Biochemistry (Moscow), Vol. 65, No. 12, 2000, pp. 1419-1423. Translated from Biokhimiya, Vol. 65, No. 12, 2000, pp. 1684-1689.

Detection of Apolipoprotein A-I, B, and E Immunoreactivity in the Nuclei of Various Rat Tissue Cells

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Received March 15, 2000
Revision received July 6, 2000

Abstract—Proteins with apolipoprotein A-I immunoreactivity were detected in the fraction of non-histone acidic proteins isolated from nuclei of various rat tissue cells. These proteins were detected in the brain, liver, kidney, lung, heart, skeletal muscle, testis, spleen, and bone marrow. In the same fraction from liver nuclei, proteins with apoB and apoE immunoreactivity were also detected. The composition of these proteins was studied by immunoblotting. ApoA-I immunoreactivity in the liver nuclei is due to two proteins. One 28-kD protein corresponds to the mature form of the plasma pool of apoA-I and another 14-kD protein is the product of limited proteolysis of apoA-I. The highest content of apoA-I immunoreactivity was detected in transcriptionally active chromatin and nuclear matrix. ApoB immunoreactivity is due to six proteins with molecular weights from 15 to 100 kD. ApoE immunoreactivity is due to a single protein corresponding to the 35-kD form of plasma apoE. Proteins with apoA-I, apoB, and apoE immunoreactivity may be involved in the regulation of transcriptional activity of chromatin.

Key words: nuclei, chromatin, lipoproteins, apolipoprotein A-I, apolipoprotein B, apolipoprotein E, immunoreactivity

Blood lipoproteins can be very important for structural and functional organization of eukaryotic chromatin. Experiments with ^{125}I-labeled HDL, LDL, and VLDL demonstrated that the protein component of LP could be detected in the liver nuclei 30 min after intravenous administration [1]. First, apolipoproteins can transport neutral lipids, phospholipids, cholesterol and its esters, tocopherol, and other lipid-like compounds to the nuclei. The requirement for these compounds depends on the functional activity of the chromatin. In regenerating liver, DNA synthesis is associated with increased contents of neutral lipids and phospholipids in the nuclei [2]. Also, tocopherol can enhance the activity of the nuclear RNA-polymerase [3, 4]. Tocopherol contents are higher in transcriptionally active chromatin and nuclear matrix than in transcriptionally inactive chromatin [5].

Second, the protein component of LP can influence the rate of gene expression. In the presence of cortisol, HDL increased the rate of gene expression in rat liver [6]. The effect was cooperative. The tetrahydrocortisol–apolipoprotein A-I complex was important for enhancement of gene expression [7].

The goal of the present study was to detect particular proteins of HDL, LDL, and VLDL in the nuclei of cells in various tissues. ApoA-I, apoB, and apoE and products of their limited proteolysis with corresponding immunoreactivity were detected by dot blot in the non-histone acidic protein fraction.

MATERIALS AND METHODS

Wistar rats (180-200 g) were used in the study. Lipoproteins were isolated from the serum by ultracentrifugation after removal of chylomicrons [8]. The density of the initial serum was increased with dry KBr, and the serum was then centrifuged for 18-20 h at 105,000*g* using a Beckman L5-75 centrifuge equipped with a 75 Ti rotor. Thus, three major lipoprotein fractions were isolated including VLDL ($0.94 < d < 1.006$ g/ml), LDL ($1.006 < d < 1.063$ g/ml), and HDL ($1.063 < d < 1.21$ g/ml).

Lipoproteins were delipidated with cold chloroform–methanol mixture (1 : 1) and 20 ml of the mixture was added per 1 ml LP solution; the residue was extensively washed with ether and dried under a stream of

Abbreviations: HDL) high density lipoproteins; LDL) low density lipoproteins; LP) lipoproteins; VLDL) very low density lipoproteins.

