EFFECT OF HYPERALPHALIPOPROTEINEMIA ON STRUCTURAL CHARACTERISTICS OF PLASMA LIPOPROTEINS AS REVEALED BY EPR SPIN PROBES

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Recent investigations have shown that high-density lipoproteins (HDLP) can remove cholesterol from cell membranes [10], as has been demonstrated in cultures of smooth muscle cells from the rat aorta and of human fibroblasts. These properties of HDLP evidently lie at the basis of the antiatherogenic properties of the class of lipoproteins (LP). Animals with a high HDLP concentration (rats) are known to be resistant to the development of experimental atherosclerosis [11]. In man elevation of the plasma HDLP concentration (hyperalphalipoproteinemia, HALP) is known to be associated with a lower incidence of ischemic heart disease (IHD) [6, 9]. However, we do not know to what this is due: whether the HDLP concentration only or, in addition, certain structural changes in LP promoting absorption of cholesterol by the lipoprotein particle.

The object of this investigation was to study, with the aid of electron paramagnetic resonance (EPR) of spin probes, the structural characteristics of HDLP in HALP.

EXPERIMENTAL METHOD

Blood plasma from 38 men aged 40-59 years, summoned for an epidemiologic study, served as the test material. The cholesterol concentration in the HDLP was determined after precipitation of lighter LP with heparin and manganous chloride [8]. Depending on the cholesterol level in HDLP, the subjects were divided into three groups on the sample quantile principle: subjects with a cholesterol concentration of under 34 mg% formed group 1 (hypoalphalipoproteinemia – 12 persons), between 34 and 72 mg% group 2 (12 subjects), and over 72 mg% group 3 (HALP, 14 subjects).

LP were isolated by preparative ultracentrifugation [11], the phospholipid concentration was determined by the method described previously [7], and cholesterol and triglycerides were estimated on the "Technicon AP" automatic analyzer. The nitroxyl derivative of stearic acid with a piperidine ring, coupled through an ester bond, was used as the spin probe*.

A solution of the probe in ethanol (1/100 of the volume of the preparation) was added to the LP preparations, diluted with physiological saline to a total lipid concentration of 10^{-3} M. The molar ratio probe/lipid was 1:100. EPR spectra were recorded on the E-4 radiospectrometer (from Varian, USA) under the following conditions: microwave power 10 mW, frequency of high-frequency (HF) modulation 100 kHz, amplitude of HF-modulation 1-2 G, intensity of magnetic field 3240 G, range of development of magnetic field 100 G, temperature 20°C, volume of sample in the ampul 70-80 μl.

*The spin probe used was synthesized and kindly presented by Candidate of Chemical Sciences R. I. Zhdanov.

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TABLE 1. Values of Velocity Constant (K) of Reaction of Reduction of Spin Probe by Ascorbic Acid in Lipoproteins in Hyper- and Hypoalphalipoproteinemia

Lipoproteins	K, 10^{-2} mmole^{-1}min^{-1}		
	hypo-α (n = 12)	normal (n = 12)	hyper-α (n = 14)
HDLP	2,8±0,2	4,6±0,2	7,0±0,2
VLDLP	1,6±0,1	1,8±0,2	4,1±0,1

Legend. Accuracy of determination of K was ± 0.05 mmole^{-1} min^{-1}.

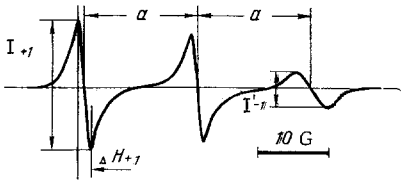

Fig. 1. EPR spectrum of spin-labeled HDLP from plasma of subjects with a normal cholesterol level.

The velocity constant (K) of the reaction of the probe with ascorbic acid (concentration 2 mM) was calculated as the tangent of the angle of slope of the straight line expressing dependence of the logarithm of the EPR signal amplitude on time.

