Chain Dynamics of Selectively Deuterated Fatty Acids in High-Density Lipoproteins Studied by Deuterium NMR

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ABSTRACT: Deuterium order parameters have been determined for ≈5 mol % selectively deuterated palmitic acid incorporated into the outer monolayer of high-density lipoproteins (HDL_3). The values are $S_{CD} = 0.38$ for [2,2-$^2H_2$]palmitic acid, 0.38 for [4,4-$^2H_2$]palmitic acid, 0.37 for [5,5,6,6-$^2H_4$]palmitic acid, 0.23 for [11,11,12,12-$^2H_4$]palmitic acid, and 0.05 for [16,16,16-$^2H_3$]palmitic acid. Comparison of the acyl chain order parameters in HDL_3 with acyl chain order parameters determined recently [Parmar, Y. I., Wassall, S. R., & Cushley, R. J. (1984) *J. Am. Chem. Soc. 106*, 2434–2435] for ≈5 mol % deuterated palmitic acid in sonicated unilamellar vesicles, composed of the same ratio of phosphatidylcholine/sphingomyelin (85/15 w/w) found in HDL_3, shows that acyl chain order in the HDL_3 monolayer is approximately 3–5 times higher than in the vesicle bilayer. The acyl chain order in the lipoprotein monolayer is approximately 1.5–2 times higher than in the bilayer of phosphatidylcholine multilamellar dispersions. Deuterium longitudinal relaxation times have been measured for deuterated palmitic acid in HDL_3, and the values $T_1 \approx 16$ ms for C^2H_2 and 170 ms for C^2H_3 groups are a factor of more than 2 times smaller than found in phospholipid bilayers.

Deuterium nuclear magnetic resonance (^2H NMR)[1] is a powerful spectroscopic technique for studying molecular motion and conformation in biological systems. It has been applied extensively to the study of the dynamic behavior of phospholipids in model bilayers and in biological membranes (Davis, 1983), as well as to the investigation of polypeptide and protein conformation and motions (Gall et al., 1981; Jelinski et al., 1980). Due to the nuclear spin $I = 1$ of the deuterium nucleus, ^2H NMR spectra and longitudinal relaxation times are dominated by quadrupolar interactions, while dipolar interactions with neighboring nuclei are much less important and can generally be ignored. Thus, in most cases of biological interest there is a straightforward interpretation of the ^2H NMR spectrum in terms of orientational order and mobility.

Very recently, it has been shown that ^2H NMR is also an excellent technique for the study of molecular order and motions in serum lipoproteins. For instance, selectively deuterated cholesteryl esters located in the hydrophobic core of reconstituted HDL have been studied, and the suggestion was made that cholesteryl esters adopt an extended conformation in the HDL particle and that the ester acyl chain possesses significant mobility (Parmar et al., 1983). In another study, small amounts (≈5 mol %) of perdeuterated fatty acids were incorporated into serum lipoproteins, and Wassall et al. (1982) demonstrated that the surface monolayer of the lipoproteins can be studied by means of ^2H NMR. Detailed information about the dynamic structure of acyl chains in lipoproteins is not possible by using the perdeuterated lipids since the resonances, with their varied line widths, are found to overlap. Hence, in the present paper we report ^2H NMR line widths and longitudinal relaxation times of selectively deuterated fatty acids incorporated into HDL_3 and compare the results with those reported for acyl chains in phospholipid bilayers.

The human serum lipoproteins HDL_2 and HDL_3 are small, almost spherical particles composed of an outer monolayer consisting of phospholipid, protein, and cholesterol, which surrounds an inner core composed of cholesteryl esters, triglycerides, and, possibly, cholesterol. The particles differ in size, with HDL_2 and HDL_3 having diameters of 85–100 and 70–85 Å, respectively. HDL_2 is isolated in the density range $d = 1.063$–1.125 g·mL^{-1} while HDL_3 is isolated between $d = 1.125$ and $d = 1.21$ g·mL^{-1}. The respective density differences of the particles arise from different proportions of lipids and apoproteins.

The present ^2H NMR results will be discussed in terms of molecular order and motions in the surface monolayer of HDL_3. It has previously been established (Stockton et al., 1976; Pauls et al., 1983) that, at low concentrations, fatty acid probes faithfully reflect the order of phospholipid acyl chains.

Materials and Methods

[1-^{14}C]Palmitic acid was purchased from New England Nuclear, Boston, MA. Palmitic acid was purchased from Matheson Coleman & Bell. Deuterium depleted water was obtained from Sigma Chemical Co. [5,5,6,6-$^2H_4$]Palmitic acid and [11,11,12,12-$^2H_4$]palmitic acid were purchased from Merck Sharp & Dohme, Canada Ltd., [16,16,16-$^2H_3$]palmitic acid was obtained from Serdary Research Laboratories, London, Ontario, and [4,4-$^2H_2$]palmitic acid was a generous gift from Dr. A. P. Tulloch, Prairie Regional Laboratory, Saskatoon, Saskatchewan. The synthesis of [2,2-$^2H_2$]palmitic acid from palmitic acid was described previously (Gorrissen et al., 1980).

