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^{13}C-NMR Spectroscopy of Human Serum High Density Lipoprotein Enriched with Labelled Phospholipids

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Summary: Native human serum high density lipoprotein (HDL) (d = 1.063–1.21g × cm^{-3}) was enriched with phosphatidylcholines labelled with ^{13}C in the polar head group ([N-$^{13}CH_3$]choline) and in the fatty acyl chains ([26-^{13}C]cholesterol) and its linoleic acid ester using the previously described exchange method (Stoffel et al. 1978). The properties of the HDL particles with the exchanged lipid classes were the same as those of the native particles (M_r, CD, fluorescence, lipid and apoprotein stoichiometry, electrophoretic mobility). The T_1-times were very similar to those obtained previously with recombined apolipoprotein-[^{13}C]lipid complexes and further support our proposals concerning lipid and apoprotein interactions in the HDL particle.

^{13}C-NMR-Spektroskopische Untersuchungen an mit markierten Phospholipiden angereichertem menschlichem Serum-High-Density-Lipoprotein

Zusammenfassung: Natives menschliches Serum-High-Density-Lipoprotein (HDL) (d = 1.063–1.21 g × cm^{-3}) wurde mit in der polaren Gruppe und in den Fettsäureketten ^{13}C-markierten Phosphatidylcholinen, [26-^{13}C]Cholesterin und dessen Linolsäureester mit der beschriebenen Austausch-Methode (Stoffel et al. 1978) angereichert. Die so erhaltenen HDL-Partikeln wiesen unverändert die Eigenschaften der nativen Partikeln auf (M_r, CD, Fluoreszenz, Lipid- und Apoprotein-Stöchiometrie, elektrophoretisches Verhalten). Es konnten exakte T_1-Zeiten gemessen werden, die mit den an Apolipoprotein-^{13}C-Lipid-Rekombinaten gemessenen in guter Übereinstimmung stehen und früher abgeleitete Modellvorstellungen unterstützen.

Key words: Native serum HDL, lipid exchange, ^{13}C-labelled phospholipids, spin-lattice relaxation time measurements.

Trivial name:
Polyenephosphatidylcholine = phosphatidylcholine from soy beans with more than 70% linoleoyl residues among its fatty acid residues.

Abbreviation:
HDL = high density lipoprotein.

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The recent development of a lipid exchange method which allows the substitution of the lipid classes present in native high density lipoproteins by a correspondingly labelled lipid species[1] induced the studies described in this communication. It is demonstrated that the mediation of the lipid exchange (phosphatidylcholine, cholesterol and cholesterol ester) by sodium cholate leaves the HDL particle unaltered as indicated by its component analysis, specific gravity, circular dichroism, fluorescence spectroscopy, agarose electrophoresis, immunodiffusion, molecular weight.

It appeared of considerable interest to compare the ^{13}C-lipid-apoprotein interactions monitored by the spin lattice relaxation time in these HDL particles with those previously obtained by different recombination experiments with apoproteins and lipids[2,3].

Materials and Methods

Polyenephosphatidyl-[*N*-$^{13}CH_3$]choline, 2-[14-^{13}C]-linoleoyl- and 2-[11-^{13}C]oleoyl-1-stearoyl-*sn*-glycero-3-phosphocholine have been synthesized in this laboratory by established procedures[4], [26-^{13}C]cholesterol by a method analogous to that for [26-^{14}C]cholesterol[5] and [26-^{13}C]cholesteryl linoleate by acylation of cholesterol with linoleoyl chloride in the presence of dimethylaminopyridine[4]. Sodium cholate was recrystallized from ethanol. Human serum HDL was prepared by the sedimentation-flotation method between densities 1.063 and 1.21 g × cm^{-3}. It proved to be homogeneous in agarose electrophoresis.

The details of the lipid exchange method have been described previously[1].

