Lipid–Protein Interactions. Effect of Apolipoprotein A-I on Phosphatidylcholine Polar Group Conformation As Studied by Proton Nuclear Magnetic Resonance†

D.-J. Reijngoud, S. Lund-Katz, H. Hauser, and M. C. Phillips*

ABSTRACT: Spin–spin coupling constants derived from high-resolution ^{1}H NMR spectra of pure 1-myristoyl-*sn*-glycero-3-phosphocholine (MLPC) micelles and 60:1 mol/mol MLPC–human apolipoprotein A-I (apo A-I) complexes have been analyzed in order to determine the effects of apoprotein on phosphatidylcholine (PC) polar group conformation. The shift ratios of the polar group proton resonances after addition of the paramagnetic shift reagent $Fe(CN)_6^{3-}$ to the above MLPC systems, egg PC small unilamellar vesicles, and human HDL_3 have been used to compare the PC polar group conformations in all systems. The location of the largely α-helical apo A-I molecules in the complex with MLPC was deduced from its effects on the chemical shifts and spin–lattice relaxation times (T_1) of the well-resolved ^{1}H resonances from the various parts of the lipid molecules. The data are consistent with the apo A-I molecules lying in the surface of the MLPC micelle with their amphipathic, α-helical segments intercalated among the glycerophosphocholine groups of the lipid molecules so that aromatic amino acid side chains are interspersed among the lipid hydrocarbon chains. This leads to a spacing out of the glycerol backbones and immediately adjacent methylene groups of the MLPC molecules, thereby causing an enhancement of the motions affecting T_1. The presence of apo A-I at the lipid–water interface apparently does not perturb the PC polar group conformation, indicating that this conformation is determined by intramolecular effects. The preferred conformation of the phosphocholine group [Hauser, H., Pascher, I., Pearson, R. H., & Sundell, S. (1981) *Biochim. Biophys. Acta 650*, 21–51] is characterized by an almost exclusively gauche conformation of the choline group and predominantly antiperiplanar conformations about the C–C–O–P and P–O–C–C bonds. The PC molecules in MLPC micelles, MLPC–apo A-I complexes, egg PC vesicles, and HDL_3 all have this polar group conformation.

A detailed picture of the structure and dynamics of the components of the surface of lipoproteins is required in order to understand phospholipid–apolipoprotein interactions as they occur on the surface of serum lipoprotein particles. Insertion of apolipoproteins into phospholipid bilayers to form lipid–protein complexes brings about considerable changes in the physical properties of the phospholipids [for a review, see Morrisett et al. (1977)]. For instance, association of phospholipids with apolipoproteins causes changes in hydrocarbon chain packing and motion and a loss of cooperativity in chain melting of the phospholipids (Andrews et al., 1976; Morrisett et al., 1977; Stoffel et al., 1978; Gilman et al., 1981). The effects of serum apolipoprotein binding on phosphatidylcholine (PC)[1] polar group conformation have not been elucidated well, and clearly this information is required before the surface interactions of lipoproteins (e.g., with cells or enzymes) can be understood fully.

Proton NMR has been applied successfully in elucidating the structure of the glycerophosphocholine group of phospholipids in solution, as a monomer or as a small micelle (Hauser et al., 1978, 1980). In these studies, the conformation of the polar group was derived from spin–spin coupling constants of the protons in the glycerol backbone and the choline moiety. Apo A-I forms small micellar complexes with lysophosphatidylcholines (Gwynne et al., 1975; Haberland & Reynolds, 1975; Jonas & Krajnovich, 1977) which are suitable for structural analysis by means of the above approach. Here we describe the phospholipid polar group conformation with and without apo A-I obtained by means of an analysis based on spin–spin coupling constants derived from high-resolution ^{1}H NMR spectra of a model system comprising 1-myristoyl-*sn*-glycero-3-phosphocholine [myristoyllysophosphatidylcholine (MLPC)] and human apo A-I. The resonances of the glycerophosphocholine protons in this conformation have characteristic shift ratios for the paramagnetic NMR shift reagent $Fe(CN)_6^{3-}$. Comparison of the shift ratios for lyso-PC and PC molecules in micelles, vesicles, and human high-density lipoprotein (HDL_3) indicates that a common polar group conformation is maintained in all these systems.

Experimental Procedures

Materials

Total HDL ($1.063 < d < 1.21$ g/mL), HDL_2 ($1.063 < d < 1.125$ g/mL), and HDL_3 ($1.125 < d < 1.21$ g/mL) were isolated from outdated human plasma from the blood bank by using sequential ultracentrifugation (Havel et al., 1955). Total HDL was delipidated with 3:2 (v/v) ethanol:diethyl ether at 0 °C (Scanu & Edelstein, 1971), and apo A-I was isolated by using a modification of the original method of Scanu et al. (1969); the protein was chromatographed on Sephacryl

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[1] Abbreviations: apo A-I, human serum apolipoprotein A-I; CD, circular dichroism; cmc, critical micelle concentration; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MLPC, 1-myristoyl-*sn*-glycero-3-phosphocholine (myristoyllysophosphatidylcholine); NMR, nuclear magnetic resonance; PC, phosphatidylcholine; T_1, spin–lattice relaxation time; T_2, spin–spin relaxation time; TSS, 3-(trimethylsilyl)-propanesulfonic acid, sodium salt; $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)-aminomethane.