Preparation of High-Density Lipoprotein Containing Deuterated Fatty Acids. HDL_3 ($d = 1.125$–1.21 g·mL^{-1}) was isolated from fresh (<3 days old) human blood serum by ultracentrifugation (Havel et al., 1955). The amount of protein present in the HDL_3 solution was quantified according to the method of Lowry et al. (1951) using egg albumin as a standard. The concentrations of lipoprotein solutions were

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[1] Abbreviations: ^2H NMR, deuterium nuclear magnetic resonance; HDL, high-density lipoprotein.

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Table I: ^2H Longitudinal Relaxation Times (T_1), Line Widths (W), and Order Parameters (S_{CD}) for Selectively Deuterated Palmitic Acid in HDL_3 (Temperature ≈ 25–27 °C)

chain position	T_1 (ms)[a] at 38.8 MHz	T_1 (ms)[a] at 61.4 MHz	W (Hz)[a]	S_{CD}[b]
2	14		300	0.38 ± 0.07
4	14		300	0.38 ± 0.07
5 (6)	16	23	280	0.37 ± 0.07
11 (12)	18		120	0.23 ● 0.04
16	170	190	6	0.05 ± 0.01

[a] Uncertainty in T_1 and W is approximately ±10%. [b] Based on $\tau_c \approx 4.8 \times 10^{-8}$ s.

increased to levels suitable for NMR experiments (typically ≈ 12 mg of protein/mL) by using Amicon ultracentrifugation membrane cones (CF 25). Exchanges with deuterium-depleted water (typically five) were performed to reduce the residual ^2HOH NMR signal. Deuterated fatty acid was incorporated by incubating lipoprotein solutions with a thin film of the selectively deuterated palmitic 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 for approximately 1 h. 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 with radioactivity determined by using a Beckman LS-8000 scintillation counter with liquifluor scintillation cocktail.

Electron Microscopy. Lipoprotein particles, with and without incorporated fatty acid, were negatively stained with 2% ammonium molybdate, pH 8.0, placed on 200-mesh Formvar carbon-coated grids, and allowed to air-dry. The specimens were examined in a Philips 300 electron microscope operating at 80 kV.

Nuclear Magnetic Resonance. Deuterium NMR experiments were carried out at 38.8 MHz using a Nalorac 5.9 T superconducting magnet and home-built spectrometer. Data collection and Fourier transformation of the free induction decays were performed on a Nicolet BNC-12 computer. Experiments at 61.4 MHz were performed on a Bruker WM400 spectrometer. Longitudinal relaxation times T_1 were measured at both frequencies by the inversion–recovery method (Vold et al., 1968). The sample temperature was approximately 25–27 °C.

Phosphorus-31 NMR spectra were recorded, without proton decoupling, at 102.2 MHz on the home-built spectrometer.

Spectral parameters are given in the legends to the appropriate figures.

Results

Deuterium NMR spectra of selectively deuterated palmitic acid incorporated into HDL_3 are depicted in Figure 1. Single Lorentzian lines provide a good fit to all of these spectra. It is apparent that the widths at half-height W of the ^2H NMR resonances of Figure 1 depend on the position of the selective deuteration, the values measured being shown in Table I. Clearly, the line widths are highest and nearly constant between C_2 and C_6 of the acyl chain and then decrease progressively until reaching their minimum value at C_{16}. This behavior is similar to that observed for deuterated fatty acids incorporated into phospholipid unilamellar vesicles (Stockton et al., 1976).

Longitudinal relaxation times T_1 at 38.8 MHz for deuterated fatty acids in HDL_3 are shown in Table I. These relaxation times are short (≤18 ms) for all the C^2H_2 segments,

