# Action of α-Phospholipase $A_2$ on Human Serum High Density Lipoprotein-3: Kinetic Study of the Reaction by $^{31}P$ Nuclear Magnetic Resonance Spectroscopy†

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ABSTRACT: $^{31}P$ nuclear magnetic resonance spectroscopy ($^{31}P$ NMR) was used to monitor the hydrolysis of phospholipids in human serum high-density lipoprotein-3 ($HDL_3$) by α-phospholipase $A_2$ purified from *Crotalus adamanteus* venom. The $^{31}P$ NMR spectra obtained at regular intervals during incubation of $HDL_3$ with the enzyme indicated that phosphatidylcholine was completely converted into lysophosphatidylcholine. The proton-decoupled line widths of the phosphatidylcholine in untreated $HDL_3$, and of the lysophosphatidylcholine produced by the enzymatic digestion of the lipoprotein particle, were ca. 7.5 Hz over the entire course of the reaction. Moreover, the chemical shifts of phosphatidylcholine and lysophosphatidylcholine (+1.11 and 0.64 ppm, respectively, with 0.0 ppm assigned to 85% $H_3PO_4$) were invariant under these conditions. From these studies, it was determined that the α-phospholipase $A_2$ catalyzed hydrolysis of phosphatidylcholine in $HDL_3$ follows first-order kinetics, in confirmation of the results of N. M. Pattnaik et al. ((1976) *J. Biol. Chem. 251,* 1984–1990), obtained by pH-stat titration. It was also found that the reaction had an average experimental first-order rate constant, $k_{exp}$, of $3.35 \times 10^{-5}$ $s^{-1}$ for initial enzyme concentrations, $E_0$, between 5.87 and $8.0 \times 10^{-9}$ M and for initial substrate concentrations, $S_0$, between 4.1 and $9.5 \times 10^{-3}$ M (based on the phosphatidylcholine concentration of the $HDL_3$). The value for $k_{cat}$ ($K_I/K_m$) was 31 $s^{-1}$ at 28 °C, in close agreement with that derived from the data of Pattnaik et al. ((1976) *J. Biol. Chem. 251,* 1984–1990). We conclude that essentially all of the phosphatidylcholines in $HDL_3$ are located at, or in rapid equilibrium with, the surface of the lipoprotein particle. We further conclude, on the basis of the constant line widths and chemical shift positions of the $^{31}P$ NMR signals, that the microenvironment of the phosphorus in the polar head groups of the $HDL_3$ phospholipids probably does not change as a result of the enzymatic digestion.

The lipid composition of the various classes of human serum lipoproteins has been determined. In all classes, phospholipids, specifically phosphatidylcholine (PC),[1] are the predominant polar lipid components (for recent reviews, cf. Scanu & Kruski, 1975; Morrisett et al., 1975). Despite this abundance of compositional information, neither the function nor structural arrangement of the phospholipids in these complexes is well understood.

Several laboratories have been investigating the structural arrangement of the components of $HDL_3$ and of other serum lipoproteins by means of a wide array of chemical and physical techniques (cf. Morrisett et al., 1975). Several lines of evidence indicate that the phospholipids are oriented on the surface of the particles in such a way as to maximize hydrophilic interactions between these components and the surrounding aqueous milieu (Henderson et al., 1975; Assmann et al., 1974; Shipley et al., 1972; Finer et al., 1975; Pattnaik et al., 1976; Verdery & Nichols, 1975; Segrest et al., 1974; Stoffel et al., 1974). This surface distribution of phospholipids and polar regions of proteins has been incorporated into various structural models (Verdery & Nichols, 1975; Segrest et al., 1974; Stoffel et al., 1974; Assmann & Brewer, 1974; Laggner et al., 1973).

