text{CRO}_2$-acyl group in I but to a $>\text{CR}_2\text{O}_2$H group in I.

The protons $H_4$ and $H_5$ also show a downfield shift in the presence of an adjacent acyl group. They are bound to a $>\text{CRO}_2$-acyl group in I and give multiplets at 4.15 and 4.08 ppm, respectively, whereas for II they are bound to an $>\text{CRO}_2$H group and show a multiplet at 3.69 ppm. The assignment of the glycerol portion of 3-palmitoyl-$sn$-glycero-2-phosphorylcholine is only tentative, because the overlap between the choline methylene groups and the glycerol protons obscures the multiplet structure of the latter. The signals at $\ldots$
about 3.72, 4.27, and 4.37 ppm integrate to 4:2:3. By analogy the
multiplets at 3.72 and 4.37 ppm should contain the choline
methylene protons. When the proton H4 is decoupled, the
protons H4 and H5 appear to be an AB quartet with J_H4 = 12.2
Hz. This might be explained by hydrogen bonding between
the hydroxyl group and the vicinal phosphate in IIIb. In
contrast, the -CH2OH group in IIa collapses to a singlet upon
decoupling H5. This equivalence of H4 and H5 in IIa is an
indication of free rotation, presumably because of the absence
of hydrogen bonding. In both cases, a chemical shift of about
3.7 ppm is consistent with a -CH2OH group. Because of the
broadness of the overlapping choline CH2OH signal, the
multiplet structure of H4 and H5 in IIIb could not be deduced.
A satisfactory appearance of the simulated spectrum was,
however, obtained with δ(H4) = 4.27 ppm, δ(H5) = 4.26 ppm,
and J_H4 = 11.5 Hz. These shifts are consistent with a
-CH2OH-acetyl group.

Although the above results show that all three positionally
isomeric lysophosphatidylcholines can be distinguished by 1H
NMR, there are several factors that make this method less
suitable for kinetic studies: (i) Because of signal overlap,
kineetrical measurements would have to rely upon the appearance
or disappearance of one distinct signal rather than on ratios.
(ii) The peaks of interest are the smallest in the spectrum and are
therefore very easily distorted, especially by the presence
of a large water peak. The H2 proton in IIa is very close to
the spinning side bands of HDO. The 1H NMR spectra serve,
however, for a structural determination of the three isomeric
phospholipids which together with their different methods of
enzymatic preparation allow the 31P NMR spectral data to
be assigned unequivocally.

Kinetics of Acyl Migration. Figure 3 shows typical 31P
NMR spectra obtained during the acyl migration reaction.
Most dramatically, 2-palmitoyl-sn-glycero-3-phosphorylcholine
(I1a), which had been prepared by the action of lipase, is
converted into 1-palmitoyl-sn-glycero-3-phosphorylcholine (Ia)
over time (Figure 3B). Table II gives a summary of the 31P
NMR chemical shifts of the lysophosphatidylcholines and other
related compounds. In Figure 3A, the rearrangement
starting from isomer Ia to give the equilibrium mixture con-
taining Ia and IIa is shown. It is found, as expected, that an

![Figure 3](image-url)

**Figure 3:** 31P NMR spectra obtained after the times indicated of
(A) 30 mM 1-palmitoyl-sn-glycero-3-phosphorylcholine (Ia) at pH
7.0 and (B) 20 mM 2-palmitoyl-sn-glycero-3-phosphorylcholine (IIa),
of which some had migrated to Ia during its preparation, at pH 7.0.

