BIOPHYSICS AND BIOCHEMISTRY

Changes in Surface Charge of Low-Density Lipoproteins during Oxidative Modification

R. K. Aidyraliev, O. A. Azizova, M. M. Mirrakhimov, and Yu. M. Lopukhin*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 8, pp. 164-166, August, 2001
Original article submitted April 17, 2001

The negative surface charge of low-density lipoproteins increased during their oxidative modification induced by autooxidation at 37°C. The degree of changes depended on the time of autooxidation: the surface charge remained practically unchanged after short-term oxidation (6-h incubation), but then progressively increased and after 24-h oxidation it 2-fold surpassed the initial level. Long-term incubation of low-density lipoproteins in the presence of EDTA inhibiting lipid peroxidation did not change their surface charge. These changes probably contribute to atherogenic activity of oxidized low-density lipoproteins. The degree of oxidative modification of low-density lipoproteins was precisely estimated using fluorescence probes.

Key Words: *low-density lipoproteins; oxidative modification; surface charge; fluorescence probes*

High blood content of low-density lipoproteins (LDL) is a risk factor for atherosclerosis. Modified lipoproteins possess considerable atherogenic properties [4,9]. Modified LDL are characterized by increased negative surface charge [8]. It was hypothesized that lipid peroxidation (LPO) contributes to modification and atherogenic activity of LDL [7]. However, little is known about the content of modified LDL in the blood of patients with dyslipoproteinemia. Fluorescence probes highly sensitive to fine physicochemical changes in LDL can be used for measurements of modified LDL content [2]. It is known that oxidative modification modulates both intrinsic fluorescence of LDL and fluorescence of probes incorporated in these particles. Previous studies showed that fluorescence of the anionic probe ANS decreases during oxidative modification [5]. Here we estimated changes in the surface charge of modified LDL using different charged fluorescence probes.

MATERIALS AND METHODS

Plasma LDL were isolated from healthy donors by ultracentrifugation [6] and dialyzed against a 500-fold volume of 10 mM Tris buffer (pH 7.4) containing 0.28 M sucrose for 15 h.

LDL were autoxidized by incubation at 37°C under constant mixing. The following fluorescence probes were used: 1-anilinonaphthalene-8-sulfonate (ANS, Serva), 4-(p-dimethylaminostyryl)-1-hexylpyridinium p-toluenesulfonate (DSP-6), and 4-(p-dimethylaminostyryl)-1-dodecylpyridinium p-toluenesulfonate (DSP-12) synthesized at the Institute of Organic Synthesis (Latvian Academy of Sciences). Fluorometric measurements were performed on an F-3000 spectrophoto-

National Center of Cardiology and Therapy, Kirghiz Ministry of Health, Bishkek; *Institute of Physicochemical Medicine, Russian Ministry of Health, Moscow. ***Address for correspondence:*** cardio@elcat.kg. Aidyraliev R. K.

0007-4888/01/1322-0751$25.00

meter (Hitachi) using a standard 1-cm quartz cuvette at a slit width of 5 nm. The intensity of ANS fluorescence (F) was measured at 470 nm (excitation wavelenght 370 nm). Fluorescence of DSP-6 and DSP-12 was measured at 550 nm (excitation 480 nm). The solution contained 20 μM ANS, 5 μM DSP-6, and 5 μM DSP-12. LDL concentration in the cuvette was 0.04 g phospholipid/liter. The measurements were performed in buffer solutions with high (F_1) or low ionic strength (F_2) containing 0.28 M sucrose, 10 mM Tris, and 2 mM EDTA (low ionic strength, pH 7.4) or 1 M NaCl, 10 mM Tris, and 2 mM EDTA (high ionic strength, pH 7.4).

The intensity of LPO was estimated by the concentration of malonic dialdehyde (MDA, μmol/g LDL phospholipids) [1]. Phospholipid concentration was measured as described elsewhere [10]. Horizontal electrophoresis was performed in 1% agarose gel (Chemapol, pH 8.6) [3].

RESULTS

In solutions with low ionic strength molecules of the solvent weakly shield the surface charge of lipoprotein particles. Under these conditions fluorescence probes provide more accurate measurements of the surface charge of LDL particles. After oxidation of LDL for 2, 4, and 6 h the intensity of ANS fluorescence in the solution with low ionic strength slightly decreased compared to nonoxidized LDL (by 11% over 6 h). After long-term oxidation (20 and 24 h) fluorescence intensity decreased by 3, 7, and 5 times, respectively (Table 1). In the solution with high ionic strength changes in the intensity of ANS fluorescence were less pronounced. The maximum decrease in this parameter was 44% of the control. These changes were observed during the increase in negative surface charge of LDL. Simultaneous measurements of F_1 and F_2 allow to estimate quantitative changes in LDL surface charge [2]. There was a linear relationship between the surface charge and $\ln(F_1/F_2)$. The surface charge remained practically unchanged during 6-h oxidation, but increased 1.95- and 2.18-fold after oxidation for 20 and 24 h, respectively.