^{13}C-NMR spectroscopy: Proton-noise-decoupled ^{13}C-NMR spectra were obtained at 22.63 MHz with a Bruker WH-90 pulse spectrometer operating in the Fourier transform mode. An internal deuterium field frequency lock was used. The free induction decay signals were obtained following a 24 μs-pulse applying a 180°-*t*-90° pulse sequence[6] to obtain the spin-lattice relaxation measurement (T_1), where *t* is the delay time between the 180° and 90° pulses. The inversion recovery technique was used to determine T_1 according to $M_o - M_z = 2\,M_o \times \exp(t/T_1)$. M_o is the amplitude of the fully relaxed spectrum (free induction decay at $t = \infty$), M_z (given in arbitrary units) corresponds to the amplitude of a partially relaxed spectrum. Sample tubes of 10 mm diameter were used. Samples were carefully flushed with purified argon gas and a gas-tight piston just above the liquid surface protected against liquid-vapor interchange.

The Bruker ST 100/700 variable temperature controller adjusted the temperature with an accuracy of ± 1 °C.

Results and Discussion

^{13}C-labelled phosphatidylcholine enriched with the ^{13}C isotope in the polar head group (the choline group) or different positions of the fatty acyl chains (the hydrophobic part of the zwitterionic molecule) was incorporated into the HDL-particles. Also [26-^{13}C]cholesterol, [26-^{13}C]-cholesteryl linoleate and cholesteryl-[14-^{13}C]-linoleate were introduced by the exchange procedure.

Fig. 1 gives an example of the T_1-determination and demonstrates the favourable signal to noise ratio due to the enrichment and the exchange technique used in this studies. The T_1-data of all exchange experiments are summarized in the table. Comparing the data described here and obtained with HDL particles, the integrity of which remained intact during the lipid exchange procedure[1], with those derived from recombination studies using isolated apolipoproteins and ^{13}C-labelled phospholipids[2,3] we can draw the following conclusions:

1) The electron microscopic ultrastructure, stoichiometry, specific gravity, circular dichroism, intrinsic fluorescence and agarose electrophoresis of the HDL particles in which phosphatidylcholine, cholesterol and some cholesterol esters have been exchanged against ^{13}C-labelled species remained unaltered[1].

2) The spin-lattice relaxation time (T_1) of the choline methyl groups in the polar head group of lecithin remained constant. It is almost identical in the native HDL particle, the exchanged HDL particle, the cholate/phosphatidylcholine micelles and phosphatidylcholine liposomes. In the HDL particle, the choline group exhibits the same unrestricted rotation as the polar head groups arranged in the surfaces of liposomes and cholate/lecithin micelles.

3) The spin-lattice relaxation time in liposomes of ^{13}C-enriched position 14 of the linoleic acid chain in lecithin refers to a rather free mobility, which is also observed in the cholate micelles.

Table. Spin-lattice relaxation times (T_1-times in ms) of specifically ^{13}C-labelled phosphatidylcholines and cholesterol integrated into human high density lipoprotein (HDL) by the cholate-exchange method, and in native HDL, liposomes and cholate-lipid micelles.

PC = phosphatidylcholine, CE = cholesterol ester.

Lipid species	Chemical shift	T_1-time [ms]				
	[ppm]	Liposomes	Lipid-apo HDL	Cholate lipid micelles	Exchanged lipid in HDL	Native HDL
[*N*-^{13}CH$_3$]PC	54.0	430	462	453	452	450
18:0/[14-^{13}C]18:2-PC	27.0	700	438	632	268	218
18:0/[11-^{13}C]18:1-PC	27.0	210	147	303	182	218
[26-^{13}C]Cholesterol	23.0	760	–	766	560	–
[14-^{13}C]18:2-CE	–	–	–	–	209	–
18:2-[26-^{13}C]CE	–	–	–	–	485	–

However, in the presence of the apolipoproteins, T_1 decreases considerably. The fatty acyl chains of the exchanged phosphatidylcholine are more strongly immobilized in the HDL particle (268 ms) than those of the enriched lecithin reassociated with delipidated apolipoproteins (438 ms)*. The T_1 = 218 ms of the resonance at 27 ppm relative to tetramethylsilane is an average of all allylic carbon atoms of linoleic and oleic acid (C-14, C-11, C-8). Analogous changes in the mobility of the central aliphatic segments of oleic acid were measured in the HDL particle. Although they were less distinct than in the liposomal arrangement, they were very distinct from the freely mobile chains in cholate micelles. Compared with the aliphatic region of the liposomal bilayer, the oleoyl chains of lecithin in the cholate micelles apparently are very loosely packed with a high degree of disorder and little interaction. On the other hand, the disorder induced by the two *cis*-double bonds of linoleic acid in lecithin is comparable in liposomes and cholate micelles.

