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STRUCTURE OF LIPOPROTEIN (a) STUDIED BY SPIN-LABELING

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SUMMARY

Lipoprotein (a) (Lp(a)), isolated from human serum was spin-labeled at protein and lipid sites with maleimide and stearic acid spin-labels (I(m/n)). Its structural properties were compared to the properties of low density lipoprotein (LDL).

Spin-labeled stearic acids suggest a similar overall structure for Lp(a) and LDL. Temperature dependence of the ESR spectra of spin-labeled proteins in Lp(a) indicates the presence of some constraint in the surface structure of Lp(a) that is not present in LDL. This constraint can be removed by digestion of Lp(a) with trypsin. Digested Lp(a) became more like LDL.

INTRODUCTION

Lipoprotein (a) which occurs as a distinct population of serum lipoproteins has an apolipoprotein characteristic of apolipoprotein B (apo B) in low density lipoprotein (LDL), and an additional factor, the Lp(a) specific antigen (1). Lp(a) is considerably larger than LDL (2) containing more protein and about six times more sialic acid than LDL (3). Nothing is known about its structure, although, because of its chemical composition one could speculate that it might be similar to LDL.

The spin-labeling technique is specially useful in obtaining information on the structural differences between Lp(a) and LDL. Spin-labels introduced into the

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lipoprotein particle reflect the orientational and dynamic properties of both proteins and lipids in the complex. In the spin-labeling study of LDL reported recently (4), a reversible thermotropie change in the surface structure of the particle was observed with both spin-labeled proteins and lipids. It was pointed out that the observed effect can be related to a thermotropic transition in the particle core (4,5,6).

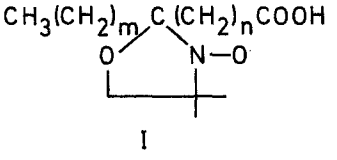
The constraints in the surface of the lipoprotein particle can influence the physical state of the lipids in the LDL interior, i.e. the structure of the core can be correlated with the organisation of the protein part (7). As Lp(a) and LDL share a distinct protein component (apo B) which is located on the surface of the particle (5,6,7,8), it is of interest to compare the structural properties of these lipoproteins by spin-labeling method under the same buffer condition. A buffer of rather high ionic strength 1.6 M NaBr was needed to obtain the stable lipoprotein (a) solutions.

EXPERIMENTAL PROCEDURES

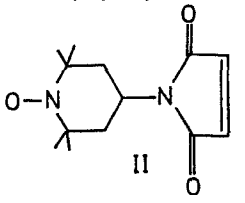
Preparation of the lipoproteins was performed by combined methods of precipitation, ultracentrifugation, and gel chromatography; analysis of different fractions were carried out as described earlier (1,2). Lp(a) was isolated from the density of 1.050-1.110 g/ml and LDL of 1.020-1.063 g/ml. To stabilize Lp(a) in its monomer form and to prevent intermolecular interactions leading to the formation of aggregates in 0.1 M Tris HCl (pH 7.6), the solutions additionally contained 1.6 M NaBr. Lipoprotein concentrations were not less than 2%. Lp(a) was concentrated under a nitrogen stream to the concentration of 6%, before it was finally purified by gel exclusion chromatography on Biogel A 5 M column (1.5x60)cm. Collection of the Lp(a) peak, recording the eluting material at 280nm, was done by hand to prevent dropping and dapping of the eluting solution into tubes, so that enough concentrated stable

solutions were received.Lp(a) was incubated with trypsin (protein-enzyme ratio 50 : 1) (SERVA) at about 22°C for two hours. All experiments were performed within three weeks of preparing the lipoproteins.

Lipoproteins were spin-labeled with stearic acid analogs (I) and 4-maleimido-2,2,6,6-tetramethylpiperidinooxyl (II) by the previously described methods (9,10).