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nitrogen. To isolate apoA-I and apoE, dry apolipoproteins (HDL or VLDL) were dissolved in 2.5% SDS and loaded on a Sepharose CL-6B column (1.6 × 100 cm; Pharmacia, Sweden) and eluted with 0.01 M Tris-HCl buffer (pH 8.6) containing 6 M urea, 0.01% sodium azide, and 1 mM phenylmethylsulfonyl fluoride (PMSF). The flow rate was 10 ml/h and the chart recorder speed was 6 mm/h. Sepharose CL-4B (Pharmacia) was used to isolate apoB from LDL. The elution profile was monitored with a Uvicord 2151 (LKB, Sweden). Purity of apoA-I, apoB, and apoE was verified by polyacrylamide gel electrophoresis in the presence of SDS [9]. Protein bands were stained with 0.1% Coomassie G-250 prepared with the mixture of methanol and 10% acetic acid (1 : 1). A low-molecular-weight protein marker kit (Pharmacia) was used, including phosphorylase (94 kD), BSA (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), trypsin inhibitor (20.1 kD), and lactalbumin (14.4 kD).

To prepare polyclonal antibodies, rabbits were subcutaneously injected in the back with the solutions of apoA-I, apoB, and apoE. The first immunization was performed using complete Freund's adjuvant (Difco, USA). Each rabbit was injected with the mixture of 0.5 ml antigen (100 μg) and 0.5 ml complete Freund's adjuvant. Two subsequent immunizations (each 10 days) comprised injections of apolipoprotein solution with incomplete Freund's adjuvant, and the last immunization was performed intravenously with apolipoprotein solution without the adjuvant. IgG were purified by ammonium sulfate fractionation; 100 ml of the serum was mixed with 50 ml of saturated ammonium sulfate solution. The pellet was removed by centrifugation and redissolved in 50 ml of distilled water; then, the antibodies were re-pelleted with 25 ml of saturated ammonium sulfate solution. This procedure was repeated 4-5 times. Then the preparation was dialyzed against phosphate buffer (pH 7.4). Finally, IgG were purified on DEAE-Toyopearl 650M TSK anion-exchange resin (Toyo Soda, Japan). The antibodies were stored at −70°C. They were identified by double radial immunodiffusion [10] or immunoblotting [11]. ApoA-I was quantified by ELISA [12].

ApoA-I, apoB, and apoE were blotted by a semi-dry method [11]. Goat anti-rabbit IgG conjugated with horseradish peroxidase were used as secondary antibody (Sigma, USA).

Hepatocytes were isolated by the collagenase method [13]. Chromatin and nuclear matrix were isolated from the cells subsequent to homogenization in hypotonic solution containing 1 mM Tris-HCl, 3 mM $CaCl_2$, 5 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 1 mM PMSF, and 0.1% Triton X-100. The homogenate was incubated on ice for 5 min, and sucrose was added to 0.25 M concentration; the solution was centrifuged at 800*g* for 10 min. The pellet was washed with homogenization buffer and resuspended in 50 volumes of 2.1 M sucrose containing 1 mM Tris-HCl (pH 7.6), 3 mM $CaCl_2$, 5 mM $MgCl_2$, 1 mM DTT, and 0.5 mM PMSF; the mixture was centrifuged at 50,000*g* for 60 min in an L5-75 centrifuge (Beckman, USA) equipped with an SW-27 rotor. The nuclear fraction was washed twice with 0.25 M sucrose solution containing 1 mM Tris-HCl (pH 7.6), 3 mM $CaCl_2$, 5 mM $MgCl_2$, 1 mM DTT, and 1 mM PMSF.