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S300 columns (90 × 5 cm) by using 6 M urea, 1 M NaCl, 10 mM Tris-HCl, pH 8.6, 1 mM EDTA, and 0.02% NaN_3 as the elution buffer. The fractions corresponding to the position of apo A-I were pooled, dialyzed against distilled and deionized water, and lyophilized. Purification of apo A-I was achieved by rechromatography on a Sephacryl S200 column (180 × 1 cm) eluted with the above buffer. The peak fractions containing apo A-I were pooled, extensively dialyzed against water, and lyophilized. The protein was stored at −24 °C. The resultant apo A-I gave a single band on $NaDodSO_4$–polyacrylamide gel electrophoresis.

Chromatographically pure 1-myristoyl-*sn*-glycero-3-phosphocholine (MLPC) was purchased from Calbiochem-Behring (LaJolla, CA) and used as supplied. The following chemicals were also used as supplied: Sephacryl S200 and S300 and Sepharose CL4B from Pharmacia Fine Chemicals (Piscataway, NJ), D_2O from Stohler Isotope Chemicals (Waltham, MA), 3-(trimethylsilyl)propanesulfonic acid sodium salt (TSS) from Wilmad Glass Co., Inc. (Buena, NJ), and $K_3Fe(CN)_6$ from Pfaltz and Bauer (Stamford, CT). Other reagents were analytical grade.

Methods

Formation of Lipid–Protein Complexes. Complexes of MLPC and apo A-I were obtained by dissolving weighed amounts of each in D_2O. NaOH dissolved in D_2O was added to give a nominal pH of ~7.5. After recombination, protein was determined by the $NaDodSO_4$–Lowry method (Markwell et al., 1978), and PC was monitored by phosphorus analysis (Sokoloff & Rothblat, 1974). The size of the complex was determined on a Sepharose CL4B column (110 × 1 cm) eluted with 150 mM NaCl, 20 mM Tris, pH 7.5, 1 mM EDTA, and 0.02% NaN_3. The column was calibrated with human LDL [Stokes radius (R_s) = 96 Å], human HDL_2 (R_s = 51 Å), and bovine serum albumin (R_s = 35 Å). The void and total volumes were measured with handshaken egg liposomes and methyl red, respectively. The sizes were also monitored by negative-stain electron microscopy by using procedures described before (Goldfine et al., 1981).

Circular Dichroism. CD spectra were monitored with a JASCO 41A spectropolarimeter calibrated with *d*-10-camphorsulfonic acid. Over the range 20–70 °C, the temperature was maintained by a Haake E12 circulating pump and was monitored inside the cuvette with a Chromel–constantan thermocouple connected to a digital display (Omega, Stamford, CT). Contents of α helix in apo A-I were estimated from the approximate expression of Greenfield & Fasman (1969): % helix = $([\theta]_{208} - 4000)/(33\,000 - 4000)$, where $[\theta]_{208}$ is the molar ellipticity in degrees at 208 nm calculated according to $[\theta]_{208} = MRW\theta_{208}/(10lc)$ where θ_{208} is the observed ellipticity in degrees at 208 nm, MRW the mean residue molecular weight (=114.3; Scanu et al., 1975), l the path length in centimeters (0.1 cm), and c the protein concentration in grams per milliliter.

Nuclear Magnetic Resonance. ^1H NMR spectra were recorded at 360 and 400 MHz by using Fourier-transform spectrometers (Bruker WH-360/180 and Bruker WH-400, respectively). The digital resolution was 0.18 Hz/point, and the spin coupling constants used in the conformational analysis were derived from computer simulations of the spectra with an accuracy of ±0.2 Hz. Spectra were computed by using the ITRCAL version of the LAOCN3 program on a Nicolet B-NC12 computer equipped with a floppy disk system FDD260. Chemical shifts were measured to ±0.01 ppm from internal TSS. A $(180°–\tau–90°–PD)_n$ pulse sequence was employed for spin–lattice relaxation time T_1 measurements (Vold et al., 1968) where τ is the variable delay time between the 180° and 90° pulses, PD is the pulse delay (2.5 s), and n is the number of times the pulse sequence was repeated. T_1 values were derived from linear plots of ln signal intensity vs. τ by using a linear regression analysis. The T_1 values were usually accurate within ±10%.

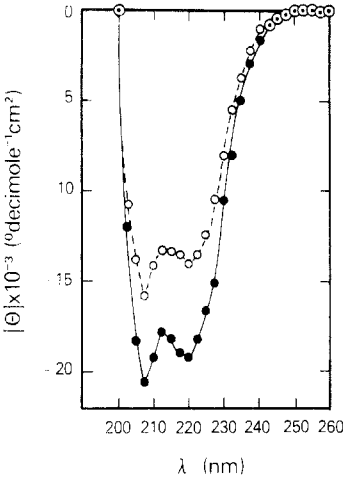


FIGURE 1: Far-ultraviolet circular dichroism (CD) spectra recorded at 37 °C of human apo A-I (○) and of a 1:60 mol/mol apo A-I–MLPC complex (●).

Results

Recombination of apo A-I with various lyso-PC molecules has been shown to lead to the formation of spherical complexes with diameters of 60–70 Å which is not much different from that of pure lyso-PC micelles (Haberland & Reynolds, 1975; Stoffel et al., 1978). Haberland & Reynolds (1975) also showed that for 1-palmitoyl-*sn*-glycero-3-phosphocholine the maximal binding to apo A-I was 95–100 mol of lipid/mol of apo A-I while the data of Stoffel et al. (1978) for various lyso-PC's indicate that up to about 70 mol of lyso-PC can bind per mol of apo A-I.