Using the technique of $^{31}P$ nuclear magnetic resonance spectroscopy ($^{31}P$ NMR), we had determined that the chemical shifts for each class of phospholipids are solvent and particle dependent (Henderson et al., 1975; Glonek et al., 1974). This was especially true for PC, the chemical shift of which was significantly upfield (ca. 0.5 to 0.6 ppm) compared with that of the other phospholipids normally present in biological materials. An investigation of the relative accessibility of the phospholipid phosphorus in human HDL and LDL to diamagnetic and paramagnetic ions led to the conclusion that most, if not all, of the phospholipid phosphorus in $HDL_3$ is accessible to these ions (Henderson et al., 1975); this is in agreement with the conclusions of Assmann et al. (1974). Yeagle et al. (1977) measured the phosphorus signal intensities of $HDL_3$ using gated decoupled $^{31}P$ NMR before and after tryptic digestion of the HDL protein. No change in the NMR signals of the phospholipids in HDL was apparent; thus, there appears to be no immobilization of the HDL phospholipids. Recently, Pattnaik et al. (1976) studied the action of *Crotalus adamanteus* α-phospholipase $A_2$ on $HDL_3$ and found that all of the phospholipids except for sphingomyelin are accessible to the enzyme and are converted into their lyso derivatives.

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[1] Abbreviations used: NMR, nuclear magnetic resonance; $HDL_3$, high density lipoprotein, subclass 3 (*d* 1.125–1.21 g/mL); PC, phosphatidylcholine; LPC, lysophosphatidylcholine; FFA, free fatty acids; SPH, sphingomyelin.

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They also observed that the reaction follows first-order kinetics and that, in spite of extensive hydrolysis, there were no significant changes in the properties of the digested particles.

In the present report, we describe studies on the structural arrangement of the phospholipids in $HDL_3$ by the use of the technique of $^{31}P$ NMR. Our purpose in this work was to follow the time course of the $\alpha$-phospholipase $A_2$ catalyzed hydrolysis of PC to lysophosphatidycholine (LPC) in $HDL_3$ so that we could (1) determine the kinetics of the reaction on the time scale of NMR and (2) define the changes which occur in the microenvironment of the polar head group phosphorus in the phospholipids before, during, and after enzymatic hydrolysis.

## Methods

*$^{31}P$ Nuclear Magnetic Resonance Spectrometry.* Pulsed Fourier transform $^{31}P$ NMR measurements were made with a Bruker HFX-5 90 MHz spectrometer operating at 36.43 MHz for $^{31}P$ (21 kG magnetic field; $^1H$ frequency of 90.0 MHz; Henderson et al., 1975). Data points of free induction decays (FID) were acquired under conditions where the 23° $^{31}P$ excitation pulse was provided in 4.5 $\mu$s. The acquisition time per FID was 832 ms; the dwell time was 200 ms which corresponds to a sweep width of 2500 Hz and a resolution limit of 1.22 Hz. Increasing the trigger time to 10 s did not result in any change in spectral intensities. As is usual with $^{31}P$ NMR, positive chemical shift values refer to signals that arise at high field strength (Crutchfield et al., 1967) and zero was assigned to the signal from 85% $H_3PO_4$ in $H_2O$. The primary standard was a capillary (1 mm diameter) containing 1.0 M methylenediphosphonic acid, $(HO)_2OPCH_2PO(OH)_2$, in $D_2O$ (pD = 9.5, $Na^+$ countercation) coaxially mounted in the sample tube. This capillary gave a signal assigned as −16.2 ppm. The magnetic field was stabilized by the deuterium signal from the capillary. Proton broad-band decoupling was routinely applied in this study. The decoupling power level was sufficient to reduce all multiplets to a single resonance signal exhibiting a line width equal to that obtained by decoupling a single proton-coupled $^{31}P$ multiplet with continuous wave irradiation at the same power level. The band width of the broad-band decoupler was approximately 1500 Hz. During approximately the first 7 h of the enzymatic hydrolysis experiments, 30 min of $^{31}P$ signal averaging (2048 accumulations) was used for each spectrum; i.e., 0–30 min for the first spectrum after the enzyme was added, 30–60 min for the second time point, and so on. At the later time points near the end of the experiments longer times were used, i.e., 60 min (4096 accumulations). The time used for a particular spectrum was taken as the time at the midpoint of the signal averaging.

