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DEUTERIUM MAGNETIC RESONANCE OF HUMAN PLASMA LIPOPROTEINS

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SUMMARY. Palmitic-d_{31} acid was incorporated into human plasma low and high density lipoproteins by incubation of lipoprotein dispersions with the fatty acid. ^2H NMR spectra were obtained and indicate that acyl chain order is higher in the surface monolayer of lipoproteins than in model phospholipid bilayer membranes. Thus the method can usefully be applied to investigate molecular motion within the monolayer of native lipoproteins.

INTRODUCTION

Human plasma HDL and LDL are approximately spherical particles with diameters of 70-100 Å and 200-300 Å respectively (1). The generally accepted structural model consists of an apolar core of cholesteryl esters, triglycerides and, perhaps, cholesterol surrounded by an amphiphilic monolayer of phospholipids, cholesterol and protein (2). Lipid mobility within lipoproteins has been shown by magnetic resonance to be considerable (3-11). However, presentation of a detailed picture of molecular motion is impossible on the basis of current data. Specific details of acyl chain ordering in either the monolayer or core have largely been inaccessible to study.

We report here the first ^2H NMR study of lipoproteins, and demonstrate the potential of the technique in providing information on any acyl chain order and dynamics within the amphiphilic monolayer of lipoproteins.

The observation of ^2H NMR spectra from deuterated fatty acids intercalated, at concentrations $\lesssim 5$ mol %, into membrane systems has been established as a reliable and non-perturbing method of probing membrane molecular motion (12,13). In the present study ~5 mol % (with respect to phospholipid) pal-

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; egg PC, egg phosphatidylcholine.

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mitic-d_{31} acid was incorporated into LDL_2 and HDL_2 (1). The experimental approach is similar to earlier work in which ESR spectra were recorded for intercalated spin labelled fatty acids (10,11), but has the advantage of avoiding the perturbations associated with the introduction of bulky ESR spin labels (14).

Linewidths in ^1H decoupled ^{31}P NMR spectra were also measured to determine whether lipoprotein size is affected by incorporation of the perdeuterated fatty acid.

MATERIALS AND METHODS

Palmitic acid was purchased from Fisher Scientific Co. and perdeuterated using a published procedure (15). [1-^{14}C]-palmitic acid was bought from New England Nuclear, Boston, Mass. Deuterium depleted water was supplied by Sigma. LDL_2 (density = 1.019-1.063 g/mL) and HDL_2 (density = 1.063-1.125 g/mL were isolated from fresh (<3 days old) human blood by ultracentrifugation (16). Protein concentrations were determined by the method of Lowry et al. (17). Crude egg PC was extracted from fresh hen egg yolks (18) and then purified by column chromatography on silica gel (19).

The concentrations of lipoprotein solutions were increased to levels suitable for NMR experiments using Amicon ultracentrifugation membrane cones (CF 25). Exchanges with deuterium depleted water (typically 5) were performed to reduce the residual ^2HOH signal. Fatty acid was incorporated by incubating lipoprotein solutions with a thin film of palmitic-d_{31} acid (~2-3 mg, representing an excess of 5 mol %) and a trace amount of [1-^{14}C]-palmitic acid in a round bottomed flask. The solution was gently agitated by hand during the period of incubation, which was up to 1 hour for ~5 mol % intercalation. The degree of incorporation was monitored by liquid scintillation counting of aliquots removed from the sample. NMR experiments were performed immediately. Association of lipoprotein and fatty acid was confirmed by column chromatography on Sepharose 4B at 4°C.

Unilamellar vesicles (5% w/v) of egg PC/palmitic-d_{31} acid (5 mol %) were prepared by sonication of a dispersion in deuterium depleted water of the lipid mixture (20).

The ^2H NMR measurements were carried out at 38.8 MHz and ~25°C with a homebuilt spectrometer and a Nalorac superconducting magnet. The spectrometer is interfaced to a Nicolet BNC-12 computer, which was used for collection and Fourier transformation of the free induction decays. The pulse length was 18 μs (flip angle = 90°) and the delay between successive pulses was ~1.5 s.

A Varian XL 100-15 NMR spectrometer, interfaced to a Nicolet 1080 computer and operating in the pulse Fourier transform mode, was employed in the ^{31}P NMR work. The free induction decays were recorded at ~25°C and a frequency of 40.5 MHz, using an external ^{19}F field frequency lock and in the presence of ^1H noise decoupling (bandwidth = 1 kHz). The pulse length was 10 μs (flip angle = 60°) and the delay between successive pulses was ~2 s. Chemical shifts were measured relative to an external H_3PO_4 (85%) reference.

