LIPID–PROTEIN INTERACTION IN PORCINE HIGH-DENSITY (HDL_3) LIPOPROTEIN

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1. Introduction

The physico-chemical properties of serum lipoproteins have been the subject of extensive studies in the past [1,2]. Particularly, the question of the nature of the lipid-protein interaction has been tackled more recently by a wide range of chemical and physical techniques [2–11]. It is hoped that such studies will contribute to a better understanding of the principles underlying lipid-protein interactions in general. In this letter a ^{1}H-n.m.r. study of porcine high density lipoproteins (HDL_3, operationally defined by ultracentrifugal flotation at densities d=1.12–1.21 g/cm^3) is presented. The results obtained with native HDL_3 are compared with data obtained with a model system consisting of dimyristoyl lecithin and total apoprotein or Apo A-I [12] prepared from porcine HDL_3.

Our n.m.r. results indicate that the prime site of interaction between the phospholipid and the apoprotein is the glycerophosphorylcholine group. The effect of the protein on the hydrocarbon chains is confined to the first few CH_2 groups consistent with a peripheral location of the apoprotein. The interaction between the lipids and the protein is weak and probably non-specific since the segmental motion of the lipid is hardly affected and similar to that prevailing in lecithin bilayers. All phospholipid polar groups are mobile and oriented at the external surface of the lipoprotein particle readily accessible to ions in the aqueous bulk phase. The apoprotein of HDL_3 is rather immobilized when present in aqueous solvents and even more so when complexed with phospholipids. It is oriented at the lipid–H_2O interface such that its polypeptide chains are intercalated between the polar groups of lipids which are arranged in patches of monolayers at the surface of the particle. The n.m.r. data are consistent with a mosaic arrangement of phospholipids and polypeptide chains at the HDL_3 particle surface. The peptide chains are largely present as α-helices as evident from CD data [13] and our n.m.r. data suggest that the long axis of these helices are oriented approximately perpendicular to the axis of the hydrocarbon chains (cf. [9, 14]). The conclusions derived for the native porcine HDL_3 are corroborated by results obtained with a model system reconstituted from dimyristoyl lecithin and apoprotein.

Our results do not support the suggestion of a specific electrostatic interaction between the zwitterionic phosphorylcholine group of lecithin and suitably arranged pairs of acidic and basic amino acid side chains of the protein [14]. Such an interaction would be expected to cause changes in molecular motion as well as chemical shift of the $N(CH_3)_3$ group not borne out by our n.m.r. experiments.

Our findings are consistent with e.s.r. [17] and X-ray small-angle scattering results [7] suggesting that the interaction of the protein with the phospholipids is confined to a rather narrow surface layer (10–15 Å from X-ray scattering experiments) of the lipoprotein particle. The n.m.r. presented here do not warrant a discussion of the interaction and spatial arrangement of cholesterol and cholesteryl esters in HDL_3.

2. Materials and methods

The methods of preparation and chemical analysis of porcine HDL_3, of total apoprotein and purified Apo A-I have all been described before [1]. Egg lecithin and dimyristoyl phosphatidylcholine (lecithin) were purchased from Lipid Products, South Nutfield,

Surrey, UK and from Koch-Light Ltd. respectively and were pure by t.l.c. standards. Lipoproteins were reconstituted from lecithins and apoprotein as described previously [16]. For ^{1}H-n.m.r. experiments the native and reconstituted complexes were dialyzed exhaustively against ^2H_2O (> 99.8% ^{2}H) containing 0.15 M NaCl, 1 mM EDTA and 0.02% NaN_3 adjusted to a nominal pH 8.6. ^{1}H-n.m.r. spectra were obtained at 26°C on Bruker 270 MHz and at 100 MHz on a Varian XL-100-15 spectrometer both instruments operating in the Fourier transform mode.