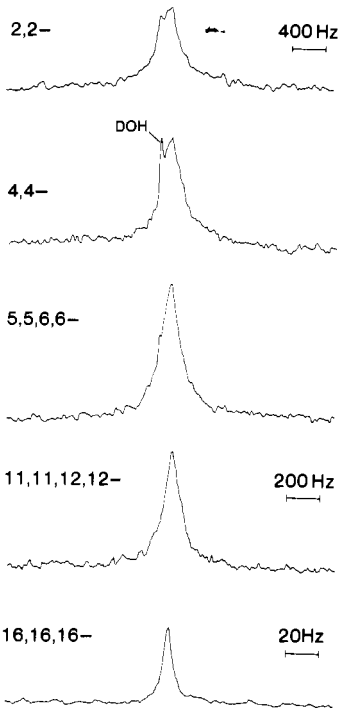

FIGURE 1: Deuterium NMR spectra for selectively deuterated palmitic acids in HDL_3. The position of the deuterium substitution on palmitic acid is given to the left of each spectrum. The plotted width of the top three spectra is the same. Spectral parameters: for 2,2-, sweep width = 5 kHz, plotted width = 4 kHz, pulse width = 19 μs (90° flip angle), number of aquisitions = 4×10^5, data points = 1024, delay before acquisition = 50 μs, and line broadening (LB) = 30 Hz. A $(180°-\tau-90°-T)_n$ sequence was used with τ = 110 ms and T = 210 ms in order to minimize interference due to residual ^2HOH. The FID was left shifted one data point to remove spectral abberations due to pulse breakthrough. For 4,4-, same as 2,2- except LB = 20 Hz. For 5,5,6,6-, same as 2,2- except LB = 20 Hz. For 11,11,12,12-, same as 2,2- except sweep width = 2.5 kHz, plotted width = 2 kHz, number of acquisitions = 1×10^5, τ = 120 ms, T = 320 ms, and LB = 8.0 Hz. For 16,16,16-, same as 2,2- except sweep width = 500 Hz, plotted width = 200 Hz, number of acquisitions = 8×10^3, T = 2.02 s, and LB = 0.5 Hz.

while at the terminal C^2H_3 segment a much longer T_1 of approximately 170 ms is measured. In addition, T_1's were measured at 61.4 MHz for [5,5,6,6-$^2H_4$]- and [16,16,16-$^2H_3$]palmitic acids in HDL_3. The results of the high field relaxation measurements are included in Table I.

In order to interpret the ^2H NMR line widths of deuterated probes incorporated into small structures such as lipoproteins and unilamellar vesicles, a knowledge of the size of these structures is necessary (Abragam, 1961a; Stockton et al., 1976). Therefore, we have obtained electron micrographs of HDL_3 containing fatty acid probes. An example is shown in Figure 2 which is the electron microscope photograph of HDL_3 particles containing [5,5,6,6-$^2H_4$]palmitic acid. From electron micrographs of HDL_3 containing [16,16,16-$^2H_3$]palmitic acid, and HDL_3 containing [5,5,6,6-$^2H_4$]palmitic acid, mean diameters of 7.4 ± 1.2 and 7.5 ± 1.4 nm, respectively, were measured. Native HDL_3 yielded a mean diameter of 7.7 ± 1.2 nm; hence, incorporation of fatty acid does not appreciably change the size of the HDL particle. In each case, approximately 200 particles were counted, and the results are in agreement with previously published work (Forte et al., 1968; Scanu, 1979).

^{31}P NMR spectra of HDL_3 containing palmitic acid are indistinguishable from those of native HDL_3, further indicating no significant size change occurs upon incorporation of fatty acid.

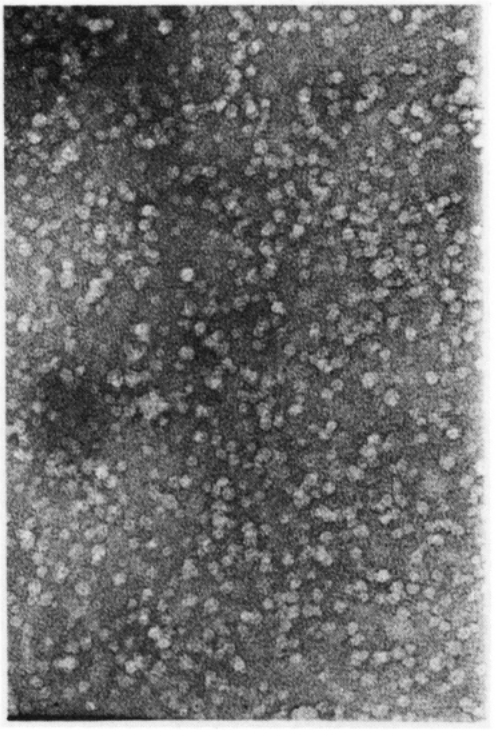

FIGURE 2: Electron micrograph of HDL_3 containing ≈5 mol % [5,5,6,6-$^2H_4$]palmitic acid. Magnification: 190 700×.

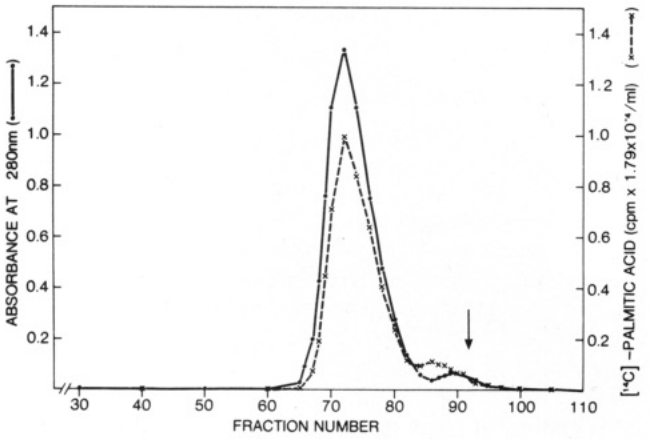

FIGURE 3: Elution profile of HDL_3 containing ≈5 mol % [5,5,6,6-$^2H_4$]palmitic acid on Sepharose 4B at 4 °C. Elution solvent was 0.15 M sodium chloride plus 0.02% sodium azide, and fractions were ≈5.2 mL each. (●) UV absorbance of HDL_3 apoprotein at 280 nm; (×) [1-^{14}C]palmitic acid (56 410 dpm). The arrow indicates the included volume position.