![Figure 4](image-url)

**Figure 4:** Typical time course of the acyl migration reaction. The
reaction shown is the rearrangement of 2-palmitoyl-sn-glycero-3-
phosphorylcholine (Ia) to an equilibrium mixture of 90% 1-palmito-
yl-sn-glycero-3-phosphorylcholine (Ia) and 10% 2-palmitoyl-sn-
glycero-3-phosphorylcholine (I1a) at pH 8.0. I and Ie are the intensities
of the 31P NMR peak of compound I1a (peak heights) at time t and
at equilibrium, respectively. I_e is the sum of the intensities of peaks
Ia and IIa.

identical equilibrium mixture is obtained starting from either
compound. The equilibrium mixture was determined between
pH 6.2 and 9.0 and was found to be nearly independent of pH
within experimental error and to consist of about 90% Ia and
10% IIa. This value was used in rate calculations throughout.

The rates were calculated by assuming a pseudo-first-order
reversible reaction A ⇌ B with a forward rate constant k_f
and a backward rate constant k_b. A typical time course is shown
in Figure 4. A plot of ln (I - I_e)/(I_e - I_a) gives a straight
line with slope k_f + k_b. Here I is the concentration of one
isomer at time t, I_e is its equilibrium concentration, and I_e is
the sum of the concentrations of both isomers. By use of the
relation Keq = k_f/k_b, K_f and k_b can then be immediately
obtained. At pH greater than 2, no competing reactions such
as phosphoryl migration or hydrolysis could be observed on
the same time scale. Figure 5 shows a plot of log k_f vs. pH.
The slope in the basic region is about 0.92; we attribute the
small difference from unity to experimental error and a possible
contribution of general acid/general base catalysis by the
buffer. From these data, a second-order rate constant of
catalyzed acyl migration of about k_a = 160 M⁻¹ s⁻¹ can
be calculated. The pH-rate profile is similar to that of a
typical ester hydrolysis (Kirby, 1972). The second-order rate
constant of base-catalyzed acyl migration of lysophosphatidylcholine is about 1.8 × 10⁻² M⁻¹ s⁻¹ (C. R. Kensil and E. A. Dennis, unpublished results), so that the acyl mi-
The quantity of the basic catalyst p-(dimethylamino)pyridine increases the rate of migration about 3-fold. Clearly, acylations in the chemical synthesis, 2-palmitoyl-sn-glycero-3-phosphorylcholine (IIa) was subjected to the conditions of the reacylation procedure for lysophosphatidylcholine of Khorana and co-workers (Gupta et al., 1977): CHCl₃ as a solvent and a stoichiometric amount of p-(dimethylamino)pyridine. In this assay, the temperature was 25 °C. The results are shown in Figure 6 where it can be seen that the reaction in CHCl₃ is rather slow, but the addition of the phosphoryl migration, if it occurs at all, is very much slower than acyl migration and apparently smaller than the base-catalyzed value. The rate constant for acylation was not investigated further, the rate constants should be viewed as approximate only.

For determination of the rate constants for phosphoryl migration, analogous experiments were carried out by starting from 3-palmitoyl-sn-glycero-2-phosphorylcholine (IIIb), because if it occurred, this compound would rearrange to Ib, which then would undergo rapid acyl migration to form some IIb as well. At pH 5.0, 7.0, and 9.0, there was no noticeable rearrangement taking place over 12 h. At pH 9.0, some hydrolysis of the fatty acyl group was observed. Only at pH 1.0 did several reactions take place, including hydrolysis of the choline group and migration of the phosphate in the product, acyl hydrolysis and migration, and a small amount of phosphoryl migration, but peak overlap and several unidentified peaks prevented us from obtaining a rate constant. At basic pH, however, the phosphoryl migration, if it occurs at all, is clearly very much slower than acyl migration and apparently even slower than acyl hydrolysis.