*$\alpha$-Phospholipase $A_2$.* The preparation of $HDL_3$ was done as previously reported (Henderson et al., 1975; Pattnaik et al., 1976). An electrophoretically homogeneous $\alpha$-phospholipase $A_2$ purified from the venom of *C. adamanteus* according to the method of Wells & Hanahan (1969) was a gift from Dr. Betty W. Shen, Department of Biochemistry, University of Chicago. Total phospholipid–phosphorus concentrations were measured as described by Kirkpatrick & Bishop (1971).

*Extraction of Lipids.* The $HDL_3$ lipids were extracted with a 50-fold volume of chloroform/methanol (2:1, v/v) (Nelson, 1972) before and after enzymatic hydrolysis with $\alpha$-phospholipase $A_2$. After filtration, the extracted solvent was evaporated under $N_2$ and the extracted lipids were redissolved in a known amount of benzene and stored at −10 °C for lipid analysis as described below.

*Chromatography.* Thin-layer chromatography (TLC) of the extracted lipids was carried out on commercially prepared silica gel G analytical plates (Brinkmann Instruments, Inc., Des Plaines, Ill.). The plates were first developed in chloroform/methanol (1:1, v/v) and dried for 15 min at 100 °C before use. The solvent system used was chloroform/methanol/water (65/25/4, by volume; Wagner et al., 1961).

The lipids were visualized with iodine vapor. The total phosphorus content of the lipid spots corresponding to PC, LPC, and sphingomyelin (SPH) was determined by scraping the corresponding areas of the silica gel into test tubes. After color development in the phosphorus determination procedure (Kirkpatrick & Bishop, 1971), the test tubes were spun for 30 min in a desk top centrifuge. The supernatant liquid was removed with a Pasteur pipet and the absorbance at 830 nm was determined on a Gilford Model 210 D spectrophotometer. Standards were run with and without added silica gel to determine what effect silica gel had on known phosphorus values. The slopes of the standard curves differed by only 6% so that, normally, standards were run without any added silica gel.

*Enzymatic Reaction.* The 4-mL enzymatic reaction mixture used for the $^{31}P$ NMR studies contained: $HDL_3$ ($4.1 \times 10^{-3}$ M to $9.5 \times 10^{-3}$ M with respect to PC); $CaCl_2$ ($7 \times 10^{-3}$ M); and $\alpha$-phospholipase $A_2$ ($5.9 \times 10^{-9}$ M to $8.0 \times 10^{-9}$ M of the dimeric enzyme) in 0.15 M NaCl containing $1.7 \times 10^{-3}$ M EDTA, pH 7.2. This low concentration of EDTA was added to the reaction mixture to complex any contaminating metal ions which could cause inhibition of the enzymatic hydrolysis. The incubation temperature was 28 °C, measured as the temperature of the NMR sample probe in the spectrometer. The reaction was stopped by adding an excess of EDTA with respect to the calcium ions present in the incubation mixture.

*Kinetic Analysis.* The areas of the $^{31}P$ NMR peaks from PC and LPC in $HDL_3$ were previously found by both continuous-wave $^{31}P$ NMR (Glonek et al., 1974) and pulsed Fourier transform $^{31}P$ NMR (Henderson et al., 1975) to be proportional to their chemically determined molar percentages; therefore, the relative amounts of these phospholipids could be determined directly from the NMR spectra. The percentages of phosphorus were determined for each spectrum by normalization of each peak after correction for the constant amount of SPH, the peak of which overlaps that of LPC. The relative amounts of PC and LPC were converted to molarity based on the measured starting phospholipid concentration. The total amount of PC plus LPC phosphorus did not change significantly during the course of the reaction.

The time course of the enzymatic digestions was analyzed according to the integrated first-order rate equation:

$$2.303 \log PC_t = -k_{exp}t + 2.303 \log PC_0 \qquad (1)$$

where $k_{exp}$ is the experimental first-order rate constant, $t$ is the time in seconds, and $PC_0$ and $PC_t$ are the concentrations of phosphatidylcholine present initially and at time $t$, respectively. In all cases, the values for $PC_t$ used in calculations were derived from the integrated peak areas obtained from $^{31}P$ NMR analysis.