Another important question which must be answered is whether the palmitic acid actually incorporates into the lipoprotein particles. In Figure 3 we show the results of gel permeation chromatography at 4 °C of HDL_3 that had been incubated with [5,5,6,6-$^2H_4$]palmitic acid containing a trace of [1–^{14}C]palmitic acid. An aliquot of 1.2 mL of the labeled HDL_3 solution was applied to a Sepharose 4B column (2.6 × 100 cm) and elution performed, at 4 °C, with a solution composed of 0.15 M sodium chloride plus 0.02% sodium azide, pH 7.6. The void volume (141 mL) was determined with Blue Dextran; average molecular weight equals 2.0×10^6. Total column volume (478 mL) was measured from the bed height under a constant head of pressure. Approximately 85% of the carbon-14 counts in the original solution are eluted and are all found coincident with the HDL_3 particles as monitored by UV spectroscopy (OD at 280 nm). The small peak to the right of the HDL_3 peak in Figure 3 contains ≈4.5% (OD) or 6%

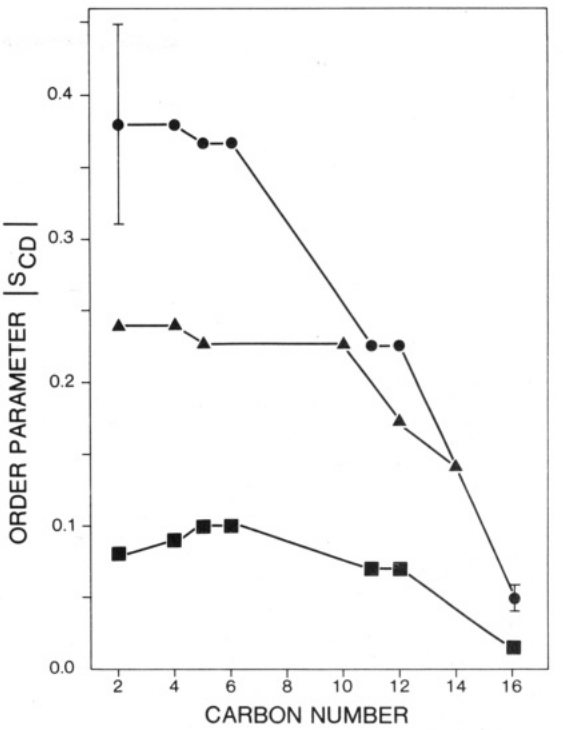

FIGURE 4: Plot of the absolute value of the order parameter $|S_{CD}|$ vs. acyl chain position for selectively deuterated palmitic acid incorporated into HDL_3 (●) and into egg phosphatidylcholine/sphingomyelin unilamellar vesicles (■) (Parmar et al., 1984). Temperature ≈ 25–27 °C. The S_{CD} values for selectively deuterated stearic acid in egg phosphatidylcholine multilamellar liposomes at 30 °C (▲) (Stockton et al., 1976) are also included.

(radioactivity) of the total eluted intensity. Part of the small peak intensity is no doubt due to "tailing" of the HDL_3 component plus possible contamination due to serum albumin. However, even assuming all of this peak is due to albumin, any fatty acid bound to so small an amount of this protein will contribute negligibly to the NMR signals in Figure 1.

We conclude the fatty acid is located in the surface monolayer of the lipoprotein particle on the basis of its amphiphilic nature. Earlier experiments on the ascorbate reduction of the ESR spin-label 12-doxylstearic acid (Keith et al., 1973) and energy-transfer experiments with fluorescent fatty acids (Schroeder et al., 1979; Sklar et al., 1980) support this conclusion.

From the ^2H NMR line shapes in Figure 1 order parameters S_{CD} for the C–^2H bonds were calculated (see Discussion). A plot of S_{CD} vs. position of substitution on the palmitic acid chain is shown in Figure 4.

Discussion

Small (≤5 mol %) amounts of selectively deuterated palmitic acids have been incorporated into HDL_3 particles. Electron microscopy studies and ^{31}P NMR of HDL_3 indicate that the size of the structures agrees well with that reported in the literature (Scanu, 1979; Glonek et al., 1974; Henderson et al., 1975) and the structures are not appreciably altered by the fatty acid incorporation. Our results agree with those of Wassall et al. (1982), who showed that the ^{31}P NMR spectra of HDL_2 containing the perdeuterated fatty acid [$^2H_{31}$]palmitic acid are identical with ^{31}P spectra for native HDL_2.