4) The advantage of our lipid exchange technique lies in the possibility of exchanging cholesterol and cholesterol esters to get some insight into their interaction by observing the relaxation behaviour of specifically ^{13}C-enriched positions. Whereas the side chain of [26-^{13}C]cholesterol is as freely mobile in phosphatidylcholine/cholesterol liposomes (molar ratio 4:1) as in cholate micelles (cholate/phosphatidylcholine/cholesterol, 11:6:1), the presence of HDL-apoproteins reduces the T_1-time very remarkably. The interaction of the aliphatic side chain with the apopolypeptide chains may lead to the observed restriction in mobility. Fig. 1 exemplifies the spin-lattice relaxation time measurement of [26-^{13}C]-cholesterol in cholate micelles (a) and in HDL particles (b).

5) The incorporation of cholesteryl linoleate labelled in the fatty acyl chain (C-14) and the sterol side chain (C-26) provided the first insight into the interactions of cholesterol esters. The dramatic reduction of the spin-lattice relaxation time of C-14 in the fatty acyl residue to T_1 = 209 ms can only be explained by a strong inhibition of conformational changes (*trans-gauche*, kink formations) of the chain due to the interaction between the fatty acyl chains of the phospholipid monolayer in the shell and/or very likely by interaction with the apolar surface of the amphipathic helices pointing to the interior of the particle. The immobilization of the side chain of the cholesterol nucleus could reflect the tight packing of the sterol nuclei in the central compartment of the HDL particle. Fig. 2 represents the set of partially relaxed Fourier-transformed spectra yielding the T_1-times of [26-^{13}C]chol-

* This may express a tighter packing of the native HDL particle as compared with those particles reconstituted from apoHDL and phosphatidylcholine only lipid species.

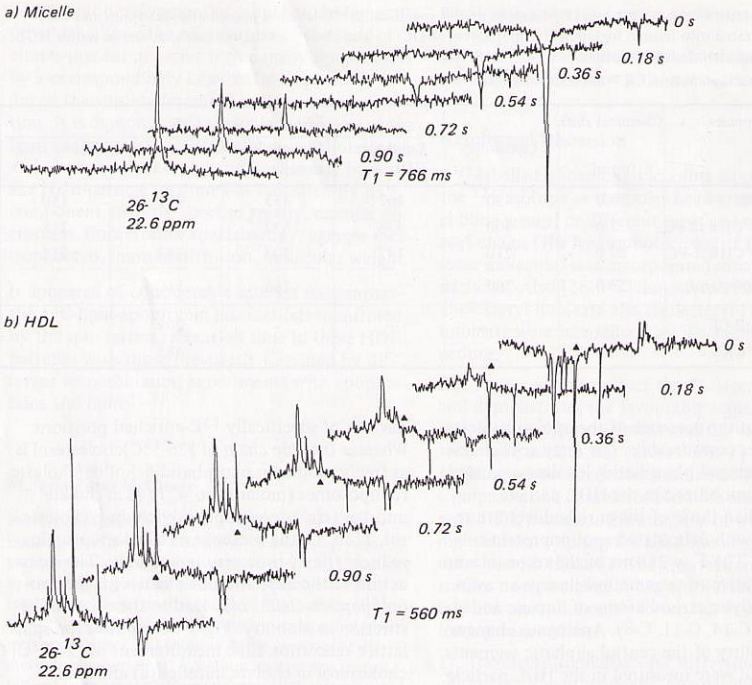

Fig. 1. ^{13}C-NMR spin-lattice relaxation time measurement of [26-^{13}C]cholesterol incorporated into a) cholate/phosphatidylcholine/cholesterol micelles (molar ratio 11:6:1) in 20% D_2O/80% H_2O (T_1 = 0.76 s) or b) high density lipoprotein particles, 20% D_2O, 0.01M Tris·HCl, pH 7.4 (T_1 = 0.56 s).
Numbers at the right side of the set of spectra represent the delay time t (s) in the 180°–t–90° pulse sequence.

