Low Ionic Strength Promotes Association of Circulating Modified LDL in Human Blood

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The resistance to association of circulating multiply-modified low-density lipoproteins (LDL) isolated from human blood and characterized by a decreased content of sialic acids in comparison with native LDL was studied by analysing light transmission fluctuations. LDL association was stimulated by decreasing environmental ionic strength. It is established that circulating modified LDL are less resistant to association than native LDL. Association of LDL in a medium with low ionic strength was irreversible. Probably, increased capacity to irreversible association determines the atherogenic properties of circulating modified LDL subfraction.

Key Words: *LDL; modified LDL; lipoprotein association; lipoprotein aggregation; atherosclerosis*

At the early stages, atherosclerosis is characterized by accumulation of intracellular lipids in the vascular wall. These lipids are delivered by circulating low-density lipoproteins (LDL) [2,6]. Recently, so-called modified LDL capable of inducing intensive accumulation of lipids in cultured human smooth muscle cells and macrophages were almost simultaneously identified in several laboratories [4,5, 15]. The contents of sialic acid, cholesterol esters, phospholipids, antioxidants in these LDL were decreased, but the content of lysophospholipids was increased. In comparison with native LDL (nLDL), these particles were characterized by high specific density, greater negative surface charge, modified NH_2-groups in the protein, and high oxidation and aggregation capacity [3, 13]. Similarly to most LDL modified in different ways *in vitro*, cmLDL have two common features: they stimulate lipidosis at the cellular level and are characterized by increased association capacity [7-9,13-15]. Moreover, many studies showed that accumulation of lipids in cells increases with increasing the mean size of LDL particles [13-15]. This fact suggests that changes in physical and chemical properties of cmLDL *in vivo* can impair their resistance to association. cmLDL associates are more efficiently captured by intimal cells and stimulate the development of the early stages of atherosclerotic degeneration of the vessels.

Resistance of the disperse systems in water medium is mainly determined by solvation shell, whose stability depends on ionic strength of the medium [1]. Our aim was to examine the effect of decreased ionic strength of the medium on the resistance of nLDL and cmLDL to association and to assess reversibility of these changes.

MATERIALS AND METHODS

The total fraction of LDL was isolated by two-step ultracentrifugation in a NaBr density gradient [10].

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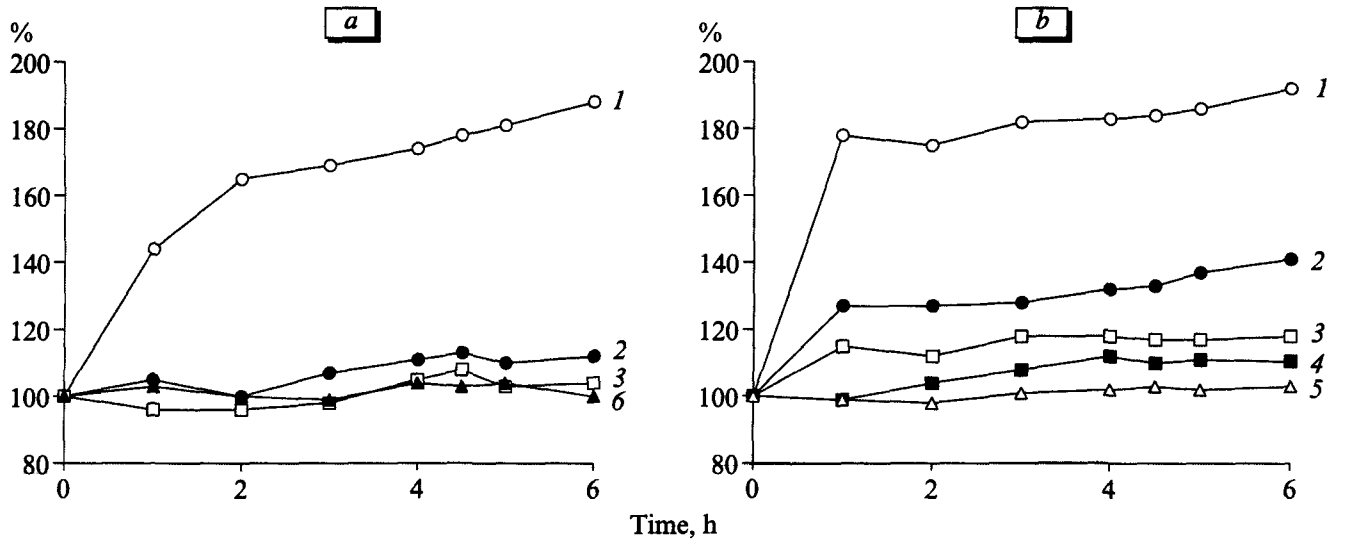