I



II

Spin-labeled stearic acids (I(m/n)) (SYVA, Palo Alto, Calif.) were incorporated into lipoproteins from a film of spin-labels prepared at the bottom of epruvets by evaporation of methanol solvent under N_2 atmosphere. The buffer solution of lipoproteins was gently shaken over the film at 30°C for several minutes.The maleimide spin-label (II) (SYVA, Palo Alto, Calif.) was incubated with lipoproteins for at least 24 hours in the refrigerator. Unbound spin-labels were removed by exhaustive dialysis against buffer until ESR spectra remained unchanged upon subsequent changes of the buffer. If some aggregates appeared during prolonged dialysis, the material was centrifuged at 10 000 rev/min for 10 minutes.

ESR spectra were taken with the VARIAN E-3 spectrometer equipped with a Varian variable temperature control unit. Temperature was monitored by a thermocouple located above the sample at the top of the microwave cavity.

ESR spectra of spin-labeled fatty acids were analysed by order parameter S_3 according to the equation(11)

$$S_3 = \frac{T_{II}-T_I}{T_{zz}-T_{xx}} \cdot \frac{a_N}{a_N'}$$

where a_N' is correction for polarity

$$a_N' = \frac{1}{2}(T_{II} + 2T_I),$$

T_{II} and T_I are hyperfine splittings parallel and perpendicular to the normal of the phospholipid surface.

The maleimide spin-label binds covalently to ε-amino groups of lysines or sulphdryl groups of cysteins in the protein moiety. ESR spectra of spin-labeled proteins were analysed using the ratio of the lineheights of the signals for weakly (h_w) and tightly (h_t) immobilized spin-labels according to Gotto and coworkers (9) who pointed out that this parameter can be used to describe empirically lipid-protein interaction in lipoproteins (Fig. 1).

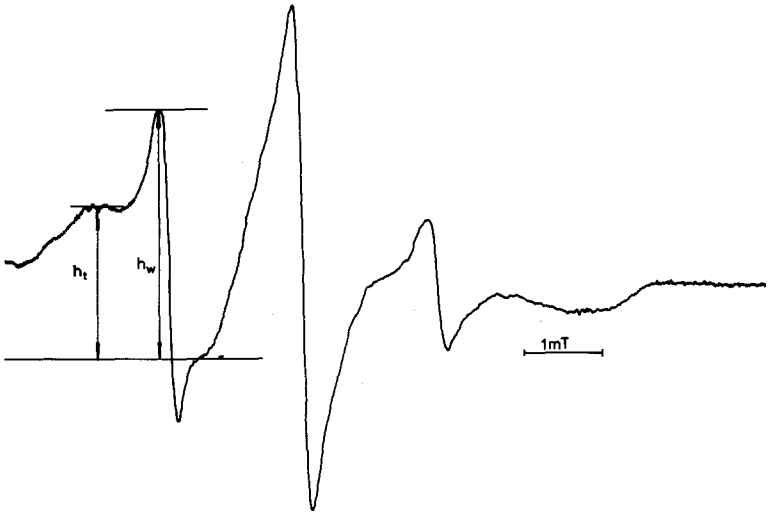


Fig. 1 ESR spectrum of maleimide spin-labeled proteins in Lp(a) taken at 20°C. The ratio of the lineheights for weakly (h_w) and tightly (h_t) immobilized binding sites of the spin-labels can be related to the lipid-protein interaction (10).

RESULTS AND DISCUSSION

Lipoprotein (a) is fairly stable in 1.6 M NaBr Tris at pH 7.6 and such high ionic strength may by itself induce changes in the surface structure of the lipoprotein particle. Therefore, we compared LDL and Lp(a) spin-labeled at both protein and lipid sites under these buffer conditions.

ESR spectra of spin-labeled proteins in Lp(a) and in LDL were of similar shape (Fig. 1). The lineheight ratio (h_w/h_t) in the spectra differed more between two different lipoprotein preparations than between Lp(a) and LDL of the same preparation.

When the ratio (h_w/h_t) was studied as a function of temperature with LDL, a strong and reversible increase in the lineheight ratio was observed at 25-30°C in accordance with previous finding under different buffer conditions(4).

