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Effect of Native and Modified Apolipoprotein A-I on DNA Synthesis in Cultures of Different Cells

I. F. Usynin, A. N. Dudarev, S. M. Miroshnichenko, T. A. Tkachenko, and A. Yu. Gorodetskaya

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Culturing of bone marrow cells in serum-free RPMI-1640 medium for 24 h was accompanied by a decrease in the rate of [^{3}H]-thymidine incorporation into DNA. Addition of native apolipoprotein A-I (apoA-I) or plasma LDL and HDL to the culture medium increased this parameter. In contrast to native apoA-I, its modified form decelerated DNA synthesis in bone marrow cells. A similar inhibitory effect of modified protein was observed in cultures of human embryonic kidney cells (HEK293) and in rapidly proliferating mouse macrophage cell line ANA-1. The only exclusion was human myeloid cell line U937: neither native nor modified apoA-I affected DNA synthesis in these cells. Thus, the regulatory effects of apoA-I are tissue-specific; this protein can produce either stimulatory or inhibitory effect on DNA biosynthesis in cells depending on its conformation.

Key Words: *apolipoprotein A-I; DNA synthesis; bone marrow cells; continuous cell lines; cell culture*

According to epidemiological data, reduced blood level of HDL is associated with a high risk of coronary heart disease [11], autoimmune diseases [18], diabetic retinopathy [26], and Parkinson's disease [25]. On the contrary, the increase in blood level of HDL under the effect of drug therapy accelerates cholesterol efflux from foam cells and reduces the risk of cardiovascular diseases [10]. Along with participation in reverse cholesterol transport from peripheral tissues to the liver, HDL also produce a regulatory effect on the functions of various body cells. For instance, mitogenic effect of HDL was demonstrated in cultures of lymphocytic, endothelial, and tumor cells [9,20]. The regulatory effects of HDL are determined by the presence of a protein component — apolipoprotein A-I (apoA-I) — on the surface of lipoprotein particle. It was found that apoA-I stimulates secretion of prostaglandin E2 [30] and apolipoprotein E in macrophages [28]. Sharp intensification of the expression of placental lactogen in human trophoblast cells under the effect of apoA-I was reported [12]. Experiments on fibroblast cell culture showed that apoA-I interaction with ATP-dependent cassette transporter (ABCA1) activates intracellular signaling pathways, including Cdc42 [21]. Our previous studies on isolated liver cells demonstrated that apoA-I stimulates protein biosynthesis in macrophages [3,23], while in a complex with steroid hormones stimulates protein and DNA biosynthesis in hepatocytes [3,4]. It is known that post-translational modification of HDL and apoA-I changes their functional properties. For example, oxidative modification of apoA-I induced by myeloperoxidase impairs the capacity of apoA-I to accept cholesterol from macrophages [13]. Moreover, thus modified apoA-I loses its anti-inflammatory properties and acquires proinflammatory ones [27].

The goal of the study was to compare the regulatory effects of native and modified apoA-I on DNA biosynthesis in the primary culture of rat bone marrow cells and in cultures of continuous cell lines.

MATERIALS AND METHODS

Bone marrow cells were isolated by a standard method [1] from the femoral bone of male Wistar rats (body

Laboratory of Mechanisms of Intercellular Interactions, Research Institute of Biochemistry, Novosibirsk, Russia. ***Address for correspondence:*** ivan.usynin@niibch.ru. I. F. Usynin

weight 180-200 g). Erythrocytes and lymphocytes were removed from bone marrow cells suspension by counter-flow centrifugation in a JE-5.0 eluting rotor of Avanti J-26XP (Beckman Coulter) centrifuge at 2500 rpm and flow rate of 14 ml/min. The resulting cell suspension containing no more than 3% erythrocytes and 5% lymphocytes was cultured in 24-well plates (1.0×10^6 cells per well) in a serum-free RPMI-1640 medium (PanEco) in CO_2 incubator (Cole Parmer) at 37°C for 24 h. Continuous cell lines HEK293, ANA-1, and U937 were cultured under similar conditions for 24 h. Animal experiments were performed with strict adherence requirements of European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986) and Regulations for Handling Experimental Animals (Ministry of Health of the Russian Federation, Order No. 267 of June 19, 2003)