Fig. 1. Kinetic curves of light transmission fluctuations in suspension of native (*a*) and modified circulating LDL (*b*) at various ionic strength of the incubation medium. Ionic strength (in mM): *1*) 1.1; *2*) 4; *3*) 20; *4*) 80; *5*) 160; *6*) LDL-free control.

nLDL and cmLDL subfractions were separated by affinity lectin-chromatography on *Ricinus communis* agglutinin-agarose column (Boehringer Mannheim, GmbH) [13].

The degree of LDL association was assessed by analysis of light transmission fluctuations (LTF, laser beam, λ=780 nm) [12]. This method is based on the fact that the relative dispersion of optical density fluctuations is determined by stochastic number of particles met by the laser beam, which in its turn depends on the mean size of these particles, *i.e.* on the degree of association. To study the kinetics of LDL association, aggregates were preliminary removed by filtration through a 0.45 μ filter (Nalgene). LDL were incubated at 37°C in isotonic phosphate buffer (IPB: Gibco, Paisley, in g/l): 0.2 KCl, 0.2 KH_2PO_4, 8 NaCl, 1.15 Na_2HPO_4, pH 7.4 with 1 mg/ml EDTA or in the medium with decreased ionic strength. The ionic strength was decreased by diluting IPB with distilled water. In all cases, pH of the incubation medium was 7.4. Concentration of LDL was 0.2 mg protein/ml. After certain time intervals, the cuvette with incubated sample was placed into the measuring chamber of a two-channel laser aggregometer (Model LA220, Biola) to record LTF.

To assess the degree of irreversible association of nLDL and cmLDL under conditions of low ionic strength, the samples were preliminarily passed through a 0.45 μ filter and dialyzed for 6 or 24 h at 4°C against distilled water. Then the dialysate was replaced with fresh IPB with 1 mg/ml EDTA, and the dialysis was carried out again for 24 h at 4°C. LTF was recorded before and after dialysis.

The LTF data for nLDL and cmLDL samples were analyzed statistically at $p<0.05$.

RESULTS

A good solubility and stability of LDL in water medium is explained by peculiarities of their structure. Nonpolar lipids of LDL nucleus are screened from hydrophilic surrounding by the polar groups of phospholipids and apoB-protein exposed into water medium [2,6]. The most important stabilizing factor of these particles is the solvation shell. Disturbances in this shell decrease repulsion forces between the particles and promote their association. Formation of the solvation shell depends on surface physicochemical properties. It can be hypothesized that cmLDL and nLDL, whose surfaces markedly differ by their physicochemical properties [13], also differ in the re-