esteryl [14-^{13}C]linoleate. The data presented in this communication are in full agreement with our previous results and conclusions on reconstituted HDL particles. They support the conception of the model proposed earlier[7]: Phospholipids and cholesterol form monolayer-like lipid patches of a limited surface area (~ 20% of the HDL particle surface), the rest of the HDL surface being covered by the expanded apolipoprotein chains. The hydrophilic side of the numerous α-helices in apolipoprotein A-I and A-II of this shell-like assembly is oriented towards the aqueous environment, whereas the apolar regions are prone to interactions with fatty acyl chains of phospholipids and cholesterolesters and the cholesterol side chain as well.

Chemical evidence for the surface alignment of the apoproteins will be presented in a forthcoming report[8].

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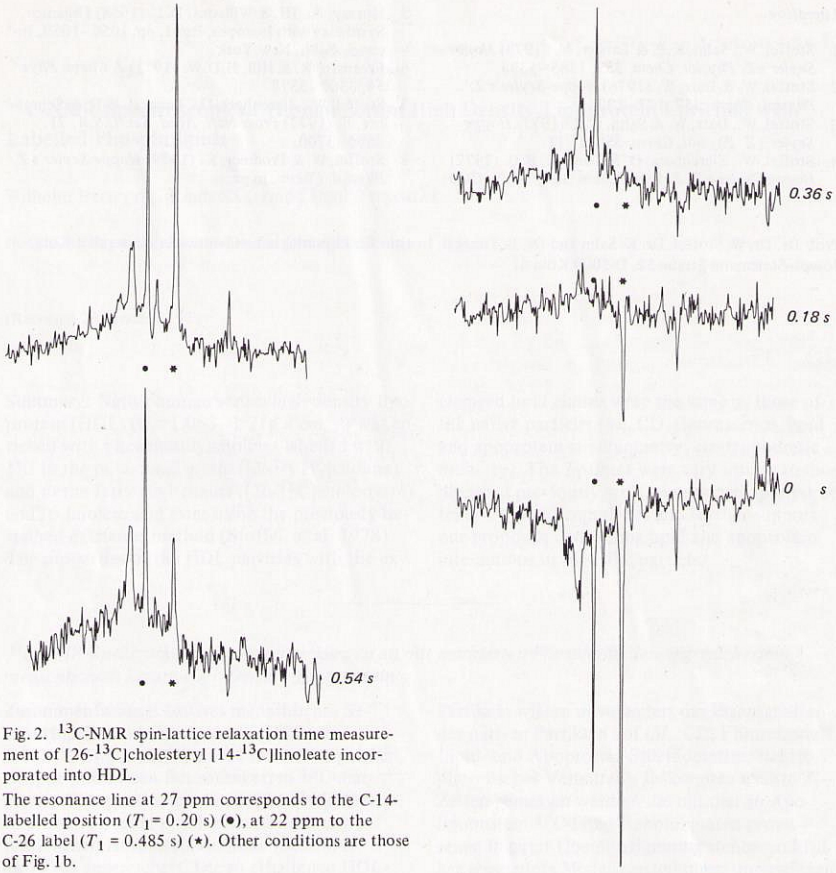

Fig. 2. ^{13}C-NMR spin-lattice relaxation time measurement of [26-^{13}C]cholesteryl [14-^{13}C]linoleate incorporated into HDL.

The resonance line at 27 ppm corresponds to the C-14-labelled position (T_1 = 0.20 s) (•), at 22 ppm to the C-26 label (T_1 = 0.485 s) (⋆). Other conditions are those of Fig. 1b.

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