For studies on the acyl migration under conditions of chemical synthesis, 2-palmitoyl-sn-glycero-3-phosphorylcholine (IIa) was subjected to the conditions of the reacylation procedure for lysophosphatidylcholine of Khorana and co-workers (Gupta et al., 1977): CHCl₃ as a solvent and a stoichiometric quantity of the basic catalyst p-(dimethylamino)pyridine at 25 °C. The results are shown in Figure 6 where it can be seen that the reaction in CHCl₃ is rather slow, but the addition of this amount of catalyst increases the rate of migration about 3-fold. Clearly, acylations in the sn-1 position without migration are not possible, and acylations in the sn-2 position will also be accompanied by some acyl migration. For example, when Khorana and co-workers (Gupta et al., 1977) acylated compound la with this catalyst at twice the concentration reported 5% migration in the product. The reaction took 24 h. From the data in Figure 6, 5% migration would be predicted by assuming the same equilibrium mixture as in aqueous solution. Thus the synthesis of mixed acyl phospholipids with absolute positional purity is very difficult to obtain under these conditions.
of lipase (Rhizopus arrhizus) on an identical substrate mixture can be seen. Lipase has been shown to selectively hydrolyze esters of fatty acyl groups with a primary hydroxyl group in phospholipids (de Haas et al., 1965; Slotboom et al., 1970a,b; Fischer et al., 1973). In this study we have also confirmed the structure of the product as IIA by 1H NMR. Again the reaction was run under conditions such that is was complete before noticeable acyl migration took place.

The specificity of phospholipase A₂ toward the monomeric phospholipid dibutyrylphosphatidylcholine was also examined. As can be seen in Figure 8, initially only one peak is generated, and only after the reaction is complete did a new small reso-

nance between the chemical shifts of dibutyrylphosphatidylcholine and 1-butyryl-sn-glycero-3-phosphorylcholine appear at 0.44 ppm. A peak at this position is also generated by the action of lipase on the same compound. Furthermore, both peaks are found if the lysophosphatidylcholine fraction from a silicic acid column of the products from dibutyrylphosphatidylcholine synthesis is examined. Unfortunately, 2-butyryl-sn-glycero-3-phosphorylcholine could not be obtained in pure form, because lipase works on dibutyrylphosphatidylcholine so poorly that even at enzyme concentrations of 5 mg/mL the enzymatic reaction was slower than the acyl migration. Therefore, we could not obtain a quantitative dependence of the migration rate on chain length. It can be estimated qualitatively, however, that the migration is faster for the shorter butyryl moiety, similar to observations in monoglycerides (Serdarevich, 1967).

Discussion

Mechanism of Acyl and Phosphoryl Migration. The data in Figures 4 and 5 show that the acyl migration is a reaction first order in lysophospholipid and base or acid. This is consistent with an intramolecular rearrangement as demonstrated previously for the acyl migration in monoacylglycerides for both the acid-catalyzed (Doerschuk, 1952; Bevan et al., 1953; van Lohuizen & Verkade, 1960) and base-catalyzed cases (Wolfenden et al., 1964). It is probable that this reaction also follows the mechanism proposed originally by Fischer (1920) (Figure 9).

Phosphoryl migration must be very much slower than acyl migration at alkaline pH as none was observed, although our experiments do suggest that some phosphoryl migration does occur at acidic pH. In previous observations of phosphoryl migration of phosphate diesters of ribose only acid-catalyzed, but no base-catalyzed, migration was found (Brown et al., 1956). Experiments on phosphoryl migration and hydrolysis of glycerophosphorylcholine (Baer & Kates, 1948) were interpreted in terms of phosphoryl migration of the phosphorylcholine group catalyzed by both acid and base, but the rearranged unhydrolyzed product was never isolated. More detailed experiments by Serdarevich (1967) suggested that migration does not occur in the unhydrolyzed glycerophosphorylcholine in either acid or base as only rearranged glycerophosphate was observed. With lysophosphatidylcholine in acid, the loss of the choline group appears to be most favorable in analogy to the case with glycerophosphorylcholine,
but there does appear to be some phosphorylethanolamine migration. Further work would be required to sort out the rate constants for formation of all of the possible hydrolysis and migration products at acidic pH. When phosphoryl migration has been studied, it has been shown to occur intramolecularly via a cyclic intermediate (Baer & Kate, 1948; Hawthorne & Kemp, 1964). From a mechanistic viewpoint, acid catalysis but not base catalysis would be expected for phosphoryl migration because even if formation of the trigonal bipyramidal intermediate occurs in base, pseudorotation of it (see Figure 9) could be expected to be energetically unfavorable since it would bring an oxygen anion from an equatorial to an apical position (Dennis & Westheimer, 1966; Holmes, 1980).