The isolated nuclei were resuspended in solution containing 0.28 M NaCl, 1 mM Tris-HCl (pH 7.9), 2 mM EDTA, and 1% Triton X-100 and centrifuged at 25,000*g* for 30 min. The gel pellet of chromatin was washed three times with solution containing 10 mM Tris-HCl (pH 7.2), 2 mM EDTA, and 1 mM PMSF. Transcriptionally active (TA) and transcriptionally inactive (TIA) chromatins were isolated as described [14] using DNAse II from Serva (Germany). To prepare nuclear matrix, the nuclei were resuspended in solution containing 0.25 M sucrose, 5 mM Tris-HCl (pH 7.6), 3 mM $CaCl_2$, 5 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, and 30 units/ml DNAse I (Serva) so that the final DNA concentration was 2.0-2.5 mg/ml. The mixture was incubated at 4°C for 16-18 h, then 20 volumes of 2.12 M NaCl and 1.1% Triton X-100 were added. Nuclear matrix was pelleted by centrifugation at 40,000*g* for 30 min [14]. Proteins of the nuclear fractions were electrophoresed through 12.5% polyacrylamide gel in the presence of SDS [9]. DNA contents in the fractions were determined as described [15].

RESULTS AND DISCUSSION

Dot blot was used to detect various apolipoproteins in liver nuclei. Dot assay revealed immunoreactivity of apoA-I, apoB, and apoE in the liver nuclei (Fig. 1). The term immunoreactivity is used because not only intact apolipoproteins can be present in the nuclei, but also products of their limited proteolysis can be detected.

In the rat liver nuclei, we detected a protein with molecular weight corresponding to that of the mature plasma pool of apoA-I and a low intensity immunoreactive band corresponding to a 14-kD protein that could be a product of limited proteolysis of mature apoA-I (Fig. 2). The presence of other apolipoproteins or their fragments was demonstrated with specific antibodies. The apoE immunoreactivity band had the same electrophoretic mobility in 12.5% gel as that of the mature plasma apoE (35 kD). Immunoreactivity of the nuclear proteins was the highest towards the anti-rat apoB antibodies. Initial apoB-100 and its other major form B-48 are not detected in the nuclear fraction, and the signal corresponds to the products of apoB proteolysis. Up to six various fractions were detected by immunoblot, and their molecular weights varied significantly. The fraction with the highest molecular weight was about 100 kD, and the fraction with the lowest molecular weight was about

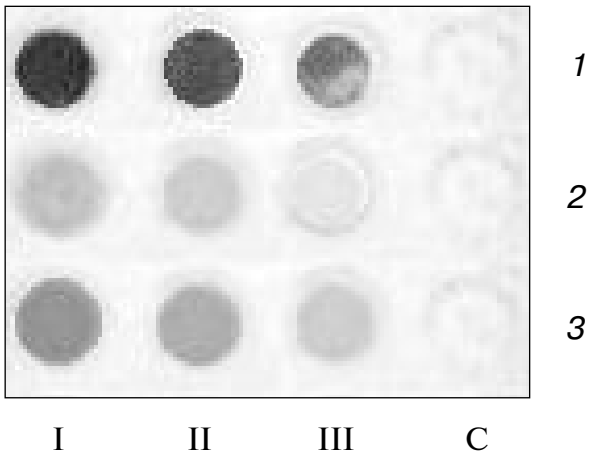

Fig. 1. Dot immunoanalysis of apoA-I, apoB, and apoE immunoreactivity in liver nuclei in the fraction of acidic non-histone proteins. I, II, and III) dilutions 1 : 1, 1 : 2, and 1 : 10; C) control; *1*) apoA-I immunoreactivity; *2*) apoB immunoreactivity; *3*) apoE immunoreactivity.

15 kD. The 60-kD fraction (molecular weight slightly lower than that of the serum albumin) had the highest intensity.

Only a few recent publications have given evidence suggesting that cellular genetic machinery can be regulated by lipoproteins or their protein components. For example, LDL induced expression of the egr-1 gene; this gene enhanced proliferation of the human umbilical vein endothelial cells [16]. LDL dose-dependently stimulated incorporation of [^{3}H]thymidine in DNA and increased Ca^{2+} concentration in endothelial cells. Oxidized LDL induced Ca^{2+}-dependent apoptosis in cultured ECV-304 human endothelial cells [17].