RESULTS AND DISCUSSION

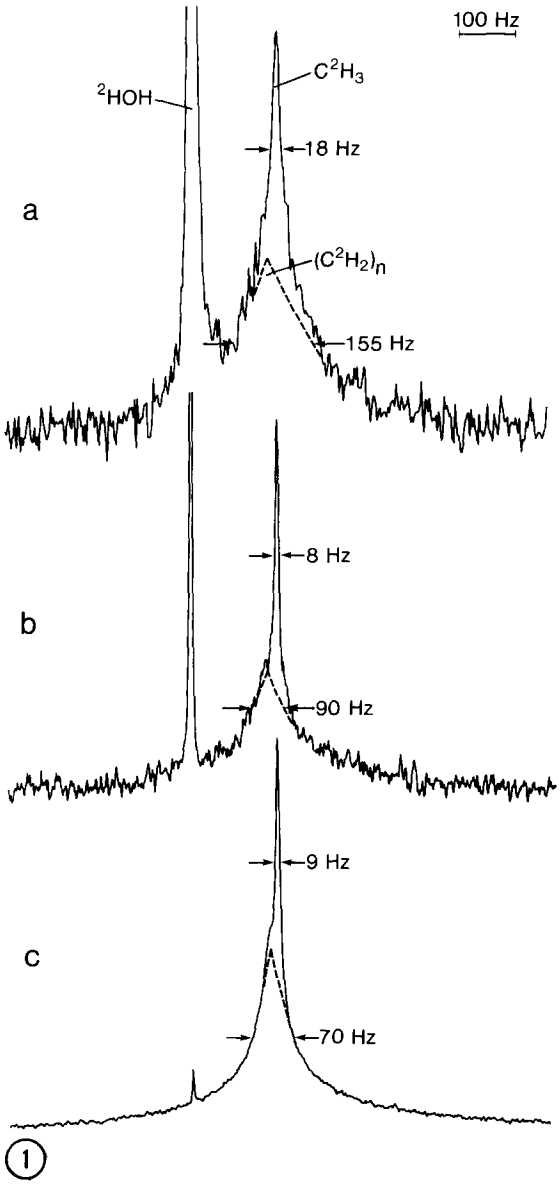
^2H NMR spectra recorded with dispersions of LDL_2, HDL_2 and, for comparison, egg PC vesicles containing ~5 mol % palmitic-d_{31} acid are presented in Fig. 1. In the three spectra, the palmitic-d_{31} acid signal can be, as illustrated, separated into two components , viz. a relatively narrow Lorentzian line due to the terminal C^2H_3 group superposed upon, and shifted ~15 Hz upfield from, a much broader composite line due to $(C^2H_2)_n$. The widths at half height, $\Delta\nu_{1/2}$, of the components are shown on the spectra. The lineshapes for LDL_2 and HDL_2 are considered to reflect molecular motion within the amphiphilic surface monolayer of the lipoproteins. Such a location for incorporated fatty acid is assumed in view of its amphiphilic nature. The assumption is supported by ascorbate reduction experiments with doxyl stearic acid (10) and energy transfer experiments with fluorescent fatty acids (21,22).

The ^1H noise decoupled ^{31}P NMR spectra shown in Fig. 2 for LDL_2 and HDL_2 samples containing ~5 mol % palmitic-d_{31} acid are similar to previously published spectra (7-9). As in the earlier reports, the upfield resonance at -0.8 ± 0.1 ppm in both spectra is assigned to phosphatidylcholine whilst the downfield resonance at -0.2 ± 0.1 ppm is assigned to sphingomyelin. The spectra and, moreover, the linewidths of the two resonances are essentially identical to those obtained before incorporation of the fatty acid. This indicates that neither the size of the lipoprotein particles nor the conformation of the phospholipid head groups was appreciably modified by added fatty acid. The width of the phosphatidylcholine and sphingomyelin lines is 12 ± 1 Hz for LDL_2 (Fig. 2a) and 6 ± 1 Hz for HDL_2 (Fig. 2b).

Particle tumbling is expected, as with vesicles (12), to be the isotropic motion largely responsible for line narrowing in the ^2H NMR signals observed with LDL_2 and HDL_2. For a C-^2H bond, the linewidth may then be related to order within the monolayer of lipoproteins by

$$\Delta\nu_{1/2} \approx \frac{9\pi}{20} \left(\frac{e^2qQ}{h} \right)^2 S_{CD}^2 \tau_t$$

where $\left(\frac{e^2qQ}{h}\right)$ is the quadrupolar coupling constant, S_{CD} is the order para-