Fatty acids incorporated at low levels (≤5 mol %) are known to be reliable probes of the dynamic behavior of phospholipid acyl chains in model membranes (Stockton et al., 1976) and the surface monolayer of lipoproteins (Wassall et al., 1982). Moreover, in a recent, comprehensive study, Pauls et al. (1983)

found that order parameters of as much as 20 mol % deuterated palmitic acid incorporated into dipalmitoylphosphatidylcholine multilamellar liposomes was within 10% of those of the phospholipid itself. Further support of the reliability of fatty acid probes for the phospholipid acyl chain order in HDL is provided by comparing the incorporation of [11,11,12,12-$^2H_4$]palmitic acid and a phosphatidylcholine substituted at the *sn*-2 position with [11,11,12,12-$^2H_4$]palmitate into HDL_2 (Y. I. Parmar, unpublished results). Order parameters determined for the two systems were 0.23 and 0.21, respectively.

Fatty acids intercalated into planar model membranes undergo fast anisotropic motion which is axially symmetric about the normal to the bilayer surface. The degree of anisotropy for a carbon–deuterium bond may be characterized by the order parameter S_{CD}:

$$S_{CD} = \frac{1}{2}\langle 3\cos^2\theta - 1\rangle \quad (1)$$

where θ is the angle between the C–^{2}H bond and the symmetry axis for the molecular motions and the angular brackets denote a time average over all orientations of the molecule. It should be realized that S_{CD} depends not only on the extent of angular fluctuations undergone by a C–^{2}H bond but also on the mean orientation of the bond with respect to the bilayer normal (Seelig, 1977). The C–^{2}H order parameter may be described in more detail by

$$S_{CD} = S_{mol}S_{geom} \quad (2)$$

where S_{mol} is the molecular order parameter of the acyl chain segment (Seelig, 1977) and

$$S_{geom} = \frac{1}{2}(3\cos^2\beta - 1) \quad (3)$$

where β is the average angle the C–^{2}H bond makes with the instantaneous segmental chain orientation. In the case of fatty acids intercalated into a liquid-crystalline phospholipid bilayer, both C–^{2}H bonds in a selectively deuterated C^2H_2 segment are motionally equivalent and possess the same average orientation ($\beta = 90°$, $S_{geom} = -1/2$). Order parameter S_{CD} is then a direct measure of the orientational order (*Seelig*, 1977; Allegrini et al., 1983).

Particle tumbling in solution and lateral diffusion of deuterated fatty acids within the vesicle bilayer or lipoprotein surface monolayer result in a narrow ^{2}H NMR resonance. In the absence of any other motions, the width at half-height W of the Lorentzian line shape is given by (Abragam, 1961b)

$$\pi W = M_2\tau_e \quad (4)$$

where M_2 is the rigid lattice second moment and τ_e is the effective correlation time of the isotropic motions. The second moment is related to the static quadrupolar splitting $\Delta\nu_Q$ [$\Delta\nu_Q \approx 126$ kHz (Burnett & Müller, 1971)] by

$$M_2 = \frac{4}{5}\pi^2\Delta\nu_Q^2 \quad (5)$$

Anisotropic acyl chain reorientations within the vesicle bilayer or lipoprotein monolayer reduce the second moment from its rigid lattice value to a residual second moment M_{2r}. Under the assumption that the rate of the local molecular reorientations is much faster than the isotropic motions described by τ_e, the line width may then be expressed in the form

$$\pi W = M_{2r}\tau_e + \frac{1}{T_1} \quad (6)$$

where $1/T_1$ is the spin–lattice relaxation rate. Inspection of eq 6 reveals that the first term on the right-hand side is due to the effectively isotropic motions while the second term represents the effect of the faster local molecular motions where we have substituted the experimentally derivable term $1/T_1$ for $1/T_2$ (i.e., extreme narrowing conditions apply for the fast local motions). Thus, the possibility that slow anisotropic motions are present is not considered. Making the additional assumption that the molecular motions within a vesicle bilayer or lipoprotein monolayer are axially symmetric about the normal to the particle surface, i.e., the radial direction, we can write the following relationship:

$$M_{2r} = \frac{4}{5}\pi^2\Delta\nu_r^2 \quad (7)$$

where $\Delta\nu_r$ is the quadrupolar splitting that would be observed if the effectively isotropic motions were not present and

$$\Delta\nu_r = \frac{3}{4}\left(\frac{e^2qQ}{h}\right)|S_{CD}| \quad (8)$$

Substitution of eq 7 and 8 into eq 6 yields the equation (Stockton et al., 1976)

$$W = \frac{9\pi}{20}\left(\frac{e^2qQ}{h}\right)^2 S_{CD}^2\tau_e + \frac{1}{T_1} \quad (9)$$