The slopes of the semilogarithmic plot of log $PC_t$ against $t$ were determined by least-square analysis and values for $k_{exp}$ were obtained. The values of the catalytic rate constant, $k_{cat}$ ($K_I/K_m$), in the absence of albumin were calculated from the rate equation for product inhibition (Pattnaik et al., 1976):

$$v_t = \frac{ds}{dt} = \frac{k_{cat}E_0S}{(K_m/K_I)S_0} = k_{exp}S \qquad (2)$$

where $K_m$ and $k_{cat}$ are the kinetic parameters of the uninhibited reaction and $K_I$ is the dissociation constant of the en-

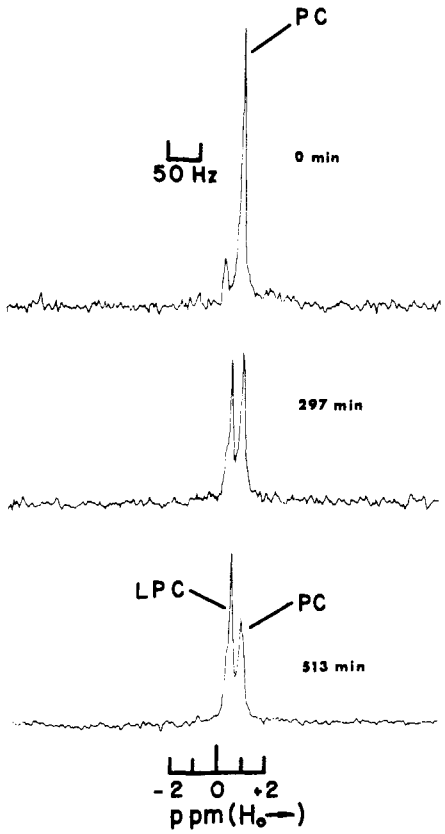


FIGURE 1: Changes in the $^{31}P$ NMR spectrum of $HDL_3$ produced by digestion with $\alpha$-phospholipase $A_2$ from *C. adamanteus*. PC, phosphatidylcholine; LPC, lysophosphatidylcholine. The upper spectrum was obtained at zero time (i.e., before addition of the enzyme), the middle spectrum after 297 min of enzyme digestion, and the bottom spectrum after 513 min of incubation at 28 °C (the temperature of the spectrometer sample probe). The enzyme reaction mixture contained, in 4 mL: $HDL_3$, $9.3 \times 10^{-3}$ with respect to PC; $CaCl_2$, $7 \times 10^{-3}$ M; and $\alpha$-phospholipase $A_2$, $5.9 \times 10^{-9}$ M; in 0.15 M NaCl containing 0.05% EDTA, pH 7.2. No bovine serum albumin was added.