In order to obtain proton NMR spectra of the complexed lipids free of signals from lipid molecules not associated with protein, we used recombinants of 1:60 mol/mol apo A-I and MLPC, which is below the amount of lipid required to saturate the protein. A Stokes radius of 37 Å was measured with a Sepharose CL4B column, and electron microscopy gave the radius of the recombinant as 34 ± 6 Å (±SD, n = 180). Since the NMR spectra were recorded at lipid concentrations of 8 × 10^{-3} M, there was practically no interference from free, monomeric MLPC molecules [cmc = (6–10) × 10^{-5} M; Hayashi et al., 1972; Jonas & Krajnovich, 1977). It should be noted that at these concentrations the solutions tended to become turbid in the course of days, due to the formation of large micelles with R_s = 70 Å [cf. Haberland & Reynolds (1975)]. Consequently, solutions of apo A-I–MLPC complexes were made up immediately before use. As can be seen from Figure 1, interaction of apo A-I with MLPC at a molar ratio of 1:60 increases the α-helix content from 34 to 59% [cf. Stoffel et al. (1978)]. This recombinant was stable between 20 and 50 °C as measured by the effect of temperature on the α-helix content of apo A-I; raising the temperature above 55 °C caused a progressive unfolding of the α helix.

^1H NMR spectra (360 MHz) of MLPC and of the 1:60 mol/mol apo A-I–MLPC complexes at 37 °C are shown in parts A and B, respectively, of Figure 2. Expanded regions of the glycerol backbone protons are presented in parts C and D, respectively, of Figure 2. High-resolution spectra of the polar group resonances recorded at 400 MHz and 50 °C,

Table I: Chemical Shifts[a] of the ^1H Resonances of 1-Myristoyl-*sn*-glycero-3-phosphocholine in the Absence and Presence of Apolipoprotein A-I

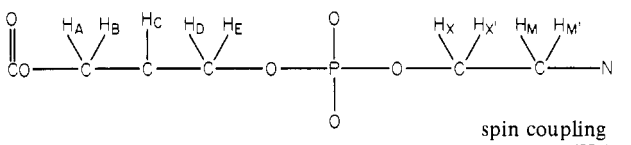
resonance	MLPC	60:1 mol/mol MLPC-apo A-I
$-CH_3$	0.86	0.83
$-(CH_2)_{10}-$	1.28	1.22
$-CH_2CCO$	1.59	1.53
$-CH_2CO$	2.38	2.33
$-CH_2OCO$: H_A[b]	4.10	4.08
H_B	4.17	4.15
CCHC	4.02	4.02
CH_2OP (glycerol): H_D	3.84	3.85
H_E	3.94	3.93
CH_2OP (choline)	4.30	4.31
CH_2N	3.67	3.66
$N(CH_3)_3$	3.22	3.22

[a] Chemical shifts are expressed in ppm ±0.01 downfield from TSS. [b] See Table II for designation of these protons.

together with a computer-simulated spectrum, are shown in Figure 3. When the temperature is raised from 21 to 50 °C, the only spectral change observed was a decrease in line width. The assignment of the lipid resonances was carried out as described previously (Hauser et al., 1978, 1980) and is summarized in Table I. The chemical shifts of the proton resonances from the glycerol and choline groups were derived from the computer simulation of the spectra. Comparison of the δ values observed in the absence and presence of apo A-I indicates that upfield shifts are induced by the protein; the shift changes are largest for the hydrocarbon chain resonances while within the error of the measurement the protein has no effect on the chemical shifts of the protons of the MLPC polar head-group signals (Figure 4A). It is clear from a visual inspection of Figure 3 that the spin–spin coupling constants responsible for the splitting of the polar group signals must be very similar in MLPC micelles and the apo A-I–MLPC complex. The spin coupling constants for MLPC in the complex derived from the computer simulation of the spectra (Figure 3) are summarized in Table II; they are practically identical with those of pure MLPC reported previously (Hauser et al., 1978). Furthermore, from the comparison of the spectra of MLPC and apo A-I–MLPC complexes (Figures 2 and 3), it is clear that apo A-I induces differential line broadening. There is some broadening of the glycerol and the CH_2 choline resonances as well as the first CH_2 group in the hydrocarbon chain while, within the error of the measurement, there is no effect on the line width ($\nu_{1/2}$) of the $(CH_2)_{10}$ and the $N(CH_3)_3$ signals. In the absence of apo A-I, $\nu_{1/2}$ = 2.2 and 12.1 Hz for the $N(CH_3)_3$ and $(CH_2)_{10}$ resonances, respectively; in the presence of apo A-I at a molar ratio of 1:60, the equivalent figures are 2.7 and 11.9 Hz. Based upon relative intensities and intensity measurements against an internal standard of sodium acetate, within experimental error, all of the lipid protons contribute to the spectrum.