DSP-6 and DSP-12 probes have similar composition, but differ in the structure of uncharged radicals. In DSP-6 this radical is less hydrophobic than in DSP-12 probe. Therefore, binding of DSP-6 to LDL particles more strictly depends on their surface charge (compared to DSP-12 probe). The measurements were performed at low ionic strength. The intensity of DSP-12 fluorescence remained practically unchanged after 6-h oxidation, while DSP-6 fluorescence increased by 20% (Table 1). After long-term oxidation (20 and 24 h) fluorescence of DSP-12 and DSP-6 probes sharply increased. These results attested to accumulation of negative surface charge on LDL particles.

TABLE 1. Fluorescence of Probes and Electrophoretic Mobility of LDL in Agarose Gel after Incubation at 37°C in the Absence (Numerator) or Presence of 0.01% EDTA (Denominator)

Parameter	Incubation, h						
	0	2	4	6	18	20	24
MDA content, μmol/g phospholipid	1.1 / 1.0	2.5 / 0.9	3.3 / 1.1	4.2 / 1.0	— / 1.1	10.1 / —	12.0 / —
ANS							
F_1	1.0 / 1.0	0.94 / 1.0	0.90 / 1.08	0.86 / 1.07	— / 1.03	0.53 / —	0.44 / —
F_2	1.0 / 1.0	0.98 / 1.05	0.94 / 1.03	0.89 / 0.96	— / 1.0	0.27 / —	0.195 / —
$\ln(F_1/F_2)$	1.0 / 0.98	0.94 / 1.0	0.93 / 1.0	0.96 / 1.01	— / 1.0	1.95 / —	2.18 / —
DSP-6	1.0 / 1.0	1.05 / 1.0	1.14 / 1.02	1.20 / 1.0	— / 1.0	6.42 / —	8.8 / —
DSP-12	1.0 / 1.0	1.06 / 1.0	1.04 / 1.01	1.03 / 1.0	— / 1.0	2.52 / —	2.97 / —
Electrophoretic mobility	1.0 / 1.0	1.05 / 0.98	1.07 / 1.0	1.09 / 1.02	— / 1.03	1.67 / —	2.0 / —

Note. Fluorescence and electrophoretic mobility are standardized by these parameters in nonoxidized LDL.

Electrophoretic mobility of LDL oxidized for 2, 4, and 6 h changed insignificantly, but increased by 1.67 and 2 times after oxidation for 20 and 24 h, respectively (similarly to experiments with ANS probe, Table 1).

MDA concentration, fluorescence of probes, and electrophoretic mobility of LDL remained practically unchanged after incubation of LDL in the presence of 0.01% EDTA (Table 1). The data indicate that changes in LDL are associated with LPO.

Our experiments with fluorescence probes indicate that the negative surface charge of LDL increases during oxidative modification. The degree of changes depends on the intensity of LPO. Autoxidation at 37°C for 24 h leads to a 2-fold increase in the surface charge. Therefore, the use of fluorescence probes makes it possible to precisely evaluate oxidative changes in LDL.

REFERENCES

1. V. B. Gavrilov, A. R. Gavrilova, and A. M. Mazhul', *Vopr. Med. Khimii*, **33**, No. 1, 118-122 (1987).
2. G. E. Dobretsov, *Fluorescence Probes in Studies of Cells, Membranes, and Lipoproteins* [in Russian], Moscow (1989), p. 157.
3. L. A. Osterman, *Methods for Protein and Nucleic Acid Analyses. Electrophoresis and Ultracentrifugation* [in Russian], Moscow (1981).
4. V. V. Tertov, *Angiol. Sosud. Khir*., **5**, Supplement, 218-240 (1999).
5. V. E. Formazyuk, Yu. G. Osis, A. I. Deev, *et al*., *Biokhimiya*, **48**, No. 2, 331-338 (1983).
6. R. Havel, H. Eder, and J. Bragdon, *J. Clin. Invest*., **34**, 1345-1353 (1955).
7. H. P. Steinbrecher, S. Parthasarathy, D. S. Leake, *et al*., *Proc. Natl. Acad. Sci. USA*, **81**, 3883-3887 (1984).
8. V. V. Tertov, G. Bittolo-Bon, I. A. Sobenin, *et al*., *Exp. Mol. Pathol*., **62**, 166-172 (1995).
9. D. Tribble, *Curr. Opin. Lipidol*., **6**, 196-208 (1995).
10. V. Vaskovsky, E. Kostetsky, and I. Vasendin, *J. Chromatogr*., **114**, 129-141 (1975).