EXPERIMENTAL RESULTS

A typical EPR spectrum of spin-labeled LP is illustrated in Fig. 1. The main parameters of the EPR spectrum of this probe, namely the correlation time of spin of the radical (τ) and hydrophobicity (h), for different classes of LP, were examined previously [1]. During the action of ascorbic acid, which reduces the amplitude of the EPR signal because of reduction of the paramagnetic nitroxyl group of the probe, on spin-labeled LP structural differences in the LP were found, depending on the cholesterol level of HDLP in the plasma (Table 1).

The velocity of the reaction of the probe with ascorbic acid was higher ($K = 70 \cdot 10^{-2}$ mmole^{-1}min^{-1}) for HDLP isolated from plasma of subjects with HALP than for average levels of cholesterol concentration of HDLP ($K = 4.6 \cdot 10^{-2}$ mmole^{-1}min^{-1}). The kinetics of this process is illustrated in Fig. 2.

Similar changes in the velocity of reduction of the probe were found in very low density LP (VLDLP): the decrease in amplitude of the EPR signal under the influence of ascorbic acid took place most rapidly in HALP ($K = 4.1 \cdot 10^{-2}$ mmole$^{-1} \cdot$ min^{-1}) and slowest when the cholesterol level of HDLP was low ($K = 1.6 \cdot 10^{-2}$ mmole$^{-1} \cdot$ min^{-1}). Values of the other parameters of the EPR spectrum of the probe in LP (τ and h) were independent of the cholesterol level of HDLP in the plasma.

Since this probe is localized in all probability in the inner hydrophobic "nucleus" of the LP [1], differences in the velocity of reduction of the probe reflect differences in permeability of the surface layer of LP, which consists of phospholipids and free cholesterol [11], for ascorbic acid.

One reason for this may be a change in structure of the lipid regions of the surface layer of LP as a result of an increase, observed previously [7] in the cholesterol/phospholipids ratio and the relative lecithin level. However, when other probes, which are evidently built into the surface layer of the LP particle, were used no difference was found in the degree of orderliness and mobility of the fatty acid chains of the phospholipids in LP from subjects with HALP [1, 2].

Differences in the permeability of the surface layer of LP may also be due to structural changes in the region of lipid–protein contact on account of differences in the quantity [7] and also, perhaps, the composition

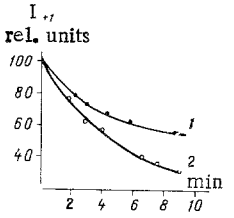

Fig. 2. Amplitude of EPR signal as a function of time for spin probes introduced into HDLP from plasma of subjects with hyper- (1) and hypoalphalipoproteinemia (2).

of the protein [5]. The ability of apoproteins to increase the permeability of the phospholipid layer for ascorbic acid (evidently because of the appearance of regions with a less dense molecular packing) has been demonstrated in model systems [15]. The presence of more loosely packed regions in HDLP from subjects with HALP, through which ascorbic acid can penetrate, may be a favorable factor for the cholesterol-acceptor properties of HDLP [10], and may also influence the reaction of lecithin-cholesterol acyltransferase (LCAT), an enzyme which catalyzes the transfer of a fatty acid from lecithin to cholesterol in HDLP [11]. The writers previously found an increase in the relative lecithin and free cholesterol level in HDLP from subjects with HALP, indirect evidence of lowering of LCAT activity.

Thd differences observed in the structure of VLDLP may be directly connected with the activity of another plasma lipolytic enzyme – lipoprotein lipase (LPL) [11], which influences accessibility of triglycerides localized in the "nucleus." This, in all probability, explains the decrease in activity of this enzyme in some patients with coronary atherosclerosis [4] with a low HDLP concentration [6], and also data [14] indicating an increase in LPL activity in HALP.

The problem of the molecular mechanism of structural differences thus revealed and of their effect on the properties of LP requires further study.

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