3. Results and discussion

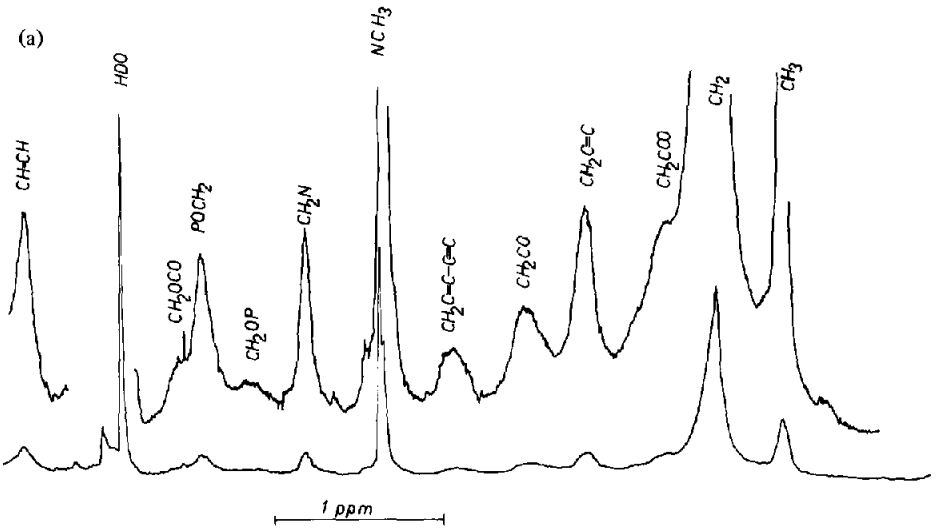
270 MHz ^{1}H-n.m.r. spectra of a sonicated egg lecithin dispersion and of native porcine HDL_3 are shown in fig.1. From a comparison of the two spectra the following points emerge.

(1) Apart from the broad, weak resonance from some aromatic amino acids the protein signals are too broad to contribute significantly to the high resolution spectrum of HDL_3, which is essentially a phospholipid spectrum, indicating that the protein in HDL_3 is fairly rigid. The ^{1}H spectrum of pure apoprotein in buffer is also characteristic of that of a protein being considerably immobilized [15]. Strong denaturing agents such as 8 M urea or trifluoroacetic acid are required to destroy the protein structure and to produce a spectrum similar to that computed from the amino acid composition. This suggests that the protein both in solution and complexed with lipids retains a high degree of secondary and/or tertairy structure. The n.m.r. data are consistent with CD results showing that 70% of the protein in HDL_3 is α-helical and delipidation decreases the α-helical content to about 50% [13].

(2) Chemical shifts measured relative to trimethylsilyl-propane sulphonate (TSS) as an internal standard are summarized in table 1. Changes in chemical shifts were only observed with the resonances of the glycerophosphorylcholine group and the first two CH_2 groups

Table 1

	H^1 resonances of lecithin	^{1}H-resonances of porcine HDL_3
CH_3 (terminal)	0.88 ± 0.01	0.88 ± 0.01
$(CH_2)_n$	1.27 ± 0.015	1.27 ± 0.01
HC = CH	5.30 ± 0.015	5.31 ± 0.01
CH_2-(C=C)$_2$	2.82 ± 0.02	2.79 ± 0.02
CH_2-C=C	2.02 ± 0.02	2.03 ± 0.02
CH_2-C-CO	1.57 ± 0.02	1.53 ± 0.02
CH_2-CO	2.38 ± 0.02	2.27 ± 0.02
CH_2OcO (glycerol)	4.41 ± 0.03	4.49 ± 0.02
CH_2OP (glycerol)	4.02 ± 0.02	4.10 ± 0.02
$POCH_2$ (choline)	4.28 ± 0.01	4.34 ± 0.01
CH_2N	3.69 ± 0.01	3.70 ± 0.01
$N(CH_3)_3$	3.25 ± 0.01	3.25 ± 0.01