DNA biosynthesis in cell cultures was evaluated by the rate of [^{3}H]-thymidine incorporation into DNA [14]. To this end, 2.0 μCi/ml [^{3}H]-thymidine was added to the culture medium 2 h before the end of the experiment and the incubation was continued at 37°C. The cells were then lysed with 0.2 N NaOH and the homogenates were transferred to Whatman 3MM cellulose filters pretreated with 10% trichloracetic acid. The filters were washed to remove [^{3}H]-thymidine excess and radioactivity was measured on a LS-650 liquid scintillation counter (Beckman Coulter) at Advanced Optical Systems Common Use Center (Institute of Experimental and Clinical Medicine; Novosibirsk). The rate of [^{3}H]-thymidine incorporation into DNA was calculated in counts per minute (cpm) per well or per 10^6 cells.

Lipoprotein fractions were isolated from human blood plasma by density centrifugation in KBr solutions [17] at 105,000*g* on an Optima L-90K (Beckman Coulter) centrifuge using a 70.1 Ti rotor (Beckman Coulter). The obtained lipoprotein fractions were dialyzed against PBS at 4°C for 24 h. To obtain native apoA-I, freshly isolated HDL fraction was delipidated with a mixture of butanol and diisopropyl ether under non-denaturing conditions [7]. ApoA-I was purified by precipitation with ammonium sulfate followed by protein renaturation by dialysis against PBS (pH 7.4) [5].

Modified apoA-I was obtained by reprecipitation of native apoA-I in ethanol solution by the method [24] in our modification. To this end, the protein was incubated in 78% ethanol in the presence of Tris buffer (pH 8.3) at -20°C for 90 min. The protein precipitate was dissolved in 6 M urea and dialyzed against PBS (pH 7.4) for 24 h. The purity of proteins was checked by PAAG electrophoresis using Thermo Fisher Scientific protein markers. Protein concentration was measured by the method of Lowry using BSA as the standard.

Analysis of the secondary structure of proteins was performed by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) [22] using a Smart Orbit attachment on infrared (IR) Fourier spectrometer Nicolet 6700 (Thermo Scientific). Optical paths of the instrument were constantly purged with dry air to remove water vapor. For quantification of the ratio of secondary structure elements in the protein, 10 μl PBS containing 7.5 μg protein was applied on a diamond crystal. After subtraction of buffer spectrum from protein spectrum and baseline correction, expansion of amide I spectral region in a wavelength range of 1600-1700 cm^{-1} was performed using Fourier deconvolution and second derivative methods [16]. The data were processed statistically using OMNIC software supplied with the instrument. The study was carried out using equipment of Spectrometric Measurements Common Use Center, Research Institute of Biochemistry.

The results are represent as the mean±standard deviation (*M*±*SD*) of three independent experiments performed in three parallels. The differences between the samples were evaluated using Mann—Whitney *U* test. The differences were significant at $p<0.05$.

RESULTS

Blood plasma lipoproteins are lipid—protein complexes. Depending on the proportion of lipid and protein components, lipoproteins have different floating density, which enables their isolation by isodensity ultracentrifugation. In this work, three lipoprotein fractions were isolated from human blood plasma: VLDL, d=0.95-1.006 g/cm^3; LDL, d=1.006-1.063 g/cm^3; and HDL, d=1.063-1.21 g/cm^3, and then, the main protein component, apoA-I, was isolated from freshly isolated HDL fraction. As functional properties of proteins are determined by their conformation, we compared the regulatory properties of native and modified apoA-I. Native apoA-I was isolated by delipidation of freshly isolated HDL fraction with butanol—diisopropyl ether mixture under non-denaturing conditions [7]. This method, in contrast to routine delipidation of plasma lipoprotein with ethanol—diethyl ether mixture, preserves the initial (native) structure of the protein and prevents its denaturation. Modified apoA-I was isolated by reprecipitation of native apoA-I in 78% ethanol [24] at pH 8.3. Both proteins had similar electrophoretic mobility (Fig. 1), which indicates the absence of considerable differences in the primary structures of native and modified apoA-I. At the same time, analysis of the ratio of secondary protein structures by IR-Fourier transform spectroscopy revealed essential conformational changes in modified apoA-I (Table 1). Thus, the content of α-helices decreased from 48% in native apoA-I to 31% in the modified protein, while

