With effect from the first few hours after injury and during the next 7 days of investigation, burn trauma is thus characterized by profound and universal pathological changes in the system of cell membranes of the liver with their disorganization and with changes in activity of membrane-bound enzymes.

LITERATURE CITED

1. V. A. Burobin, in: Methods of Investigation of Some Enzymes in Clinical Medicine [in Russian], No. 6, Moscow (1967), p. 22.
2. T. L. Zaets, E. B. Burlakova, V. K. Sologub, et al., Byull. Éksp. Biol. Med., No. 7, 60 (1980).
3. A. A. Kiyashko, S. A. Smorshok, and M. P. Gariyan, in: Pathology of Membrane Permeability [in Russian], Moscow (1975), p. 30.
4. R. V. Merkur'eva, Vopr. Med. Khim., No. 2, 35 (1982).
5. M. I. Remizova and N. I. Kochetygov, Patol. Fiziol., No. 3, 63 (1976).
6. S. A. Smorshok, K. K. Volkov, and I. L. Tyurina, in: Abstracts of Proceedings of the 9th All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian], Minsk (1981), pp. 357-358.
7. M. Anson, J. Gen. Physiol., 22, 79 (1939).
8. H. Bergmeyer (ed.), Methoden der enzymatischen Analyse, Weinheim (1974).
9. C. De Duve, Exp. Cell Res., 7, Suppl., 169 (1959).
10. L. Heppel and R. Hilmor, Methods Enzymol., 2, 540 (1955).
11. D. Nevill, J. Biophys. Biochem. Cytol., 8, 413 (1980).
12. R. Pennington, Biochem. J., 80, 649 (1961).
13. H. Reichard, Acta Chir. Scand., 126, 45 (1963).
14. R. Silber, M. Conclyn, and G. Grusky, J. Clin. Invest., 56, 1324 (1975).
15. M. A. Swanson, Methods Enzymol., 2, 541 (1955).

SPATIAL ORGANIZATION OF LOW-DENSITY LIPOPROTEINS OF THE HUMAN AORTA (A FLUORESCENT PROBE STUDY)

G. E. Dobretsov, M. M. Spirin, A. S. Kuznetsov, and A. V. Popov

UDC 616.132-004.6-092: 616.132-02:577.122.856

KEY WORDS: aorta; low-density lipoproteins; structure; fluorescent probes.

Low-density lipoproteins (LDL) constitute the principal transport form of cholesterol in organs and tissues. Interaction between plasma LDL and the wall of the aorta is currently regarded as the key to the understanding of formation of the atherosclerotic focus [5]. Several properties of LDL of the aortic wall have been investigated [7-9, 11], but the spatial organization of these lipid—protein complexes has not yet been studied.

The aim of the investigation described below was to study the spatial structure of LDL isolated from the human aorta. The method used has only recently been developed and is based on recording the transfer of energy between fluorescent probes and from protein to probes [2, 3].

EXPERIMENTAL METHOD

LDL were isolated within the density range 1.006-1.063 g/cm^3 as described previously [6] from blood plasma obtained from healthy donors and from 14 men aged 39-52 years dying accidentally and free from atherosclerosis of the intima of the aorta. The LDL fraction of the aorta used in the study had the same elution volume on gel-filtration on Sepharose 4B as

Department of Biophysics, N. I. Pirogov Second Moscow Medical Institute. Department of Biochemistry, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Klimov.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 10, pp. 45-47, October, 1983. Original article submitted November 19, 1982.

0007-4888/83/9610-1392$07.50

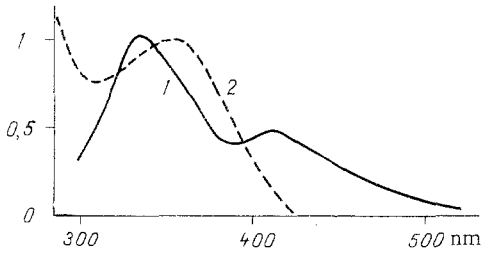

Fig. 1. Fluorescence spectrum of a-LP on excitation by light with wavelength of 286 nm (1) and excitation spectrum of fluorescence of a-LP recorded at 420 nm (2). Concentration of a-LP was 0.1 mg protein/ml. Abscissa, wavelength (in nm). Ordinate, intensity of fluorescence (in relative units). No correction introduced for spectral sensitivity of the fluorometer.

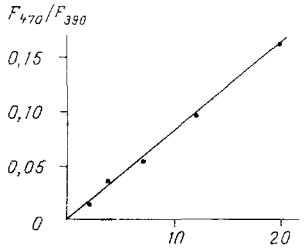

Fig. 2

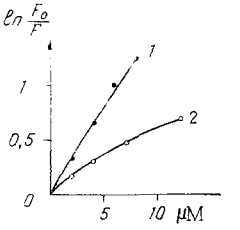

Fig. 3

Fig. 2. Degree of excimerization of pyrene as a functon of its concentration in a-LP. Concentration of a-LP was 0.1 mg protein/ml. F_{470} and F_{390}) intensity of fluorescence of pyrene at 470 and 390 nm respectively, due to pyrene excimers and monomers. Abscissa, concentration of pyrene (in μM).

Fig. 3. Quenching of fluorescence of protein of p-LP (1) and a-LP (2) as a result of transfer of energy to pyrene located in the lipid phase. Concentration of LDL 0.1 mg protein/ml. F_0 and F) intensity of fluorescence of protein at 345 nm (excitation — 286 nm) in the absence and presence of pyrene respectively. Abscissa, pyrene concentration (in μM).

plasma LDL. Henceforward the LDL obtained from the aortic wall and blood plasma will be designated a-LP and p-LP respectively.