HDL inhibited cytokine-induced (tumor necrosis factor-α; TNF-α) expression of adhesion factor-1 in human umbilical vein endothelial cells. Interestingly, HDL_3 fraction was more potent than the HDL_2 fraction [18].

According to Desanctis et al. [19], all classes of lipoproteins enhanced proliferation of T cells and natural killer cells. However, HDL (unlike all other classes of lipoproteins) had no influence on interleukin-2-induced T cell proliferation but enhanced proliferation of large granular lymphocytes 2-fold. In another work, these authors demonstrated that HDL decreased secretion of certain cytokines from the natural killer cells, including TNF-α, granulocyte/macrophage colony-stimulating factor, and interleukin-1 and interleukin-1β. Secretion of interleukin-2, interleukin-8, and interferon-γ was stimulated [20].

ApoE-dependent inhibition of proliferation of mitogen-activated T cells (CD4 and CD8) was associated with a dramatic decrease in their biological activity and cytokine secretion (for example, interleukin-2). ApoA-I and apoC-III were inactive [21]. Recently, direct evidence for a role of apoE in suppression of interleukin-2-dependent T cell proliferation was described [22]. The region with amphipathic α-helical conformation including amino acid residues 141-149 is responsible for the cytostatic and cytotoxic effects of apoE.

Voegl et al. [23] have shown that recombinant apoE-3 expressed in *E. coli* inhibited proliferation of various cell types including endothelial cells, human malignant melanomas, breast carcinoma, and Kaposi's carcinoma. Inhibition was associated with a significant decrease in DNA synthesis.

An acetylated apoE fragment (141-155) responsible for B,E-receptor binding stimulated the synthesis of androgens in theca- and interstitial cells of rat ovary [24]. This resulted in enhanced transcription of the P450-17α-hydroxylase and 17-20-lyase genes [25]. The apoE peptide (126-169) efficiently transported antisense oligonucleotides in the apoE peptide–oligonucleotide complex and selectively inhibited expression of the cholesterol ester transport protein (CETP) gene in Chinese hamster ovary cells. Over 95% of the antisense nucleotides were detected in the nuclei. Interestingly, inhibition of CETP gene expression was not observed when apoE peptide and oligonucleotide were added separately to the cells [26].

Handwerger et al. [27] have detected enhanced expression of certain genes by HDL and apoA-I; they have shown that synthesis of placental lactogen is stimulated in human trophoblasts. Lactogen synthesis was completely suppressed by cycloheximide or actinomycin D.

The exact mechanisms of various regulatory activities of lipoproteins or their protein components are not known in detail. The effect of apolipoproteins on expres-

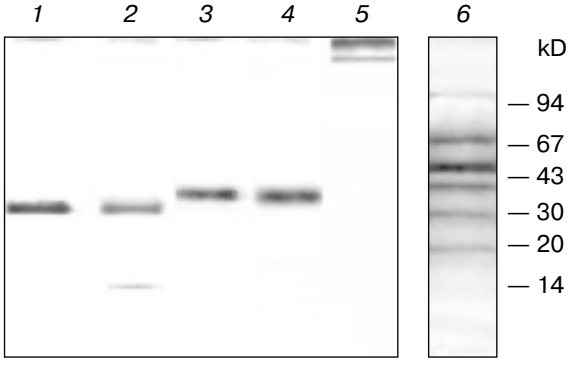

Fig. 2. Immunoblot of proteins with apoA-I, apoB, and apoE immunoreactivity in rat liver nuclei: *1*) apoA-I standard; *2*) apoA-I immunoreactivity in rat liver nuclei; *3*) apoE standard; *4*) apoE immunoreactivity in rat liver nuclei; *5*) apoB standard; *6*) apoB immunoreactivity in rat liver nuclei.