Proton spin–lattice relaxation times (T_1) for MLPC micelles and the 1:60 mol/mol apo A-1–MLPC complex are given in Table III. In MLPC micelles, similar T_1 values were measured for all protons except for the acyl chain CH_3 protons which have a significantly longer T_1 (Table III); a similar pattern has been observed by Hauser (1976) for egg lyso-PC. Addition of apo A-I induces small but significant changes in the T_1 values for the protons in several segments of MLPC (Figure 4B). The most pronounced alterations are the increase in T_1 for the protons of the glycerol backbone and nearby parts of the acyl chain and the decrease in T_1 for the terminal methyl group. No significant change could be detected in the T_1 for

Table II: Spin–Spin Coupling Constants of the Polar Group Resonances of 1-Myristoyl-*sn*-glycero-3-phosphocholine in the Absence and Presence of Apolipoprotein A-I



group	signal	spin coupling constant notation[a]	spin coupling constants (Hz) b	c
glycerol	CH_2O	$^2J_{AB}$	11.2	11.4
		$^3J_{AC}$	6.73	6.5
		$^3J_{BC}$	4.13	3.4
	CHO	$^3J_{CD}$	6.34	6.0
		$^3J_{CE}$	3.77	4.1
		$^2J_{DE}$	11.1	11.2
	CH_2OP	$^3J_{PH_D}$	6.09	5.8
		$^3J_{PH_E}$	6.07	6.0
choline	$OPCH_2$	$^2J_{XX'}$	14	14
		$^3J_{PH_X} = {}^3J_{PH_{X'}}$	6.0	6.0
		$^3J_{NH_X} = {}^3J_{NH_{X'}}$	2.5	2.5
	CH_2N	$^2J_{MM'}$	14	14
		$^3J_{MX} = {}^3J_{M'X'}$	2.5	2.5
		$^3J_{MX'} = {}^3J_{M'X}$	6.9	6.9

[a] The lettering used to designate the polar group protons of MLPC is shown in the above structure. [b] Computer fit using LAOCN3 program to MLPC spectrum (Figure 3A). [c] Computer fit to MLPC-apo A-I spectrum (Figure 3B).

Table III: Spin–Lattice Relaxation Times (T_1) of the Proton Resonances of 1-Myristoyl-*sn*-glycero-3-phosphocholine in the Absence and Presence of Apolipoprotein A-I[a]

resonance	T_1 of MLPC micelle	T_1 of MLPC-apo A-I complex (60:1 mol/mol)
$-CH_3$	0.77 ± 0.02	0.62 ± 0.01
$-(CH_2)_{10}-$	0.52 ± 0.04	0.55 ± 0.02
$-CH_2CCO$	0.44 ± 0.01	0.55 ± 0.04
$-CH_2CO$	0.47 ± 0.01	0.64 ± 0.08
$-CH_2OCO$	0.44 ± 0.04	0.65 ± 0.04
CC*H*OHC	0.54 ± 0.06	0.64 ± 0.03
CH_2OP (glycerol)	0.43 ± 0.09	0.61 ± 0.01
CH_2OP (choline)	0.53 ± 0.03	0.57 ± 0.01
CH_2N	0.49 ± 0.01	0.52 ± 0.04
$N(CH_3)_3$	0.45 ± 0.02	0.45 ± 0.02

[a] T_1 values are given in seconds ± SD.

the remaining MLPC signals. Similar effects of apo A-I on the relaxation of lyso-PC selectively enriched with ^{13}C in the $N(CH_3)_3$ group and the terminal CH_3 regions of the acyl chain were observed by Stoffel et al. (1978) by using ^{13}C NMR. These authors observed a 25–35% reduction in T_1 for the terminal part of the acyl chain without any change in the relaxation of the $N(CH_3)_3$ group.

The effects of the paramagnetic shift reagent $Fe(CN)_6^{3-}$ were compared in order to relate the glycerophosphocholine conformation in apo A-I–MLPC complexes to that in egg PC vesicles and human HDL_3. As has been shown previously [Hauser et al., 1976, 1980; for a review, see Morris & Dwek (1977)], paramagnetic shift reagents can be applied to elucidate the lipid polar group conformation. $K_3Fe(CN)_6$ can be used to good effect for conformational analysis in such systems because it does not seem to perturb the native conformation significantly. Thus, Brown & Seelig (1977) have shown that addition of $Fe(CN)_6^{3-}$ induces only minor changes in the ^{31}P chemical shift anisotropy and the ^2H quadrupole splitting of NCD_2CH_2OP and NCH_2CD_2OP in dipalmitoyl-

