THERMOTROPIC STRUCTURAL CHANGES IN PLASMA LIPOPROTEINS IN ISCHEMIC HEART DISEASE

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Plasma lipoproteins (LP), one of the principal transport systems of lipids in the blood stream, play a definite role in the pathogenesis of such common diseases as atherosclerosis and its clinical manifestation, namely ischemic heart disease (IHD) [5]. As has recently been discovered, LP of patients with IHD differ significantly from LP of healthy persons not only in chemical composition [1], but also in their physical state [3, 9]. The structural organization of the lipoprotein particles must affect interaction between LP and vascular cell receptors, i.e., must ultimately affect the lipid concentration (including cholesterol) in the blood plasma and intima of the aorta.

In the present investigation temperature dependence of the degree of orderliness of the iminoxyl derivative of stearic acid in LP of different classes from healthy persons and patients with IHD was studied by the EPR method.

EXPERIMENTAL METHOD

Very low-density (VLDL), low-density (LDL), and high-density (subfractions HDL_2 and HDL_3) lipoproteins were isolated from blood plasma of healthy persons and patients with IHD by the method in [11]. The diagnosis of IHD was based on ECG findings, physical exercise tests, or a history of myocardial infarction. The preparations obtained were dialyzed for 18-20 h at 4°C against physiological saline containing 10 mM Tris-HCl buffer, pH 7.4. Lipids were extracted from LP by the method in [10], the solvent was evaporated on a rotary vaporizer, 0.5 ml of physiological saline containing 10 mM Tris-HCl buffer, pH 7.4, was added, and, by mechanical shaking, a homogeneous dispersion of lipids was obtained, frozen, and tested in the course of 3 days. Spin-labeled stearic acid [2-(3-carboxypropyl)-2-tridecyl-4,4-dimethyl-3-oxazolidinyloxyl] (from Serva, USA), introduced into the LP preparation in the form of a solution in ethanol up to a final concentration of 5×10^{-5} M (the ethanol concentration in the sample did not exceed 2 vol. %), was used as the spin label. EPR spectra were recorded on an E-4 radiospectrometer (Varian, USA), with thermostatic control of the samples [8]. The degree of immobilization of the probe in the sample was characterized by the parameter of orderliness S [2]. To characterize hydrophobicity of the microenvironment of the probe in LP, the parameter $h = (a_w - a)/(a_w - a_h)$ was used, in which a_w, a_h, and a are constants of isotropic superfine interaction for water, heptane, and the test system respectively. In the case of the probe which we used a_w = 1.7 mT, a_h = 1.4 mT [4, 6], $a=(T_{\parallel}+2T_{\perp})/3$, where $2T_{\parallel}$ and $2T_{\perp}$ stand for distances (in mT) between the outer and inner extrema of the EPR spectrum [2]. Parameters S and h were calculated for each donor, and subjected to statistical analysis on EMG-666/B computer (Hungary) programmed in accordance with recommendations in [7].

EXPERIMENTAL RESULTS

Typical graphs showing the relationship S for the spin probe in VLDL, LDL, HDL_2, and HDL_3 in Arrhenius coordinates, calculated from EPR spectra, are given in Fig. 1. For comparison, similar relations for plasma LP from healthy persons (control curves), which were described by the writers previously [8], are given in the same figure. The course of the curves for LP of patients with IHD as functions of temperature differ significantly from the control curves. In the case of VLDL, for instance, inflections appear in the region of 14 and 32°C

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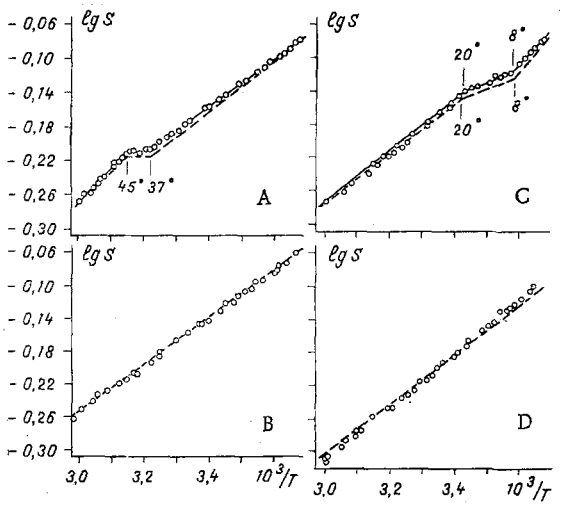

Fig. 1. Typical graphs of parameter of orderliness of spin label (S) in LP of healthy subjects and patients with IHD in Arrhenius coordinates. A) VLDL, B) LDL, C) HDL_2, D) HDL_3. Here and in Fig. 2, continuous lines denote patients with IHD, broken lines denote healthy blood donors.