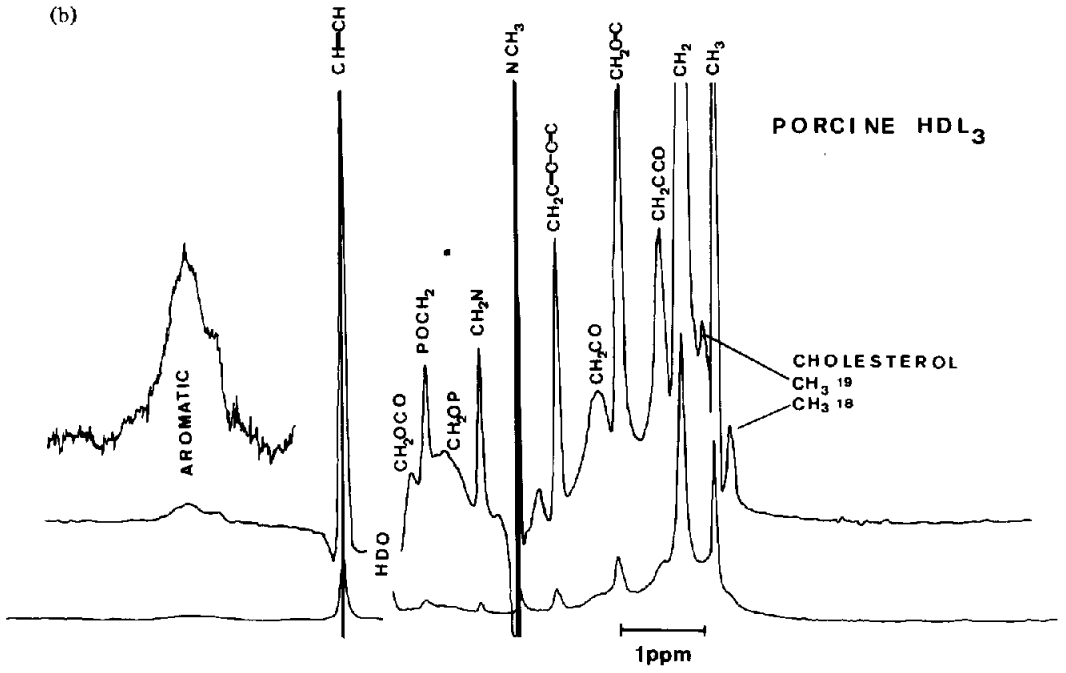


Fig.1. (a) 270 MHz proton n.m.r. spectrum of a sonicated egg lecithin dispersion (20 mg/ml ≃ 0.027 M) in ^2H_2O (nominal pH 5.5). (b) 270 MHz proton n.m.r. spectrum of native porcine high density lipoprotein HDL_3 (d = 1.12–1.21 g/cm^3) in ^2H_2O (concentration 100 mg/ml) containing 0.15 M NaCl, 1 mM EDTA and 0.02% NaN_3 adjusted to a nominal pH 8.6.

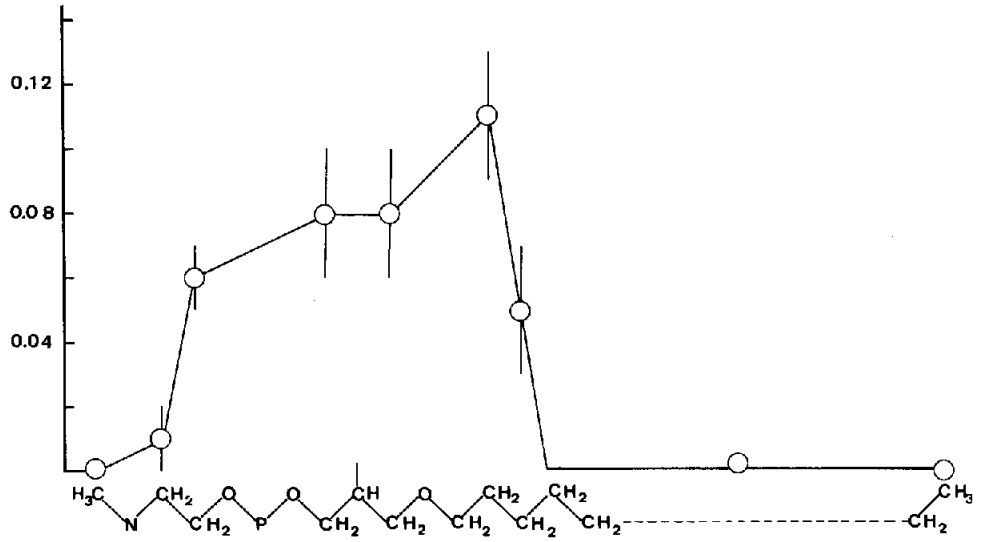


Fig.2. Changes in chemical shifts $\Delta\delta$ (ppm) of various proton resonances (shown on the abscissa) of native porcine high density lipoprotein (HDL_3). The shift changes are taken relative to the chemical shifts of the corresponding resonances observed with pure egg lecithin bilayers in ^2H_2O (see in fig.1 and table 1).

of the hydrocarbon chains. Apart from a slight shift change of the CH_2-$(C{=}C)_2$ resonance no changes were measured with the remaining hydrocarbon chain signals (table 1 and fig.2). Fig.2 shows that the maximum effect is in the region of the glycerol group and the first CH_2 group of the hydrocarbon chains. The effect decreased rapidly towards the terminal $N(CH_3)_3$ group and along the CH_2 groups of the hydrocarbon chains. Similar shift changes were observed in the ^1H spectra of lipoprotein complexes reconstituted from dimyristoyl lecithin and apoprotein (weight ratio = 2.5 : 1) [15].