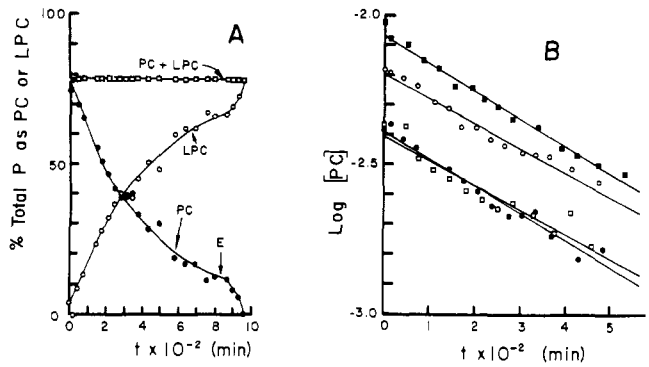


FIGURE 2: A represents the time course of a typical experiment in which $HDL_3$ was digested with $\alpha$-phospholipase $A_2$. The percentages of PC and LPC were taken from the relative areas of the respective peaks on $^{31}P$ NMR spectra such as those shown in Figure 1. E indicates the time at which additional enzyme was added. B represents a plot of log [PC] (in M) vs. time calculated from the $^{31}P$ NMR spectra of four different digestion experiments. The experimental conditions were as described in the legend of Figure 1. The average rate constant, obtained from the slopes of the lines, is $3.35 (\pm 0.45) \times 10^{-5}$ s$^{-1}$. The symbols indicate the experimental data; the lines represent the best fit for the experimental data as determined by linear regression analysis ($P \geq 0.95$). The enzyme and PC concentrations for the individual experiments were: (■) PC, $9.5 \times 10^{-3}$ M; enzyme, $8 \times 10^{-9}$ M; (○) PC, $6.5 \times 10^{-3}$ M; enzyme, $5.9 \times 10^{-9}$ M; (●) PC, $4.1 \times 10^{-3}$ M; enzyme, $5.9 \times 10^{-9}$ M; and (□) PC, $4.3 \times 10^{-3}$ M; enzyme, $6.25 \times 10^{-9}$ M.

zyme–product complex. Initial velocities were read directly from the plots of percentage PC phosphorus against $t$ as the slopes of tangents.

## Results

The NMR conditions used were selected because they result in the maximum signal-to-noise ratio per unit time. Under these conditions the phosphorus resonances are all partially saturated; however, the relative areas of the phospholipids were not affected by this saturation.

Figure 1 shows the $^{31}P$ NMR spectra of $HDL_3$ before treatment with $\alpha$-phospholipase $A_2$ and at various times during the incubation period. The reaction mixture exhibited no turbidity before, during, or after completion of digestion. In control experiments carried out under identical conditions in the absence of enzyme, the signals corresponding to PC, SPH, and LPC did not change over the course of 8 h, the length of time for a typical experiment. The sharp, narrow signal ($W_{1/2}$ = 7.5 Hz, where $W_{1/2}$ is the line width measured at the peak half-height) which was assigned to the PC phosphate appeared at +1.11 ppm, as reported earlier (Glonek et al., 1974; Henderson et al., 1975). Before hydrolysis the signal appearing at +0.51 ppm arose mainly from SPH since very little LPC was present in the intact particle. As the reaction proceeded, the relative area of the PC signal decreased, while that of LPC increased to the extent where after 500 min of hydrolysis, 58% of the PC originally present had been converted to LPC. The LPC formed appeared as a sharp, narrow signal ($W_{1/2}$ = 7.5 Hz) at +0.64 ppm, overlapping a signal already present in the intact particle at +0.51 ppm arising primarily from SPH. The line widths of the PC and LPC signals obtained at intermediate times during the enzymatic digestion were both ca. 7.5 Hz. In addition, no new signals were observed, nor did the chemical shifts of the PC and LPC signals (+1.11 and +0.64 ppm, respectively) change as a consequence of the enzymatic digestion.

It was necessary to study the time course of the entire hydrolysis in order to investigate the effect of hydrolysis on all the PC in the $HDL_3$. A plot of the percentage of total phosphorus present as PC and LPC, determined from NMR spectra such as those shown in Figure 1, against the length of incubation of $HDL_3$ with $\alpha$-phospholipase $A_2$ (Figure 2A) showed that the percentage of PC decreased exponentially as a function of time of incubation, whereas that of LPC increased exponentially. When additional enzyme was added to the incubation mixture at the time when the reaction had slowed appreciably (Figure 2A, point E), the hydrolysis was resumed at an accelerated rate. Only one reaction was allowed to go to completion, according to $^{31}P$ NMR measurements, due to the time involved (over 8 h) and to the lack of precision of the area measurements as the PC concentration fell.

The percentage of phosphorus for each $HDL_3$ component present, PC, SPH, and LPC, in the reaction mixture before and after enzymatic hydrolysis was analyzed by TLC and NMR (Table I) as outlined in the Materials and Methods section. A reproducibility of ±6% for the $^{31}P$ NMR measurements was obtained from seven measurements on a single $HDL_3$ sample. The standard deviation for each component in the various TLC determinations is given when the data were available. As can be seen, there was close correlation between the two methods for determination of the relative lipid composition of the $HDL_3$ particle, suggesting that all the phospholipids on the outside of the $HDL_3$ particles are mobile enough to be detected under the $^{31}P$ NMR conditions used in this study.