The correlation time τ_e for the effectively isotropic motions depends upon the correlation times for particle tumbling τ_t and lateral diffusion τ_d according to

$$\tau_e^{-1} = \tau_t^{-1} + \tau_d^{-1} \quad (10)$$

The term τ_t is given by the Stokes–Einstein relationship

$$\tau_t = \frac{4\pi\eta R^3}{3kT} \quad (11)$$

where η is the solvent viscosity (0.8904 cP),[2] R is the radius of the particle, k is Boltzmann's constant, and T is the absolute temperature. The other term on the right-hand side of eq 10 is the lateral diffusion time τ_d given by

$$\tau_d = R^2/(6D) \quad (12)$$

where D is the lateral diffusion coefficient for molecules in the vesicle bilayer or lipoprotein outer monolayer. We have determined the diffusion coefficients of a number of lipids in highly curved particles by the NMR method of Cullis (1976). Using ^{2}H NMR spectroscopy, we find the value of $D = (1.5 \pm 0.4) \times 10^{-8}$ cm^2 s^{-1} for phospholipid in HDL (Y. I. Parmar, unpublished results). This is lower than the value of $D = (2.5 \pm 0.6) \times 10^{-8}$ cm^2 s^{-1} measured for phospholipid diffusion in egg phosphatidylcholine vesicles at 25 °C (S. R. Wassall, H. Gorrissen, and R. J. Cushley, unpublished results). In egg phosphatidylcholine vesicles we have determined $D = (7 \pm 1) \times 10^{-8}$ cm^2 s^{-1} for [$^2H_{31}$]palmitic acid (S. R. Wassall, H. Gorrissen, and R. J. Cushley, unpublished results). By analogy with the behavior of phospholipids that diffusion is lower in HDL than in phospholipid vesicles, we find that ignoring τ_d for palmitic acid in HDL_3 introduces an error of less than 10% in $\tau_c \approx \tau_t \approx 4.8 \times 10^{-8}$ s.

The radius, 3.75 ± 0.6 nm, determined from the electron microscopy measurements for the HDL_3 particle containing

[2] We have used solvent viscosity in our calculations; however, solution viscosity might also be used since the HDL_3 solutions are not highly dilute. For the [4,4-$^2H_2$]-, [5,5,6,6-$^2H_4$]-, and [11,11,12,12-$^2H_4$]palmitic acids in HDL_3, where we have measured the solution viscosities, the values of S_{CD} in Table I are only 0.05, 0.04, and 0.02 lower, respectively.

≈5 mol % selectively deuterated palmitic acid, was used in the calculation of τ_t. It might be argued that HDL_3 radii determined by electron microscopy might be smaller than the true hydrodynamic radii; however, in a recent paper (Anderson et al., 1977), a radius of 3.90 ± 0.15 nm was determined for HDL_3 using sedimentation. This value is very close to the value of 3.75 ± 0.6 nm we determined using electron microscopy.

From the ^2H NMR line widths absolute values of the order parameters for selectively deuterated palmitic acid in HDL_3 were determined by means of eq 9. The values are listed in Table I and plotted vs. chain position in Figure 4. Uncertainties in the order parameters are also included in Figure 4 and Table I.

The order parameters for the acyl chains in the HDL_3 monolayer (Table I) have the values $S_{CD} \approx 0.38$ for positions 2–6, drop to 0.23 for positions 11 and 12, and, finally, decrease to $S_{CD} = 0.05$ for the terminal C^2H_3 group of the palmitate chain. Brainard et al. (1980) have also reported high order in HDL_3 using spin-labeled stearic acid spin-labels. Those authors obtained ESR order parameters of ≈0.7 at 25 °C for both 5-doxyl- and 12-doxylstearates. The ESR order parameters can be equated with $S_{mol} = 2S_{CD}$ (eq 2). The acyl chain order in the HDL_3 monolayer may be compared with acyl chain order in phospholipid bilayers. Since the HDL_3 particle has a small diameter, leading to a high degree of curvature for the surface monolayer, the comparison should be made with phospholipid unilamellar vesicles where the surface is also highly curved. Very recently Parmar et al. (1984) determined the ^2H NMR spectra of selectively deuterated palmitic acid in 15% w/v unilamellar vesicles composed of 80/14/6 mol % egg phosphatidylcholine/sphingomyelin/palmitic acid (selectively deuterated) in deuterium-depleted water. The mixed sphingomyelin/phosphatidylcholine system was chosen since this is the composition of phospholipids found in the outer monolayer of HDL (Assmann et al., 1974; Glonek et al., 1974).

Deuterium line widths for unilamellar vesicles cannot be represented by eq 9 because, in contrast to the HDL_3 particles, the radii of vesicles vary considerably. Consequently, in order to determine the orientational order of acyl chains in the sphingomyelin/phosphatidylcholine vesicles, theoretical ^2H NMR line shapes were calculated by taking a superposition of spectral lines, statistically weighted in intensity according to the proportion of vesicles in each of a number of size categories. To determine the statistical weighting, electron micrographs were determined for two selectively deuterated palmitic acids in egg phosphatidylcholine/sphingomyelin vesicles. The order parameters obtained in this manner for the various segments of the palmitic acid chain are $S_{CD} = 0.08$ for [2,2-$^2H_2$]palmitic acid, 0.09 for [4,4-$^2H_2$]palmitic acid, 0.10 for [5,5,6,6-$^2H_4$]palmitic acid, 0.07 for [11,11,12,12-$^2H_4$]-palmitic acid, and 0.015 for [16,16,16-$^2H_3$]palmitic acid in egg phosphatidylcholine/sphingomyelin unilamellar vesicles (Parmar et al., 1984). These values are plotted in Figure 4 vs. chain position of deuteration for comparison with the HDL_3 data.