(3) All phospholipids contribute to the ^1H high resolution spectra (fig.1) indicating that the interaction with the protein does not immobilize the lipid molecules. The line widths of the resonances from HDL_3 (fig.1b) are actually smaller than those for pure lecithin. This was also true for the spectrum of the reconstituted lipoprotein complex [15]. The Stokes radius of the HDL_3 particle determined by gel filtration on Sepharose 4-B was 56 Å. Consistent with that electron microscopy of negatively stained samples revealed spherical particles with an average radius of 55 Å. Line width calculations using the above dimensions and average values for the motional parameters of lecithin give values consistent with the experimental data. The segmental motion of the lecithin molecules in HDL_3 is therefore similar to that of lecithin in bilayers of sonicated vesicles indicating that the interaction between lipid and protein is weak and probably non-specific.

(4) The addition of paramagnetic K_3 $(Fe(CN)_6)$ to HDL_3 produced upfield shifts with the signals of the glycerophosphorylcholine group, but no chemical shift changes were observed with the resonances of the hydrocarbon chains. The shift changes of the $N(CN_3)_3$ and CH_2N resonances were easily measurable. About 100% of the peak intensity appeared to be shifted indicating that all choline containing phospholipids (~95% of the total phospholipid) are on the surface of the lipoprotein particle readily accessible to the bulky $(Fe(CN_6)^{3-}$ anion (diameter approx. 9 Å) in the aqueous phase. The magnitude of the shift changes were larger by a factor of 2–3 compared to those observed with pure lecithins (fig.3) and their order was $N(CH_3)_3 \gtrsim CH_2N > CH_2OP$ (choline) > CH_2OP (glycerol). The electron relaxation time of the ferricyanide anion is short in the n.m.r. time scale so that ferricyanide anions produce primarily shift

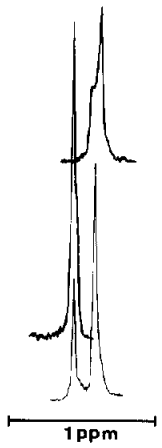


Fig.3. The proton signal of the lipid-$N(CH_3)_3$ groups of native porcine HDL_3 in the absence and presence of 0.05 M K_3 $(Fe(CN)_6)$. For comparison the upfield shift of the proton $N(CH_3)_3$ signal of egg lecithin induced by adding 0.1 M K_3 $(Fe(CN)_6)$ is also shown. Only 0.68 of the total $N(CH_3)_3$ signal intensity originating from the molecules located on the outer surface of the bilayer were shifted by the proximity of the paramagnetic $(Fe(CN)_6)^{3-}$.

changes rather than line broadening with signals from nuclei present in their close vicinity. The $N(CH_3)_3$ resonance of HDL_3 shifted in the presence of $(Fe(CN)_6)^{3-}$ was broadened (fig.3) and its line width was field dependent as reported before [11]. From its line shape and the field dependence it is clear that the broadening is due to a spread in chemical shifts. The observation of a single $N(CH_3)_3$ resonance in the absence of $(Fe(CN)_6)^{3-}$ (fig.3) the chemical shift of which is identical to that of pure lecithin (table 1) indicates that, on the n.m.r.-time scale, all $N(CH_3)_3$ groups in HDL_3 experience the same chemical environment. The latter must be very similar to that of pure lecithin. The broadening of the $N(CH_3)_3$ signal in the presence of $(Fe(CN)_6)^{3-}$ suggests that some of the $N(CH_3)_3$ groups are in a different chemical environment and exchange between those more slowly than about 0.1 S. The difference in chemical environment probably arises from some lipid molecules being present in the vicinity of the apoprotein. In such 'transition' zones the apoprotein may affect the accessibility of the $(Fe(CN)_6)^{3-}$ to the lipid polar groups and/or affect the binding of $(FE(CN)_6)^{3-}$ by a local accumulation of positive or negative charges in

the vicinity of the lipid. Both effects would produce changes in the amount of $(Fe(CN)_6)^{3-}$ bound to the lipid polar groups and thus in chemical shift $\Delta\delta$ according to the following equation:

$$\Delta\delta = (Fe(CN)_6)^{3-} \text{ bound}/ (Fe(CN)_6)^{3-} \text{ sat}\,(\Delta\delta \text{ max}).$$

where $\Delta\delta_{max}$ is the maximum shift and $(Fe(CN)_6)^{3-}_{sat}$ the amount of anion bound at saturation.

Similar observations were made when $K_3(Fe(CN)_6)$ was added to the reconstituted lipoprotein complex to be discussed in detail in [15].

The effects observed in the presence of $K_3(Fe(CN)_6)$ are consistent with a peripheral location of the protein and a mosaic surface of the HDL_3 particle consisting of apoprotein peptide chains intercalated between phospholipid polar groups. The shift experiments are not consistent with a significant proportion of apoprotein covering the lipid polar groups.

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