It has been reported that, with concentration of $PC_0$ of 1 to $40 \times 10^{-3}$ M and initial enzyme concentrations of 2 to $75 \times 10^{-8}$ M, the enzyme was saturated (Pattnaik et al., 1976). The initial enzyme and PC concentrations used in this study indi-

TABLE I: $HDL_3$ Phospholipid Composition before and after Enzymatic Hydrolysis.

| experiment[a] | component | amount present (%) TLC (±SD)[c] | amount present (%) NMR[b] |
|---|---|---|---|
| 1 before hydrolysis | PC | 73.7 ± 1.6 (2) | 76.3 |
| | SPH | 25.6 ± 1.0 (2) | 20.2 |
| | LPC | 0 (2) | 3.5 |
| 1 after hydrolysis | PC | 10.6 ± 3.3 (4) | 19.8 |
| | SPH | 16.8 ± 1.9 (4) | 20.2 |
| | LPC | 75.6 ± 5.6 (4) | 60.0 |
| 2 before hydrolysis (○) | PC | 75.1 ± 2.1 (2) | 87.4 |
| | SPH | 12.9 ± 10.1 (2) | 11.0 |
| | LPC | 11.9 ± 8.1 (2) | 1.6 |
| 2 after hydrolysis (○) | PC | 16.5 ± 6.7 (5) | 18.7 |
| | SPH | 15.4 ± 5.0 (5) | 11.0 |
| | LPC | 68.2 ± 4.0 (5) | 70.4 |
| 3 before hydrolysis (●) | PC | 69 (1) | 74.7 |
| | SPH | 16 (1) | 21.6 |
| | LPC | 14 (1) | 3.7 |
| 3 after hydrolysis (●) | PC | 27.6 ± 5.0 (8) | 0.0 |
| | SPH | 17.5 ± 6.8 (8) | 21.6 |
| | LPC | 54.9 ± 5.2 (8) | 78.4 |
| 4 before hydrolysis | PC | 72 (1) | 84.8 |
| | SPH | 18 (1) | 11.0 |
| | LPC | 10 (1) | 4.2 |
| 4 after hydrolysis | PC | 21.7 ± 3.6 (3) | 27.2 |
| | SPH | 13.3 ± 4.0 (3) | 11.0 |
| | LPC | 65.0 ± 2.0 (3) | 61.8 |

[a] The symbols in the parentheses refer to the experimental conditions given for Figure 2B. The experimental conditions for experiment 1 and experiment 4 were: (1) PC, $5.3 \times 10^{-3}$ M; enzyme, $6.3 \times 10^{-9}$ M; and (4) PC, $9.2 \times 10^{-3}$ M; enzyme, $5.9 \times 10^{-9}$ M. [b] Standard deviation for NMR determinations is ±6% as determined as described in Materials and Methods. The amount present of each component as determined by NMR is given as area percentage and not as molar percentage as is given for the TLC determinations. [c] Standard deviation; ($n$) is the number of individual determinations made.

cate that the enzyme should be saturated with substrate; however, zero-order kinetics were not followed during the reaction. In all of the experiments, the conversion of PC to LPC followed first-order kinetics within the first 500 min of the enzymatic reaction during which time 70 to 80% of the PC was converted to LPC; after that time, the first-order relationship ceased (Figure 2B). These data were obtained by conversion of the relative amounts of PC and LPC, determined as shown in Figure 2A, to molarity based on the measured starting phospholipid concentrations. The average experimental rate constant ($k_{exp}$) obtained from the $^{31}P$ NMR spectral data was 3.35 (±0.45) $\times 10^{-5}$ $s^{-1}$ for four experiments. The average catalytic rate constant ($k_{cat}$), from eq 2, is 30.9 $s^{-1}$, which is closely comparable to the value of 34.3 $s^{-1}$ derived from the data of Pattnaik et al. (1976). Their results were obtained by measurements at the same temperature by the technique of pH-stat titration, which monitors, strictly speaking, the uptake of protons, i.e., free fatty acids (FFA) released, whereas the technique of $^{31}P$ NMR follows the phosphorus moiety change, and with a different time scale (seconds compared to minutes). In addition, the $k_{cat}$ which was obtained from the initial velocity calculations was similar to the two previously mentioned values.