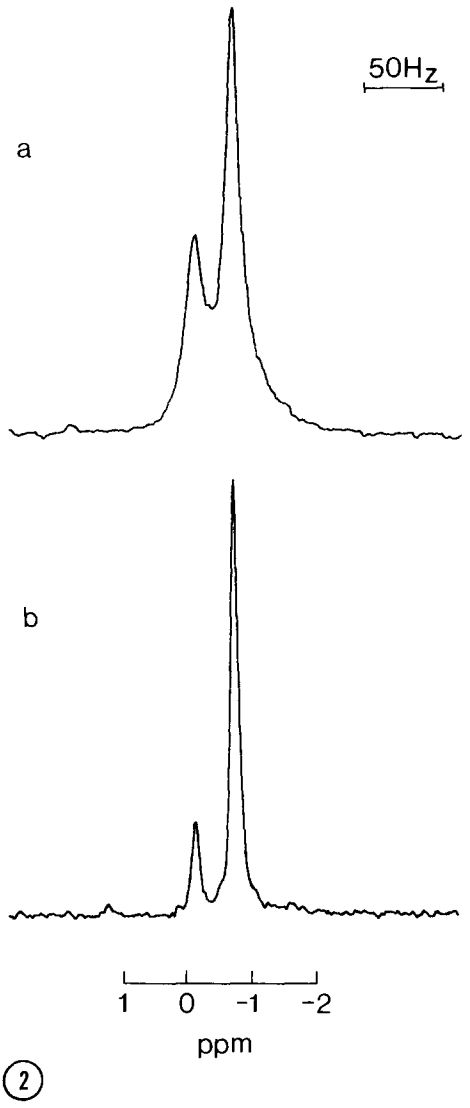


Fig. 1. ^{2}H NMR spectra for: a) LDL_2 + palmitic-d_{31} acid (7 mol %), 20 mg protein per mL solution; b) HDL_2 + palmitic-d_{31} acid (4 mol %), 13 mg protein per mL solution; c) egg PC + palmitic-d_{31} acid (5 mol %) vesicles, 5% lipid w/v. Spectral parameters: sweep width = a) 10 kHz, b) and c) 5 kHz; number of data points = 8K; line broadening = 2 Hz; number of transients = a) and b) 20000, c) 10000.

Fig. 2. ^{1}H noise decoupled ^{31}P NMR spectra for: a) LDL_2 + palmitic-d_{31} acid (7 mol %), 20 mg protein per mL solution; b) HDL_2 + palmitic-d_{31} acid (4 mol %), 13 mg protein per mL solution. Spectral parameters: sweep width = 2 kHz; number of data points = 2K zero filled to 4K; line broadening = 1 Hz; number of transients = 1024. Chemical shifts with respect to external H_3PO_4.

meter of the bond and τ_t is the correlation time for particle tumbling. The correlation time is given by

$$\tau_t = \frac{4\Pi\eta R^3}{3kT}$$

where η is the viscosity of the solution, R is the radius of the lipoprotein particles, k is Boltzmann's constant and T is temperature. On the basis of these expressions, the linewidths in Fig. 1 allow order in the surface monolayer of the lipoproteins to be compared with that in the model bilayer system of egg PC vesicles.

The comparison between LDL_2 and egg PC vesicles is particularly direct, since both types of particle have a diameter of ~210 Å (1,20). The significantly larger linewidths of 18 and 155 Hz measured for C^2H_3 and $(C^2H_2)_n$ respectively of palmitic-d_{31} acid in LDL_2 (Fig. 1a), as opposed to 9 and 70 Hz for the corresponding resonances in egg PC vesicles (Fig. 1c), clearly imply that acyl chain ordering is much higher in the surface monolayer of LDL_2 than in the egg PC bilayer. Linewidths of 8 and 90 Hz for C^2H_3 and $(C^2H_2)_n$ respectively of palmitic-d_{31} acid in the smaller HDL_2 particle (Fig. 1b), diameter ~90°Å (1), similarly indicate that order is again considerably higher in the surface monolayer of the lipoprotein than in the phospholipid model bilayer. This latter conclusion is in agreement with ESR work (11). The order parameters calculated from the C^2H_3 linewidths are $S_{CD} \approx 0.021$, 0.049 and 0.015 in LDL_2, HDL_2 and egg PC respectively. Calculation of order parameters from the $(C^2H_2)_n$ line was not performed because of the composite nature of the signal. It should be stressed, moreover, that the width at half height of this signal only reflects the behaviour of the more disordered methylene segments near the terminal methyl group of the fatty acid chain. These segments give rise to narrow resonances and so dominate the height of the $(C^2H_2)_n$ line.

Possible reasons for the higher order in the surface monolayer of LDL_2 and HDL_2 include the presence of cholesterol, which is known to increase order in egg PC bilayers (23); motional constraints imposed by partial interdigitation of molecules at the interface between amphiphilic monolayer and apolar

core; and lipid-protein interactions. In the case of HDL_2, high curvature of the monolayer may also cause tighter packing.

The present study thus demonstrates that ^2H NMR spectra may be obtained from deuterated fatty acids incorporated into lipoproteins, and that information on molecular order within the surface monolayer may be derived from the data. From the ^2H spectra of incorporated palmitic-d_{31} acid, acyl chain ordering is shown to be greater in the amphiphilic monolayer of LDL_2 and HDL_2 than in the bilayer of phospholipid model membranes. Further insight into this interesting observation will result from more extensive experiments involving the incorporation of selectively deuterated fatty acids, whereupon the complete profile of molecular ordering can be determined.

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