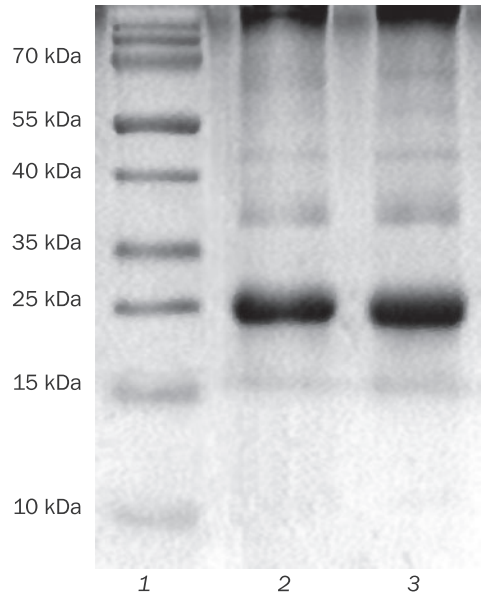

Fig. 1. Electrophoresis of native and modified apoA-I in 12% PAAG with sodium dodecyl sulfate. *1*) Standard proteins (10-180 kDa); *2*) native apoA-I; *3*) modified apoA-I.

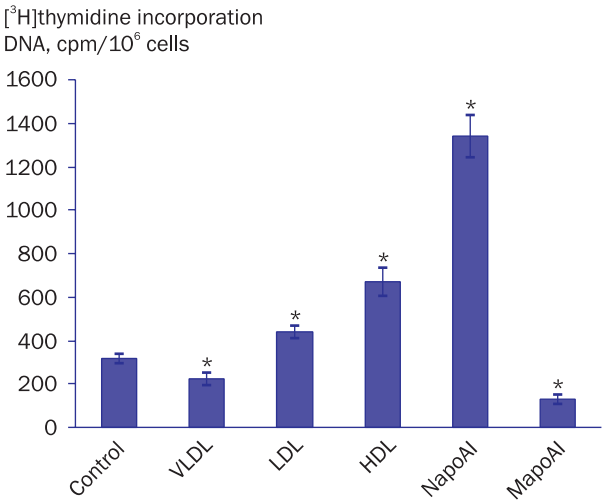

Fig. 2. Effect of different classes of plasma lipoproteins, native apoA-I (N-apoA-I), and modified apoA-I (M-apoA-I) on DNA synthesis in primary culture of rat bone marrow cells. *$p<0.05$ in comparison with the control.

the content of β-sheets after ethanol precipitation of the protein increased by 2 times (Table 1). The results attested to a partial unfolding of α-helix and destabilization of a substantial part of the secondary structure in modified apoA-I. We can hypothesize that ethanol precipitation of apoA-I disturbs protein folding at the stage of its renaturation, which results in the observed conformational changes in the protein.

The regulatory role of isolated lipoprotein fractions and apoA-I was studied on different cell types cultured in serum-free RPMI-1640 medium. The serum-free medium was used, because FCS used for culturing of animal cells contains apoA-I [29] and high density lipoproteins [15]. It was shown that culturing of bone marrow cells in serum-free medium considerably decreased the rate of DNA biosynthesis: the rate of [^{3}H]-thymidine incorporation into DNA was 3008±315 after 4 h of cell culturing, 1275±150 after 8 h, and 260±30 cpm per 10^6 cells after 24 h. In 24 h after addition of 50 μg LDL or 50 μg HDL per 1 ml culture medium, this parameter increased by 1.36 or 2.1, respectively (Fig. 2). In the presence of VLDL (50 μg/ml), the rate of DNA biosynthesis decreased by 1.4 times (Fig. 2), which agrees with previous data [31]. The inhibitory effect of VLDL is most likely determined by its protein component, apolipoprotein E, exhibiting antiproliferative activity [19].