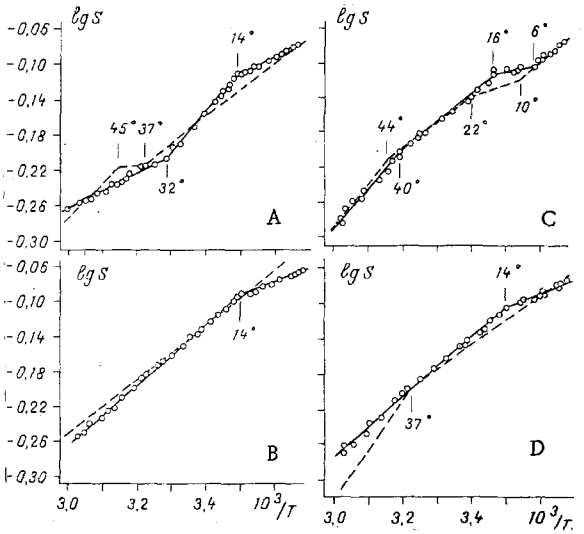

Fig. 2. Typical curves of orderliness of the spin probe in a suspension of lipids isolated from LP of healthy subjects and patients with IHD, as a function of temperature, plotted between Arrhenius coordinates. Lipids from: A) VLDL, B) LDL, C) HDL_2, D) HDL_3.

and disappear in the region of 37 and 45°C (Fig. 1A). For LDL of patients with IHD an inflection, absent in LDL of healthy subjects, appears at 14°C (Fig. 1B). In the case of HDL_2 inflections observed in HDL_2 of healthy subjects at 10, 22, and 44°C are shifted through 4-6°C toward lower temperatures (Fig. 1C). For HDL_3 an inflection was found at 14°C which was not present in the control. Meanwhile the inflection observed in HDL_3 of healthy subjects at 37°C was not recorded in HDL_3 of patients with IHD (Fig. 1D).

To shed light on the nature of the structural changes recorded in LP, the temperature dependence of orderliness of the spin probe in liposomes made from lipids of plasma LP from patients with IHD were studied. These curves are illustrated in Fig. 2. For comparison, control curves obtained with lipids from LP of healthy subjects are shown on the same figure. Clearly, dependence of the parameter S on temperature between Arrhenius coordinates for lipids of all classes of LP from patients with IHD agree satisfactorily with that for lipids from LP of healthy subjects, i.e., no significant differences are observed in the structural organization of lipids isolated from LP from patients and healthy persons. This indicates that the changes observed in the structure of LP in patients with IHD (Fig. 1) are initiated in the protein region or are due to a disturbance of protein–lipid interactions of their lipoprotein monolayer.

TABLE 1. Parameter of Hydrophobicity h (in relative units) of Spin Probe in LP and Lipids Isolated from Them, for Healthy Subjects and Patients with IHD (M ± m).

Experimental conditions	LP	Lipids
VLDL		
Control (7)	0,78±0,05	0,74±0,05
IHD (10)	0,75±0,06	0,75±0,05
LDL		
Control(7)	0,71±0,04	0,73±0,04
IHD (8)	0,71±0,05	0,70±0,03
HDL_2		
Control(7)	0,68±0,03*	0,76±0,02
IHD (8)	0,74±0,02*	0,77±0,04
HDL_3		
Control (7)	0,68±0,04*	0,75±0,05
IHD (7)	0,77±0,03*	0,76±0,03

Legend. *P < 0.001 compared with control. Measurements made at 37°C. Number of subjects tested shown in parentheses.