**Importance for Phospholipase Specificity and Phospholipid Synthesis Studies.** There are principally two methods to determine the specificity of phospholipases, namely, structural determination of the products or the use of specifically labeled substrates. Both methods directly depend on a quantitative knowledge of isomerization rates of lysophospholipids and their equilibria: in the first technique, to assess the validity of the measured isomer distribution in the product and, in the second, to quantitate the migration of the label during substrate synthesis. After the failure of a chemical product determination (Hanahan et al., 1960, and references therein), there have been no further studies to establish phospholipase specificities by product structure determination, although a pure isomer may be identified by $^1$H NMR as shown here and suggested by Chang & Lo (1975) or by oxidation with O$_2$ (Thiele, 1968). The complete chemical synthesis of specific mixed acylphospholipids was used by de Haas and van Deenen (de Haas et al., 1960; de Haas & van Deenen, 1961; van Deenen et al., 1963) to establish the specificity of pancreatic and snake venom phospholipase A$_2$. For differentiation of the residual activity against the second fatty acid, rather pure phospholipids must be used. Since current syntheses of phospholipids start from lysophospholipids generated from naturally derived phospholipids, optimum conditions for synthesis were studied.

The stability of lysophosphatidylcholine against acyl migration is highest between pH 4 and 5. Both acid and base catalyze the acyl migration. In order to obtain very pure isomers, high concentrations of enzyme should be used to generate the lysophosphatidylcholines before significant isomerization can take place, and an optimum pH should be chosen accordingly. We have found in $^{31}$P NMR a simple and rapid method to check the purity of the resulting lyso compound directly. If the specificity of phospholipases is investigated, again care should be taken to run the reaction under conditions so that the migration is much slower than the enzymatic hydrolysis. If radiolabeled phospholipids are employed, the fact that lysophospholipids are also substrates of phospholipases, however, much poorer (Slotboom et al., 1963), must be taken into account. Therefore an absolutely specific enzyme can appear to liberate the "wrong" fatty acids by two mechanisms: (i) The substrate was not absolutely specifically labeled, or (ii) migration of the acyl group of the lysophospholipid may give rise to a new substrate at very long reaction times or at high pH. However, both difficulties are overcome by the use of $^{31}$P NMR to determine the specificity. A disadvantage of the method is, however, that fairly high enzyme concentrations have to be used.

The positional specificity of lysophospholipases or of phospholipases A$_1$ and A$_2$ working on lysophospholipids is a rather difficult problem, because in the absence of direct observation of the isomerization and product formation, it cannot be distinguished whether the hydrolysis reaction goes exclusively via one isomer and rearrangement or via both isomers (van den Bosch et al., 1968). When $^{31}$P NMR is used, the occurrence of the other isomer can be directly observed, provided its concentration is not too low.

Previous $^1$H NMR experiments in this laboratory have shown that there are significant differences in the conformation of the sn-1 and sn-2 acyl chains in phospholipids, both in micelles (Roberts et al., 1978) and vesicles (De Bony & Dennis, 1981), however, not necessarily in monomers (Roberts et al., 1978; Burns & Roberts, 1980). The arrangement in the interface is such that the α-CH$_2$ group of the sn-2 chain and the carbonyl group are oriented at the interface and the α-CH$_2$ group and carbonyl group of the sn-1 chain are somewhat less exposed. For determination of whether this affects the specificity of the enzyme, the $^{31}$P NMR technique was used with the monomeric substrate dibutyrylphosphatidylcholine. The result obtained showing that only the sn-2 fatty acid chain was hydrolyzed demonstrates that the specificity for this chain is not due to the particular conformation preferred in aggregated lipids.

**References**