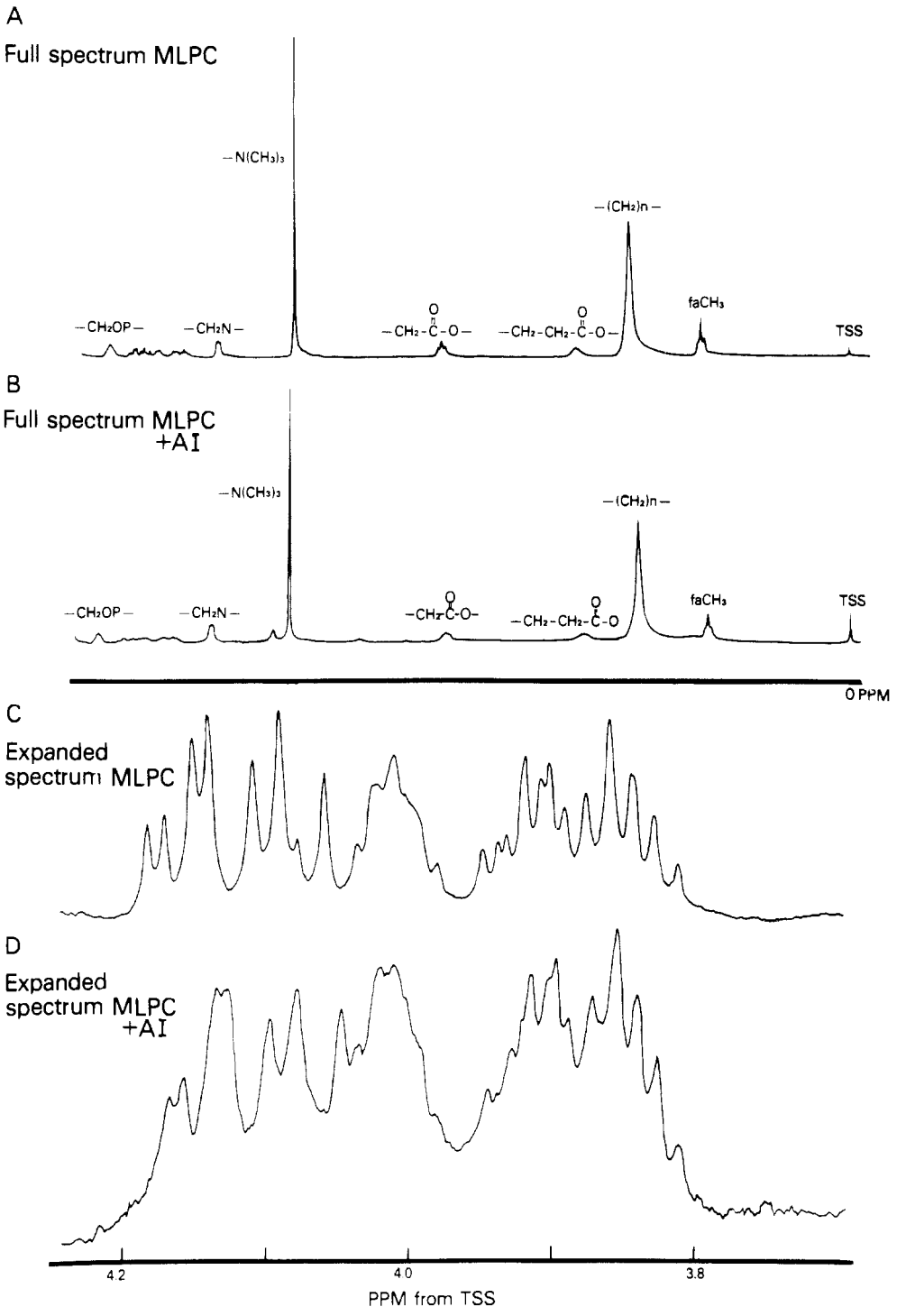


FIGURE 2: ^{1}H NMR spectra (360 MHz) recorded at 37 °C of (A) MLPC in D_2O (4.3 mg/mL = 0.0088 M, nominal pH ~7.5) and of (B) a 1:60 mol/mol apo A-I–MLPC complex in D_2O (MLPC concentration is 4.5 mg/mL, nominal pH ~7.5). Spectra A and B were processed with 0.1-Hz exponential filtering and a recycle time of 0.5 s. The expanded regions of the glycerol backbone in MLPC (C) and the apo A-I–MLPC complex (D) have the horizontal and vertical axes expanded by factors of 10 and 15, respectively.

PC. The shift changes ($\Delta\delta$) induced by $Fe(CN)_6^{3-}$ in the proton NMR spectra of MLPC and of the 1:60 mol/mol apo A-I–MLPC complex are shown in Figure 5. These shift changes are compared to those induced in the polar group signals of PC present in small unilamellar vesicles and in HDL_3. It is obvious from Figure 5 that the presence of apo A-I does not alter the shift changes ($\Delta\delta$) for MLPC induced by $Fe(CN)_6^{3-}$. Similarly, addition of $Fe(CN)_6^{3-}$ to PC as present in vesicles or in HDL_3 particles gives rise to values of $\Delta\delta$ almost identical with those observed with MLPC or egg lyso-PC micelles (H. Hauser, unpublished results). Therefore, the proteins present in the phospholipid surface of HDL_3 do not affect the shift changes or shift ratios induced by $Fe(CN)_6^{3-}$ in the polar group resonances.

Discussion

High-resolution ^{1}H NMR spectra and spin–spin coupling can be observed in phospholipids such as dihexanoyl- and lyso-PC's which form small, spherical micelles in water (Hauser et al., 1980). Initially, we attempted to use short-chain PC molecules for analysis of the polar group conformation because of their greater similarity to PC molecules occurring in lipoproteins. However, complexes of these short-chain phosphatidylcholines with apo A-1 gave spectra