Results obtained by the writers previously indicated that the spin-labeled stearic acid used as spin probe is located in LP both in the region of the lipid monolayer and in the region of protein–lipid contact, and can "sense" the presence of apoproteins in the lipoprotein monolayer of LP [8]. This is manifested as an increase in the degree of polarity of the microenvironment of the probe located in LP, compared with lipids isolated from them. In this investigation the degree of polarity of the microenvironment of the probe was investigated in LP from patients with IHD. For this purpose, the parameter of hydrophobicity h [6] was calculated: Its values vary from 1 for the most hydrophobic environment to 0 for a polar environment, as the maximal measure of which water was chosen [6]. Values of the parameter h of the probe in LP from healthy subjects and from patients with IHD are given in Table 1. Clearly values of h, within the limits of experimental error, for VLDL and LDL isolated from the plasma of patients with IHD do not differ from those for VLDL and LDL of healthy subjects. Meanwhile, in the case of HDL_2 and HDL_3 from patients with IHD the values of parameter h were higher than the control values of HDL_2 and HDL_3. It must be emphasized that in the case of the lipid phase isolated from LP no such effect was observed (Table 1). It can be postulated on the basis of these data that in IHD, there is a disturbance of protein–lipid interactions in HDL_2 and HDL_3 at the depth of penetration of the nitroxyl group in their lipoprotein monolayer (about 0.8 nm), leading to an increase in lipophilicity of the microenvironment of the spin fragment of the probe. On the one hand this may be the result of a change in the chemical composition of LP in IHD [1]; on the other hand it may be due to weakening of protein–lipid contact, as a result of which apoproteins are "extruded" on to the surface of LP, thus reducing polarity in the region of the nitroxyl fragment of the probe.

The results thus indicate significant changes in the structure of LP of all classes in IHD. These changes are evidently connected with modification of the lipoprotein monolayer [of proteins and (or) protein–lipid interactions], which probably must affect interaction between LP and membrane-bound receptors of the blood vessel cells and, ultimately, the concentration of lipid (including cholesterol) in the plasma and vessel wall.

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EFFECT OF ISCHEMIA AND POSTISCHEMIC REPERFUSION ON FATTY ACID OXIDATION IN CARDIAC MITOCHONDRIA

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Postischemic reperfusion (PIR) of the myocardium has been shown [3, 10] to help to restore the viability of its cells and its contractile function. During PIR further ultrastructural damage to the myocardial cells and a decrease in the concentration of ATP and total adenine nucleotides, as well as a sharp increase in release of cytoplasmic enzymes [12], are also observed. The effect of PIR on mitochondrial function has received very little study. Kane et al. [6] found that PIR for 2 h after occlusion of the coronary artery for 15-180 min does not restore respiration of cardiac mitochondria (Mc) with glutamate and malate. Moreover, even more marked depression of respiration than during continuous occlusion was observed after ischemia for 3 h followed by PIR. According to our data, occlusion of the coronary artery for 1 h followed by PIR for 24 h led to much lower activity of fatty acid oxidation than in the control [2]. However, it was not clear whether reperfusion promotes normalization of functions of Mc or aggravates their injury.

To study this problem the effect of continuous ischemia and PIR on activity of individual stages of the fatty acid oxidation enzyme system was compared.

EXPERIMENTAL METHOD

Rabbits weighing 2.5-3 kg were used. Myocardial ischemia was induced by continuous and temporary (1 h) occlusion of the anterior descending branch of the left coronary artery [13]. Material for isolation of Mc was taken after 1 and 4 h in the case of continuous occlusion, and 3 h after occlusion for 1 h followed by PIR. The method of isolation of Mc was described previously [2]. Respiration of Mc was recorded polarographically at 37°C in incubation medium containing 0.15 M KCl and 0.005 M KH_2PO_4, pH 7.4. Activity of the β-oxidation system was studied in the same medium by the method described by Bremer and Davis [4]. Protein was determined by a modified biuret method [1]. Reagents: palmitoyl CoA (P-CoA) was from Sigma, USA, L-carnitine and L-palmitoyl carnitine (PCar) (from Japan) were generously provided by Dr. J. W. de Jong and the firm "Otpuska," to whom the authors are grateful.

EXPERIMENTAL RESULTS

Depending on the length of their carbon chain, fatty acids are oxidized by enzyme systems of different complexity. Comparative analysis of the velocities of oxidation of P-CoA, PCar, and acetate gives information about the state of oxidative enzymes specific for short and long carbon chains.

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