Assay of apoA-I immunoreactivity in chromatin fractions of rat liver nuclei [1]

Chromatin fraction	ApoA-I content, ng/mg protein
Total chromatin	60 ± 4.5
TIA chromatin	52 ± 3.8
TA chromatin	100 ± 5.0
Nuclear matrix	110 ± 5.2

sion of the functionally important genes is poorly understood. Recent studies confirmed that cooperative effects of HDL and cortisol on hepatocyte gene expression involve liver macrophages and occur via an unknown mechanism. This mechanism may involve interaction of deproteinized DNA regions with the tetrahydrocortisol–apoA-I complex that is formed in macrophages and then penetrates into the nuclei of parenchymal cells (hepatocytes). An interaction of the complex with DNA results in breakage of hydrogen bonds between the base pairs, inducing the formation of the single-strand structures interacting with RNA-polymerase [7, 28]. This mechanism mediates enhancement of gene expression induced by cooperation of glucocorticoids (cortisol) and HDL.

According to dot blot, apolipoprotein A-I is nonuniformly distributed between chromatin fractions (table). ApoA-I contents were the highest in transcriptionally active chromatin and nuclear matrix. ApoA-I contents were 2-fold lower in total and transcriptionally inactive chromatin. An apoA-I fragment (about 14-kD) was detected only in transcriptionally inactive chromatin.

Dot immunoassay indicates that apoA-I immunoreactivity was present in the acidic non-histone nuclear protein fractions of 10 different tissues. These tissues include brain, liver, kidney, lung, heart, skeletal muscle, adrenals, testis, spleen, and bone marrow (Fig. 3). In tissues with high proliferative activity (liver, spleen, bone marrow), the immunoreactivity was higher. It was especially high in bone marrow. In tissues with low proliferative activity (heart, muscle, brain), apoA-I immunoreactivity was low. The lowest reactivity was found in the brain.

Thus, immunoreactivity corresponding to apolipoproteins A-I, B, and E was detected in the acidic nuclear non-histone protein fraction of cells from various tissues. This suggests that these proteins not only participate in the transport of various lipids to the nuclei, but also play a regulatory role associated with changes in transcriptional activity of the chromatin. The apoA-I immunoreactivity includes two bands. One corresponds to the mature

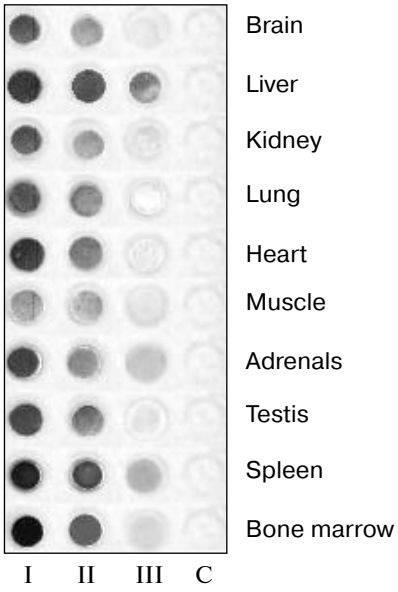

Fig. 3. Dot immunoassay of apoA-I immunoreactivity in the acidic nuclear non-histone protein fraction of various tissues. I, II, and III) dilutions 1 : 1, 1 : 2, and 1 : 10; C) control.

plasma pool of apoA-I (28 kD) and the other corresponds to a product of limited proteolysis of apoA-I (14 kD). The first band was present in transcriptionally active chromatin and nuclear matrix, and the second band was detected in transcriptionally inactive chromatin. ApoB immunoreactivity includes six bands. They correspond to various fragments of apoB-100. The shortest polypeptide has molecular weight of 15 kD, and the largest band has molecular weight of 100 kD. ApoE immunoreactivity includes a single band corresponding to the plasma apoE. A regulatory role of apoA-I was demonstrated previously [5, 7, 28]. Regulatory roles of the apoB fragments and apoE are now being studied.

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