As can be seen in Figure 4, the shapes of the order parameter profiles for the fatty acid in HDL_3 and egg phosphatidylcholine/sphingomyelin unilamellar vesicles are similar. There is an "order plateau" of almost constant S_{CD} for the first few carbons in the chain followed by a progressive decrease in order toward the terminal C^2H_3 group. However, the S_{CD} values for fatty acid in the lipoprotein outer monolayer are considerably higher (≈3–5 times) in the plateau region than for the equivalent chain position in the vesicle bilayer. We should point out that, in a recent communication, Wassall et al. (1982) proposed that the orientational order of perdeuterated palmitic acid in HDL_2 is also substantially higher than in phospholipid bilayers.

The HDL_3 order parameters may also be compared with the order parameters determined for selectively deuterated stearic acid in egg phosphatidylcholine multilamellar dispersions where the surface curvature is much lower. Order parameters calculated fro stearic acid positions 2–10 are S_{CD} = 0.23–0.24, for position 12, $S_{CD} = 0.17$, and for the terminal $-C^2H_3$ group, $S_{CD} = 0.18$ (Stockton et al., 1976). These values are approximately 1.5–2 times lower than the values for palmitic acid in the HDL_3 monolayer and are included in Figure 4 for comparison.

Possible reasons for our observation that the orientational order of deuterated palmitic acid in HDL_3 is higher than in pure phospholipid bilayers include the known presence of cholesterol in the outer monolayer (Lund-Katz & Phillips, 1981), which has been shown to increase the order parameter of fatty acids in phospholipid membranes (Stockton & Smith, 1976). Another possibility is interdigitation of the acyl chains of the surface monolayer components with the cholesteryl ester and triglyceride chains and the rigid cholesteryl moiety of the cholesteryl esters in the core. The consequent spatial restriction imposed upon the phospholipid chain motion could result in higher order in the lipoprotein outer monolayer.

It may be argued that fatty acid in HDL_3 exhibits a higher orientational order due to the presence of protein in the outer layer. Using the number of protein and phospholipid molecules per particle reported by Verdery & Nichols (1974), we obtain a molar ratio of phospholipid/protein of 14/1 for HDL_3. At such a low phospholipid/protein ratio a substantial effect of the protein on the behavior of the phospholipid chains might be expected. However, ^2H NMR studies of protein/phospholipid model membrane systems (Paddy et al., 1981; Bienvenue et al., 1982) indicate that, at least on the ^2H NMR time scale, there is no appreciable acyl chain ordering effect due to the proteins. Clearly, further research is necessary to clarify the origin of the apparently greater orientational order in the lipoprotein particle.

The ^2H NMR longitudinal relaxation times measured for selectively deuterated palmitic acid in HDL_3 (Table I) exhibit a profile in which the relaxation time T_1 is approximately constant at ≈16 ± 2 ms for positions 2–12 and rises to ≈170 ms at the 16-position. The shape of this profile, with its plateau region of approximately constant values for the upper portion of the acyl chain, is qualitatively similar to that recorded for liquid-crystalline dipalmitoylphosphatidylcholine membranes (Brown et al., 1979). In contrast, the T_1 values in HDL_3 are, apart from the terminal methyl, a factor of more than 2 times smaller than in the phospholipid bilayer. Although the difference in resonant frequency ω_0 of the two sets of measurements (38.8 MHz for HDL_3 vs. 54.4 MHz for dipalmitoylphosphatidylcholine) may be a significant contributing factor, the shorter spin–lattice relaxation times suggest that the molecular motion(s) responsible is (are) more effective in the lipoprotein monolayer than in the model membrane and/or there are additional motion(s) in the case of HDL_3. ^{13}C NMR relaxation times for HDL that are shorter than for liposomes prepared from HDL lipids have been reported previously (Stoffel et al., 1974).

Table I illustrates that the T_1's measured at 25 °C for [5,5,6,6-$^2H_4$]palmitic and [16,16,16-$^2H_3$]palmitic acids in HDL_3 increase by ≈45 and 17%, respectively, between 38.8

and 61.4 MHz. ^{13}C NMR relaxation times which increase with resonant frequency have similarly been reported for HDL (Brainard et al., 1980). The trend is qualitatively in agreement with the behavior seen in liquid-crystalline phospholipid bilayers (Brown et al., 1983). On the basis of the current ^{2}H NMR data, obtained at only two resonant frequencies, it is not possible to decide upon the exact nature of the molecular motions determining the relaxation in the lipoprotein monolayer. The situation in HDL_3 is further complicated by the possibility that particle tumbling ($\tau_t \approx 4.8 \times 10^{-8}$ s) is itself fast enough to influence the longitudinal relaxation (Parmar et al., 1983).