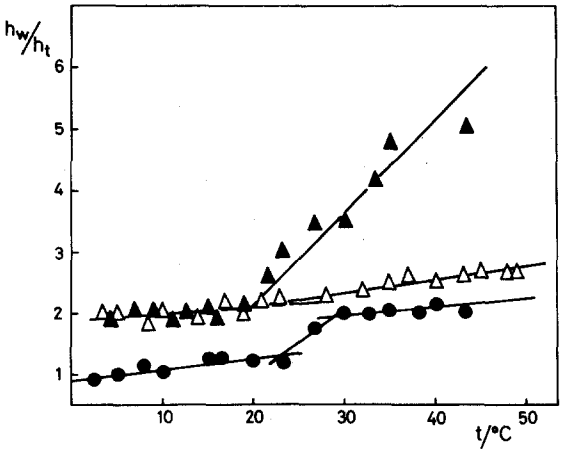


Fig. 2 Temperature dependence of the h_w/h_t ratio in the ESR spectra of maleimide spin-labeled Lp(a), LDL and trypsinated Lp(a). The ratio of the lineheights h_w/h_t is a linear function of temperature in native Lp(a) (open triangles). In LDL on the other hand, a reversible change in the h_w/h_t ratio was obtained in the temperature range of 25-32°C (closed circles) (4). In trypsin digested Lp(a) an reversible change in the lineheight ratio occured after the temperature 23°C (closed triangles).

In Lp(a) almost no change in the h_w/h_t ratio was observed with increasing temperature (Fig. 2 open triangles). A linear temperature dependence of the h_w/h_t ratio in Lp(a) may be caused by some constraint in the surface structure of Lp(a) that is not present in LDL. Then, limited tryptic digestion of Lp(a) may remove the constraint leading to a temperature dependent change in the h_w/h_t ratio. Fig. 2 (closed triangles) shows the temperature dependence of the lineheight ratio (h_w/h_t) in the ESR spectra of Lp(a) incubated previously with trypsin at room temperature for two hours. An irreversible increase in the lineheight ratio was observed above 23°C. The temperature dependence of the order parameter S_3 of spin-labeled stearic acids I(m/n) in Lp(a) and in trypsinated Lp(a) is represented in Fig. 3. An Arrenius plot ($\log S_3/T^{-1}$) similar to the

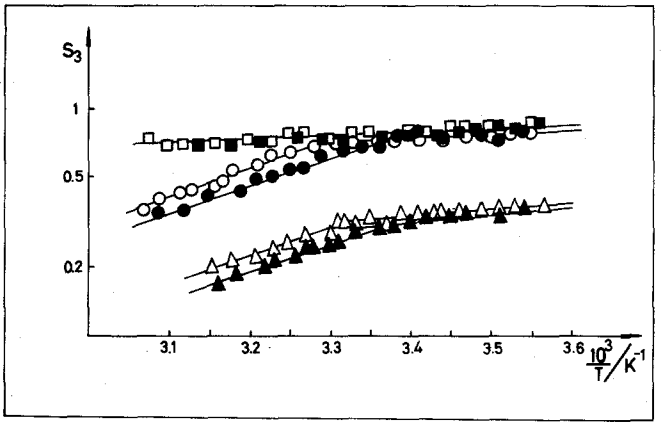


Fig. 3 Temperature dependence of the order parameter S_3 for spin-labeled stearic acid I(m/n) in Lp(a) (white marks) and in trypsinated Lp(a) (black marks) in 0.1 M Tris HCl, 1.6 M NaBr at pH 7.6 . I(12/3)-squares; I(5/10)-circles; I(1/14)-triangles.

one obtained for LDL was obtained for both native and trypsinated Lp(a). This result suggests that both native and trypsinated Lp(a) underwent change in the surface structure of the lipids similar to those observed with LDL (4). If this change was connected with a phase transition of the cholesteryl esters in the particle core a similar phase transition occured in both lipoproteins, Lp(a) and LDL.

Data with spin-labeled proteins in Lp(a) suggest presence of constraint that prevents the temperature dependent change in lipid-protein interactions. When the protein part of Lp(a) that is exposed on the surface of the Lp(a) particle was digested with trypsin, changes in lipid-protein interactions occured at temperatures similar to those found for LDL.

Therefore, although Lp(a) and LDL have a similar over-all structure, some additional constraint to the lipid-protein interaction must be present in native Lp(a).

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