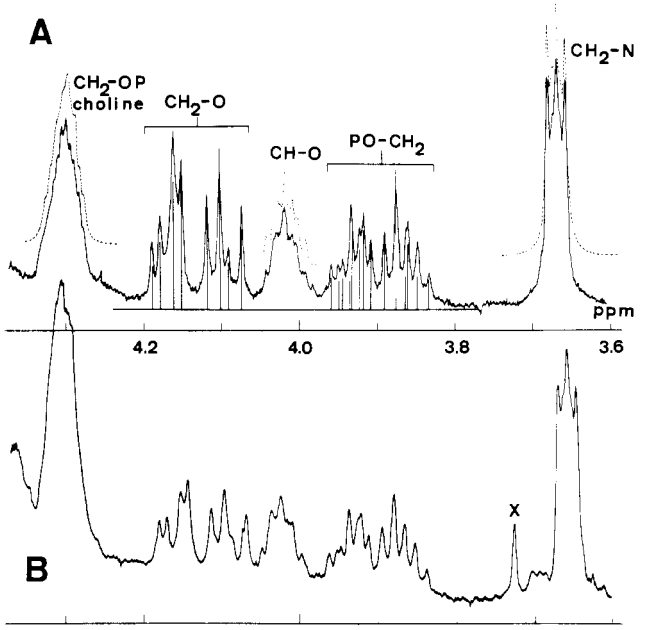


FIGURE 3: ^1H NMR spectra (400 MHz) recorded at 50 °C of (A) MLPC in D_2O (5.5 mg/mL = 0.011 M, nominal pH ~ 6) and of (B) a 1:60 mol/mol apo A-I–MLPC complex in D_2O (MLPC concentration is 5.5 mg/mL, nominal pH ~ 6); X = impurity. The dotted and stick spectra in (A) are computer simulations.

which could not be interpreted unequivocally because it was not clear to which form of the lipid to assign the spectra. In the dihexanoyl-PC–apo A-I system, the lipid can be present in several states: (a) as monomers at relatively high concentrations (cmc ~ 14 mM; Tausk et al., 1974; Hauser et al., 1980), (b) as micelles, and (c) as the apo A-I–lipid complex. In order to avoid the high free monomer concentration, we used dioctanoyl-PC which has a lower cmc, but with dioctanoyl-PC–apo A-I complexes no coupling constants could be obtained, presumably because of residual dipolar line broadening (Finer et al., 1972). For these reasons, we have used the MLPC–apo A-I system for monitoring the effects of apo A-I on the glycerophosphocholine conformation in PC molecules.

Structure of Apo A-I–MLPC Complexes. The localization of the protein moiety in both types of particle should be similar in order for the results on lipid polar group conformation obtained with apo A-I–MLPC complexes to apply directly to serum lipoproteins. For lipoproteins such as HDL, a surface localization of apolipoproteins, especially the amphipathic α helix, is well established [for a review, see Morrisett et al. (1977)], and the protein seems to be similarly situated in the apo A-I–MLPC complexes. Both the shift changes (cf. Figure 4A) and the changes in T_1 (Figure 4B) induced in the presence of apo A-I can be rationalized by placing the axis of the amphipathic α helix in the position indicated by the dashed line in Figure 4. The localization of the axis of the amphipathic helix of apo A-I in the vicinity of the acyl chain ester linkage leads to the presence of the aromatic residues Tyr and Trp in the outer portion of the apolar core of the MLPC micelle. The observed changes in the chemical shift of the protons of the acyl chain could then be due to ring current effects from these aromatic residues; similar upfield shifts have been observed when lyso-PC molecules bind to bovine serum albumin (Oakes, 1973), casein (Barratt & Rayner, 1972), or melittin (DeBony et al., 1979). The presence of the protein in the surface of the MLPC micelle spaces out the lipid molecules which leads to an increase in fast segmental motion of the glycerol backbone, as indicated by the increase in T_1 values. This conclusion is not incompatible with the observed

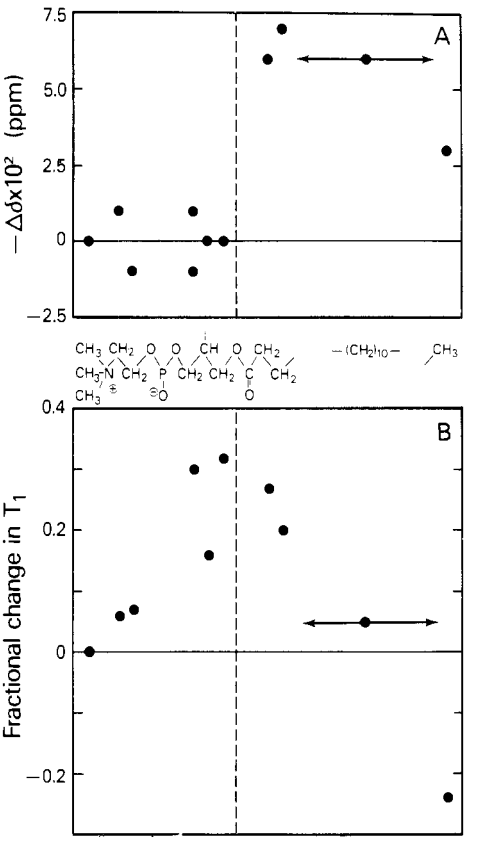


FIGURE 4: (A) Changes in chemical shift ($\Delta\delta$) of the proton resonances of MLPC induced by the presence of apo A-I. $\Delta\delta$ values were calculated from Table I by subtracting the chemical shift measured in the apo A-I–MLPC complex from that of MLPC. A positive $\Delta\delta$ indicates a downfield shift. (B) Fractional changes in spin–lattice relaxation times (T_1) of the proton resonances of MLPC induced by the presence of apo A-I. Fractional changes = $[T_1(+) - T_1(-)]/T_1(+)$ were calculated from the data in Table III where T_1 measured minus protein is $T_1(-)$ and plus protein is $T_1(+)$. In both (A) and (B), the vertical dashed line indicates the approximate position of the axis of the α helix of apo A-I.