Registry No. Palmitic acid, 57-10-3.

REFERENCES

Abragam, A. (1961a) *Principles of Nuclear Magnetism*, pp 427–441, Clarendon Press, Oxford.

Abragam, A. (1961b) *Principles of Nuclear Magnetism*, pp 424–427, Clarendon Press, Oxford.

Allegrini, P. R., Van Scharrenburg, G., DeHaas, G. H., & Seelig, J. (1983) *Biochim. Biophys. Acta 731*, 448–455.

Anderson, D. W., Nichols, A. V., Forte, T. M., & Lindgren, F. T. (1977) *Biochim. Biophys. Acta 493*, 55–68.

Assmann, G., Sokoloski, E. A., & Brewer, H. B. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 549–553.

Bienvenue, A., Bloom, M., Davis, J. H., & Devaux, P. F. (1982) *J. Biol. Chem. 257*, 3032–3038.

Brainard, J. R., Knapp, R. D., Patsch, J. R., Gotto, A. M., & Morrisett, (1980) *Ann. N.Y. Acad. Sci. 348*, 299–317.

Brown, M. F., Seelig, J., & Häberlen, U. (1979) *J. Chem. Phys. 70*, 5045–5053.

Brown, M. F., Ribeira, A. A., & Williams, G. D. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 4325–4329.

Burnett, L. J., & Müller, B. H. (1971) *J. Chem. Phys. 55*, 5829–5831.

Cullis, P. R. (1976) *FEBS Lett. 70*, 223–228.

Davis, J. H. (1983) *Biochim. Biophys. Acta 737*, 117–171.

Forte, G. M., Nichols, A. V., & Glaeser, R. M. (1968) *Chem. Phys. Lipids 2*, 396–398.

Gall, C. M., DiVerdi, J. A., & Opella, S. J. (1981) *J. Am. Chem. Soc. 103*, 5039–5043.

Glonek, T., Henderson, T. O., Kruski, A. W., & Scanu, A. M. (1974) *Biochim. Biophys. Acta 348*, 155–161.

Gorrissen, H., Tulloch, A. P., & Cushley, R. J. (1980) *Biochemistry 19*, 3422–3429.

Havel, R. J., Elders, H. A., & Bragdon, J. H. (1955) *J. Clin. Invest. 34*, 1345–1353.

Henderson, T. O., Kruski, A. M., Davis, L. G., Glonek, T., & Scanu, A. M. (1975) *Biochemistry 14*, 1915–1920.

Jelinski, L., Sullivan, C. E., & Torchia, D. A. (1980) *Nature (London) 248*, 531–534.

Keith, A. D., Melhorn, R. J., Freeman, N. K., & Nichols, A. V. (1983) *Chem. Phys. Lipids 10*, 223–236.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Lund-Katz, C., & Phillips, M. C. (1981) *Biochem. Biophys. Res. Commun. 100*, 1735–1742.

Paddy, M. R., Dahlquist, F. W., Davis, J. H., & Bloom, M. (1981) *Biochemistry 20*, 3152–3162.

Parmar, Y. I., Gorrissen, H., Wassall, S. R., & Cushley, R. J. (1983) *J. Biol. Chem. 258*, 2000–2004.

Parmar, Y. I., Wassall, S. R., & Cushley, R. J. (1984) *J. Am. Chem. Soc. 106*, 2434–2435.

Pauls, K. P., MacKay, A. L., & Bloom, M. (1983) *Biochemistry 22*, 6101–6109.

Scanu, A. M. (1979) in *The Biochemistry of Atherosclerosis* (Scanu, A. M., Wissler, R. W., & Getz, G. S., Eds.) pp 3–8, Marcel Dekker, New York.

Schroeder, F., Goh, E. H., & Heimberg, M. (1979) *J. Biol. Chem. 254*, 2456–2463.

Seelig, J. (1977) *Q. Rev. Biophys. 10*, 353–418.

Sklar, L. A., Doody, M. C., Gotto, A. M., & Pownall, H. J. (1980) *Biochemistry 19*, 1294–1301.

Stockton, G. W., & Smith, I. C. P. (1976) *Chem. Phys. Lipids 17*, 251–263.

Stockton, G. W., Polnaszek, C. F., Tulloch, A. P., Hasan, F., & Smith, I. C. P. (1976) *Biochemistry 15*, 954–966.

Stoffel, W., Zierenberg, O., Tunggal, B. D., & Schreiber, E. (1974) *Hoppe-Seyler's Z. Physiol. Chem. 355*, 1381–1390.

Verdery, R. B., & Nichols, A. V. (1974) *Biochem. Biophys. Res. Commun. 57*, 1271–1278.

Vold, R. L., Waugh, J. S., Klein, M. P., & Phelps, D. E. (1968) *J. Chem. Phys. 48*, 3831–3832.

Wassall, S. R., Treleaven, W. D., Parmar, Y. I., & Cushley, R. J. (1982) *Biochem. Biophys. Res. Commun. 107*, 429–434.