## Discussion

In a previous report, Pattnaik et al. (1976) showed that $HDL_3$ which has been treated with purified $\alpha$-phospholipase $A_2$ maintains essentially the same physicochemical and immunological properties as native $HDL_3$, even though all PC are converted to LPC and FFA. In the work described here, we used $^{31}P$ NMR to monitor the $\alpha$-phospholipase $A_2$ catalyzed conversion of PC to LPC and FFA and found the same kinetic results that Pattnaik et al. (1976) had obtained by an independent method. Phosphatidylcholine and LPC exhibited the same proton-decoupled line widths ($W_{1/2}$ = 7.5 Hz) under all experimental conditions.

NMR line widths at a given magnetic field strength are related to $T_2$ (spin–spin relaxation time) which in turn is affected by the translational and rotational mobilities of a nuclide. Other things being equal, increased line width reflects decreased mobility (Finer et al., 1975) and changes in the inhomogeneity of the magnetic microenvironment (James, 1975). The $T_2$ can be estimated from the signal line widths by the relationship (James, 1975):

$$T_2 = \frac{1}{\pi W_{1/2}}$$

Since the line width of the original PC and of the LPC formed did not change as hydrolysis proceeded, $T_2$ also probably remained constant. Thus, it appears that there were no changes in the microenvironment of the phosphates in the polar head group of PC and LPC, respectively, as a result of the enzymatic digestion. It can be concluded that the mobility of the phospholipids in the $\alpha$-phospholipase $A_2$ digested particle is probably the same as in the native $HDL_3$ complex.

Gent & Prestegard (1974), as well as Berden et al. (1974) have shown that, for phospholipid vesicles, changes in line width are directly related to changes in the vesicle size. This observation is compatible with the differences in line widths found between HDL and LDL (Henderson et al., 1975).

Therefore, the constant line widths described herein are consistent with no detectable changes in the diameter of $HDL_3$ following enzymatic hydrolysis, a fact in keeping with the data of Pattnaik et al. (1976) and small angle X-ray scattering data (L. P. Aggerbeck, personal communication.)

If the phosphate resonances account for most or all of the phospholipids present, it appears that the PC molecules present in $HDL_3$ before and after enzyme hydrolysis have the same degree of shielding of the PC-phosphorus nuclide; the same appears to be true for LPC. The data concerning line width, signal position, and number of signals all are indicative that, on the time scale of the NMR technique, there are no pools of PC having different environments.

The kinetic analysis of our data, which were collected on a time scale of minutes agrees with that of Pattnaik et al. (1976), derived from pH-stat measurements on a time scale of seconds. By both techniques, and on different time scales, the reaction followed first-order kinetics and had identical values of $k_{cat}$. It follows that $^{31}P$ NMR can be utilized effectively to provide kinetic data on soluble lipoproteins.

In conclusion, our studies confirm the enzymatic studies of Pattnaik et al. (1976) and support the concept that PC are located at or are in rapid equilibrium with the surface of the particle as indicated by the accessibility of the PC to $\alpha$-phospholipase $A_2$. Furthermore, as judged by line widths, chemical shifts, and the absence of additional signals, the $^{31}P$ NMR spectra indicate that the removal of the fatty acid from the 2 position of the PC molecules in $HDL_3$ does not perturb the microenvironment of the polar head group of PC. Our results also suggest that, in $HDL_3$, the polar head groups of the LPC produced have similar microenvironments as the polar head groups of PC in the native particle, suggesting that the LPC formed, and the PC originally present probably have a similar structural role.

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