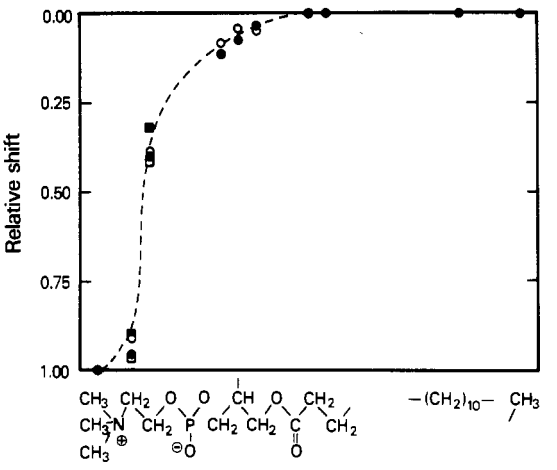


FIGURE 5: Effect of $K_3Fe(CN)_6$ on the chemical shifts of the proton resonances of the PC polar group. The changes in chemical shifts ($\Delta\delta$) induced by $Fe(CN)_6^{3-}$ are normalized to the largest shift change observed with the $N(CH_3)_3$ signal (upfield shifts of 0.16–0.20 ppm for all systems studied); in this way, relative pseudocontact shifts are readily expressed as fractions of the $N(CH_3)_3$ shift. (●) MLPC; (○) 1:60 mol/mol apo A-I–MLPC complex; (■) sonicated aqueous dispersion of egg PC consisting of small unilamellar vesicles; (□) human HDL_3.

slight line broadening of the resonances from the glycerol backbone protons because T_1 and T_2 are sensitive to different molecular motions in phospholipid systems (Kainosho et al.,

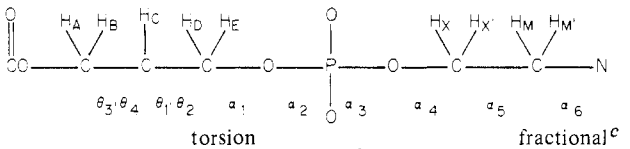
1978). Fast motions such as chain isomerization dominate the spin–lattice relaxation rate (T_1) whereas spin–spin relaxation (T_2) is most affected by slower motions involving reorientation of local segments. Intercalation of the apo A-I helices among the glycerol backbones of the molecules could easily decrease the cooperative, segmental motion in this region, thereby causing the observed line broadening. Despite the spacing out of the lipid molecules, T_1 of the protons of the acyl chain terminal methyl group decreases considerably (Figure 4). Recent calculations of Dill & Flory (1981) on the molecular organization of micelles provide a basis for explaining the anomalous decrease in T_1 of the methyl group. In a spherical micelle, only a few of the hydrocarbon terminal methyl groups can be present in the center of the micelle, and the majority of these groups are distributed midway between the center of the micelle and the outer surface. Rapid exchange between these localizations than contributes to the high value of T_1. The apo A-I molecules located in the surface of a MLPC micelle with the aromatic residues in the apolar core of the micelle impede the free motion of the terminal CH_3 group by confining it to the center of the apolar core of the micelle, thereby decreasing T_1.

Given the surface localization of apo A-I, the composition of an apo A-I–MLPC complex can be calculated on the basis of surface area considerations. Assuming that apo A-I is essentially all α helical and completely embedded in the surface of the complex, it can be calculated that for one apo A-I molecule the total surface area is 0.37×10^4 Å^2 [245 residues with an average residue surface area of 15 Å^2 (Phillips & Sparks, 1980; Shen & Scanu, 1980)]. Sixty MLPC molecules with a surface area of 52 Å^2/molecule (Hauser et al., 1981) occupy a total surface area of 0.31×10^4 Å^2. Since the Stokes radius of the spherical apo A-I–MLPC complex is 37 Å, the total surface area of the complex (1.7×10^4 Å^2) is equivalent to the surface area of two apo A-I molecules and 120 molecules of MLPC. A similar composition was derived by Stoffel et al. (1978) on the basis of molecular weight considerations.

In summary, our data are consistent with the apo A-I molecules lying in the surface of the MLPC micelle with their amphipathic, α-helical segments intercalating among the glycerophosphocholine groups of the lipid molecules. This leads to a spacing out of the glycerophosphocholine groups and immediately adjacent acyl chain methylene groups with an enhancement of the rapid motions affecting proton spin–lattice relaxation. The apolar amino acid side chains protrude into the apolar core of the micelle and alter the acyl chain packing. A decrease in cooperativity of the methylene group segmental motion is expected which is consistent with the decrease in cooperativity of the gel to liquid-crystalline transition observed on adding apo A-I to dimyristoyl-PC bilayers [e.g., see Andrews et al. (1976)]. The apolar interactions observed between MLPC molecules and apo A-I are consistent with previous investigations of the interaction of lyso-PC molecules with proteins.

MLPC Polar Group Conformation. In the presence of a protein, all the positions which a lipid molecule can occupy may not be equivalent because, in the extreme, they can either be in close contact with the protein or be completely surrounded by lipid molecules. For estimation of the distribution of lipid and protein molecules in the 60:1 mol/mol MLPC–apo A-I complex, an apo A-I molecule can be represented by a rectangle with an average width of 16 Å; this number has been computed for α helices (Tall et al., 1977). For these rectangles to have a surface area equivalent to that of apo A-I (0.37×10^4 Å^2), their total length must be 230 Å, which leads to a circumference for the rectangle of 500 Å. If a MLPC molecule is represented by a disc of 8-Å diameter, 62 MLPC molecules can be packed around the circumference of an apo A-I molecule. This indicates that in the apo A-I–MLPC complex described here all MLPC molecules are in close contact with the protein.