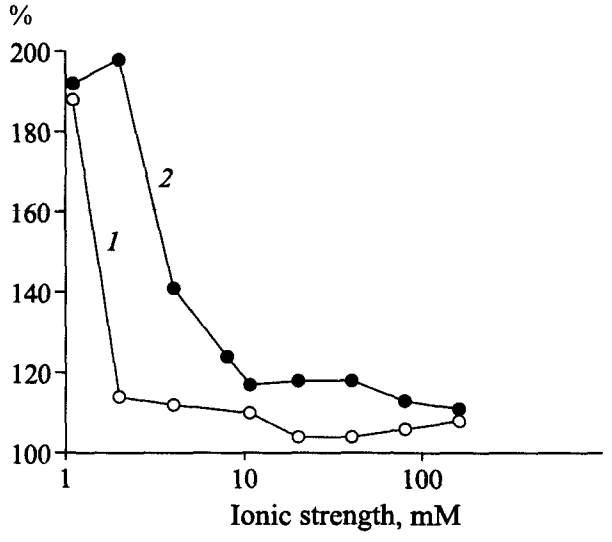

Fig. 2. Light transmission fluctuation in suspension of native LDL (*1*) and modified circulating LDL (*2*) incubated for 6 h at 37°C as a function of ionic strength.

TABLE 1. Effect of Decreasing Ionic Strength in Incubation Medium by Dialysis Against Distilled Water Followed by Its Restoration by Dialysis Against IPB on LTF in nLDL and cmLDL Suspensions ($M \pm m$)

LTF measurement	LTF, rel. units	
	nLDL	cmLDL
Before dialysis (control)	33±5	39±4
Experiment 1		
after 6-hour dialysis against distilled water	97±10*	133±19*,**
after 24-hour dialysis against IPB	121±21*+	188±33*,*+
Experiment 2		
after 24-hour dialysis against distilled water	165±8*++	217±12*,*++
after 24-hour dialysis against IPB	425±23*+,++	517±3*,*+,++

Note. $p<0.05$ compared to the *control, **nLDL, +values after 6-hour dialysis against distilled water, ++experiment 2.

sistance to association under conditions, when their solvation shells are modified by changes in ionic strength.

When incubated in isotonic medium (IPB, pH 7.4), neither nLDL, nor cmLDL significantly associated over 6 h (the data are shown only for cmLDL, Fig. 1, *b*, 5). By contrast, LTF, and hence, the mean size of nLDL and cmLDL increased under conditions of decreased ionic strength. At the same ionic strength of the incubation medium, cmLDL associated more rapidly than nLDL. For example, one-hour incubation at ionic strength of 1.1 mM 1.78-fold increased the size of cmLDL (Fig. 1, *b*, *1*), while the size of nLDL particles increased only 1.44-fold (Fig. 1, *a*, *1*).

After 6-h incubation at any ionic strength, the mean size of cmLDL particles was somewhat greater than that of nLDL (Fig. 2). The pronounced increase in the size of nLDL (1.88-fold) was observed only in the case, when ionic strength of the incubation medium was decreased to 1.1 mM (Fig. 2, *2*). The resistance of cmLDL to association pronouncedly decreased with the decrease in ionic strength of the medium. For example, after 6-h incubation at the ionic strength of 4 mM, their mean size increased by 1.4 times, while similar incubation at 2 mM increased this parameter by 1.9 times (Fig. 2, *2*).

During dialysis against distilled water, the decrease of ionic strength initiated association of LDL (Table 1). After 6- or 24-h dialysis, the size of nLDL increased by 2.9 and 5 times, respectively. Under these conditions, the mean size of cmLDL increased more pronouncedly — by 3.4 and 5.6 times, respectively.

Restoration of ionic strength did not decrease LTF and, consequently, the mean size of nLDL and cmLDL. Moreover, 24-h dialysis against IPB significantly increased LTF for both types of LDL, the effect being more pronounced for cmLDL (Table 1). These data confirm irreversible character of association of nLDL and cmLDL particles caused by low ionic strength of the incubation medium.

Thus, cmLDL are less resistant to association than nLDL after corruption of their solvation shell in a medium of low ionic strength. This association was irreversible, at least under our experimental conditions. Probably, such irreversibility results from peculiarities of physicochemical properties of the surface of cmLDL [3,10,13]. Bearing in mind that the efficiency of LDL uptake by cells increases with increasing the size of these particles [3,12-14], we can hypothesize that the ability of cmLDL to accumulate cholesterol in cultured smooth muscle cells isolated from human aortic intima and in macrophages [3,11,15] results from predisposition of these particles to association.

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