Protein was determined by Lowry's method with the addition of sodium dodecylsulfate [4], and phospholipids were determined as phosphorus [12]. The phospholipid/protein ratio for a-LP and p-LP was 1.28 and 1.22 respectively

The fluorescent probes 1-anilinonaphthalene-8-sulfonate (ANS; from Serva, West Germany), pyrene (from Fluka, Switzerland), 4-dimethylaminochalcone (DMC) and 7-(2-anthryl)-heptanic acid (A6K) [4] were used. The A6K probe was generously provided by A. P. Kaplun (M. V. Lomonosov Moscow Institute of Fine Chemical Technology).

The technique of measurement of fluorescence was described previously [3]. In every case the LDL were in solution in 0.01 M Tris-HCl buffer, pH 7.4, and they were used within 3-5 days of isolation.

EXPERIMENTAL RESULTS

Both a-LP and p-LP had spontaneous fluorescence with a maximum near 340 nm (Fig. 1), due to tryptophan residues. However, a second maximum close to 420 nm also was found in the fluorescence spectrum of a-LP (Fig. 1). The excitation spectrum of this fluorescence had a maximum at 355 nm (Fig. 1, curve 2), which is characteristic of lipid peroxidation (LPO) products [10].

The quantum yield of fluorescence of ANS was 3.8 times lower in a-LP than in p-LP. We know that LPO products are quenchers of fluorescence of ANS [1]; quenching, moreover, must be the result of direct contact of the ANS molecule (located on the surface of the LDL particle) with LPO products.

The viscosity of lipoprotein lipids was estimated from the degree of excimerization of pyrene. The straight line on the graph showing the degree of excimerization of pyrene as a function of its concentration (Fig. 2) indicates that this probe was uniformly distributed in the lipid phase of a-LP and was not bound with protein. Similar results were obtained previously for p-LP [2]. The viscosity of the lipid phase at 20°C was about the same for both a-LP and p-LP.

The arrangement of protein in the a-LP particle was determined from the efficiency of energy transfer from tryptophan residues to the lipid probe pyrene. The results are given in Fig. 3. The mean quantum yield of tryptophan fluorescence in a-LP and p-LP was 0.05 and 0.13 respectively, and the radius of energy transfer was 2.65 and 3.10 nm. The average slope of the curve of quenching of tryptophan fluorescence by pyrene in a-LP was thus $\theta = 0.58 \pm 0.15$. According to the theory put forward previously [2], this means that tryptophan residues were at an average distance $\bar{x} = 0.5 \pm 0.5$ nm from the surface of the water—lipid boundary in a-LP on the side of the aqueous phase. Tryptophan residues in p-LP were buried in the depth of the lipid phase by 1.2 ± 0.2 nm, in agreement with data obtained previously for plasma LDL [2]. Curve 2 in Fig. 3 has much greater curvature than curve 1; consequently, different tryptophan residues in a-LP lie at different distances from the surface of the water—lipid phase boundary and dispersion of this distribution is higher than in p-LP. Protein on the surface of the lipoprotein particle in a-LP evidently lies in a thicker layer than in p-LP.

The total surface area of a-LP was measured by energy transfer from the A6K probe to the DMC probe (as described previously for plasma LDL [3]). It was found to be $(0.89 \pm 0.06) \cdot 10^6$ cm^2/liter when the concentration of phospholipids in a-LP was 0.1 g/liter. This concentration of phospholipids should occupy about $0.63 \cdot 10^6$ cm^2/liter (0.65 nm^2 for each phospholipid molecule). Cholesterol also can occupy not less than one-third of $0.63 \cdot 10^6$ cm^2/liter. Hence, we obtain that the fraction of the surface of a-LP covered by protein does not exceed 20%. In p-LP it was 30-40%, in agreement with the value obtained previously [2].

The results thus indicate that LDL in the aortic wall and in blood plasma differ somewhat. The lipid phase of a-LP contains LPO products which are not found in p-LP. The protein in a-LP is evidently removed from the lipid phase and covers a smaller part of the surface in a thicker layer than in p-LP.

LITERATURE CITED

1. Yu. A. Vladimirov and G. E. Dobretsov, Fluorescent Probes in the Investigation of Biological Membranes [in Russian], Moscow (1980), p. 243.
2. G. E. Dobretsov, M. M. Spirin, and I. M. Karmanskii, Biokhimiya, No. 4, 622 (1980).
3. G. E. Dobretsov, M. M. Spirin, and Kh. G. Ferster, Biokhimiya, No. 1, 47 (1982).
4. G. E. Dobretsov, M. M. Spirin, O. V. Chekrygin, et al., Bioorg. Khim., 7, No. 4, 606 (1981).
5. A. N. Klimov, in: Dyslipoproteinemias and Ischemic Heart Disease, ed. E. I. Chazov and A. N. Klimov [in Russian], Moscow (1980), pp. 22-24.
6. A. S. Kuznetsov, Vopr. Med. Khim., 25, 343 1979).
7. M. Hanig, I. R. Shinoff, and A. D. Lowy, Science, 124, 176 (1956).
8. H. F. Hoff, Artery, 6, 178 (1979).
9. W. Hollander, Exp. Mol. Pathol., 25, 106 (1976).
10. V. Malshet, A. Tappel, and V. Burbs, Lipids, 9, 328 (1974).
11. E. S. Smith and R. Slater, Atherosclerosis, 25, 71 (1970).
12. V. E. Vaskovsky, E. Y. Kosetsky, and I. M. Vasendin, J. Chromatogr., 114, 129 (1975).