Table IV: Minimum Free Energy Conformations and Rotamer Populations of the Polar Group of 1-Myristoyl-*sn*-glycero-3-phosphocholine in the Absence and in the Presence of Apolipoprotein A-I



bond	torsion angle[a]	staggered[b] conformation	fractional[c] population
R_1COOCH_2–CHOH	θ_3 (θ_4)	antiperiplanar (synclinal)	0.48
		synclinal (–synclinal)	0.37
		–synclinal (antiperiplanar)	0.15
HOCH–CH_2OP	θ_1 (θ_2)	–synclinal (synclinal)	0.42 (0.49)
		antiperiplanar (–synclinal)	0.44 (0.10)
		synclinal (antiperiplanar)	0.14 (0.41)
$CHCH_2$–OP	α_1	±synclinal	0.20
		antiperiplanar	0.80
PO–CH_2CH_2	α_4	±synclinal	0.20
		antiperiplanar	0.80
$POCH_2$–CH_2N	α_5	±synclinal	1.0
		antiperiplanar	

[a] For the notation and definition of torsion angles, see Hauser et al. (1981). [b] The terminology used to describe the approximate conformation of two substituents across a simple X–Y bond has been discussed before [see Figure 3 of Hauser et al. (1981)]. Synclinal or gauche = 60 ± 30°; antiperiplanar = 180 ± 30°. [c] For torsion angles θ_1 and θ_2, two sets of values were obtained with $J_{CD} > J_{CE}$ and $J_{CD} < J_{CE}$ (in parentheses).

Within experimental error, identical spin–spin coupling constants are observed for MLPC in micelles and in complexes with apo A-I (Table II). This finding indicates that whatever motionally averaged conformation MLPC molecules have it does not change significantly when the lipid is in close contact with the apolipoprotein. Table IV summarizes the minimum free energy conformations and rotamer populations for MLPC in the absence and presence of apo A-I, as determined by methods described previously (Hauser et al., 1978). The details of the motionally averaged conformation of MLPC present in micellar dispersions have been discussed before (Hauser et al., 1978), and these considerations are also applicable to the apo A-I–MLPC complex. The main features of this "solution" conformation may be summarized as follows [see Table IV in this paper; Figures 8 and 9 in Hauser et al. (1981)]. The ^{1}H NMR data indicate that there are two different, almost equally populated, conformations allowed about the glycerol C_1–C_2 bond (torsion angles θ_3 and θ_4). One conformation (θ_3 = antiperiplanar, θ_4 = synclinal) is consistent with the conformation found in the crystal structure of phospholipids [for a review, see Hauser et al. (1981)]. The second conformation with θ_3 = synclinal and θ_4 = –synclinal is consistent with that found in the single crystal structure of a diacylglycerol derivative (Watts et al., 1972). Around the second glycerol bond, C_1–C_2 (next to the phosphate group with torsion angles θ_1 and θ_2), there seems to be some preference for the conformation θ_1 = –synclinal and θ_2 = synclinal; this is the conformation found in the single crystal structures of both dilauroylphosphatidylethanolamine (Hitchcock et al., 1974) and 1-palmitoyl-*sn*-3-phosphoethanolamine (Pascher

et al., 1981). Finally, the conformation of the N–C–C–O choline group is almost exclusively ±synclinal (gauche) with the torsion angles α_1 and α_2 antiperiplaner.

The data obtained with the apo A-I–MLPC complex, PC vesicles, and human HDL_3 by using $Fe(CN)_6^{3-}$ as a shift reagent suggest that the description of the glycerophosphocholine moiety obtained for MLPC applies to all systems studied. This agrees well with ^1H NMR data on the conformation of the lipid polar head group on PC in solution and in micelles (Hauser et al., 1980). The increased flexibility around the glycerol C_1–C_2 bond (torsion angles θ_1 and θ_2) in PC compared to lyso-PC [cf. Table IV and Table IV of Hauser et al. (1980)] cannot be resolved by using $Fe(CN)_6$ as a shift reagent. Furthermore, it is clear that neither the lipid–protein stoichiometry nor the proximity of the PC molecule to the apo A-I molecule is critical since the same conformation seems to be maintained in PC–water interfaces with no protein (e.g., PC vesicles) or with protein present (e.g., HDL_3). The latter particle contains ~13 phospholipid molecules per apoprotein molecule which is less than the 60:1 molar ratio of the MLPC–apo A-I complex, but still the average conformation of the glycerophosphocholine group appears to be the same.

In conclusion, the presence of apolipoprotein at a phospholipid–water interface does not perturb the phosphatidylcholine polar group conformation which is determined by intramolecular effects. It also has little effect on the dynamics of the lipid molecules. As far as phospholipid conformation is concerned, the primary effect of amphipathic α-helix-forming apolipoproteins at the surface of lipoprotein particles is spacing out of the phospholipid molecules. The finding that the insertion of apo A-I does not significantly change the polar group conformation of the lipid suggests that electrostatic forces do not play a major role. Our results point to hydrophobic interactions being the dominant stabilizing force.

Acknowledgments

We thank J. Collins and J. Gryn for expert technical assistance, B. Goren for artwork, and Dr. George MacDonald for valuable discussion. Some of the spectra were obtained at The Middle Atlantic NMR Facility at the University of Pennsylvania which is supported by National Institutes of Health Grant RR-542.

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