Native apoA-I, similar to HDL, stimulated DNA biosynthesis in bone marrow cells. In the presence of 20 μg/ml apoA-I, the rate of [^{3}H]-thymidine incorporation into DNA was higher by 4.2 times than in the control (without additives) and by 2.0 times than in cells incubated in the presence of 50 μg/ml HDL (Fig. 2). Our findings suggest that the stimulating effect of HDL is determined by its main protein component, apoA-I. As was shown in our previous studies, HDL phagocytosis by isolated liver macrophages does not lead to their full degradation, but is followed by apoA-I release (resecretion) to the extracellular medium with preservation of its immunological properties [3]. Resident macrophages present in the bone marrow

TABLE 1. Proportion of Components of Secondary Structure of Native and Modified apoA-I According to IR-Fourier Transform Spectroscopy

Secondary structure components	Native apoA-I		Modified apoA-I	
	absorption maximum, cm^{-1}	proportion, %	absorption maximum, cm^{-1}	proportion, %
α-Helix	1652	48±5	1655	31±3*
β-Sheet	1696, 1623	15±3	1699, 1620	28±2*
β-Turn	1680, 1670	18±4	1682, 1669	21±4
Random coil	1638	19±5	1637	20±4

Note. *$p<0.05$ in comparison with native apoA-I.

TABLE 2. Effect of Native and Modified apoA-I on DNA Synthesis in Cultures of Different Cell Lines

Cell culture	[^{3}H]-thymidine incorporation into DNA, cpm/well		
	control	native apoA-I	modified apoA-I
HEK 293	18 794±828	20 282±702	13 579±680*
ANA-1	12 883±967	13 166±816	2483±241*
U937	31 202±1297	30 982±954	32 079±678

Note. *p<0.05 in comparison with the control.

cell culture can also resecret biologically active apoA-I after adsorption of HDL. In contrast to bone marrow cells, in the cultures of rapidly proliferating cell lines, native apoA-I did not significantly affect the rate of DNA biosynthesis (Table 2).

Modified apoA-I inhibited DNA biosynthesis in bone marrow cells. In 24 h, the rate of [^{3}H]-thymidine incorporation into DNA in the presence of modified apoA-I (20 μg/ml) was 2.5-fold lower than in the control, and 10-fold lower than in cells incubated with native protein (Fig. 2). A similar inhibitory effect of modified protein was found in continuous cell cultures. In the presence of modified apoA-I, the rate of [^{3}H]-thymidine incorporation into DNA in human embryo kidney HEK293 cell culture decreased by 1.4 times, and in the mouse macrophages cell line ANA-1 culture by 5.2 times (Table 2). Taking into account that [^{3}H]-thymidine is incorporated into cell DNA during replicative DNA synthesis (*i.e.* during S phase of the cell cycle) [8], the results indicate the involvement of apoA-I in the regulation of cell proliferation. The observed antiproliferative effect of modified apoA-I opens a way for further studies on antineoplastic action of this protein and its potential use in the therapy of various tumors. The only exclusion was human myeloid cell line U937: neither native nor modified apoA-I affected DNA synthesis in these cells. The observed differences can be attributed to low expression of apoA-I receptors on the membrane of U937 cells and inability of these cells to produce extracellular matrix essential for apoA-I binding [6].

Thus, the results obtained in our study on the rate of [^{3}H]-thymidine incorporation into DNA in cultures of different cell types in the presence of native or modified apoA-I indicate that apoA-I produces tissue-specific regulatory effects and, depending on the protein conformation, can either stimulate or inhibit cell proliferation.

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