BIOPHYSICS AND BIOCHEMISTRY

Interaction of Apolipoproteins A-I and E in the Regulation of DNA, RNA, and Protein Biosynthesis in Cultured Rat Hepatocytes

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Experiments on hepatocyte culture showed that apolipoprotein A-I—tetrahydrocortisol complex increases the rate of DNA, RNA, and protein biosynthesis measured by radioactive label incorporation. Apolipoprotein E acted as competitor of the apolipoprotein A-I—tetrahydrocortisol complex and abolished biological activity of the latter. We hypothesize that this mechanism of regulation plays an important role in processes of intracellular regeneration and proliferation.

Key Words: *hepatocytes; apolipoproteins A-I and E; tetrahydrocortisol; biosynthesis of protein and nucleic acids*

Apolipoproteins A-I and E (apo A-I and apo E) were detected in rat hepatocyte nuclei. Both proteins were present in the fraction of acid nonhistone proteins participating in the regulation of gene expression [2,3]. It was found that apo A-I increases transcription activity of chromatin. In complexes with reduced forms of steroid hormones apo A-I interacts with GC-rich DNA sites. The reduced Δ^4-3-keto group of A-ring of the steroid initiates break of hydrogen bonds in GC pairs. Then, this break is extended due to hydrophobic interaction between nitrogen bases and hydrophobic sites of apo A-I. RNA polymerase interacts with single-stranded DNA sites and triggers gene expression followed by activation of protein biosynthesis [1,5,6].

Apo E is known to inhibit proliferation of various cell types including tumor cells. Studies of the effect of apo E on differentiation of human adenocarcinoma HT29 cells and distribution of β-catenin showed that apo E participates in the maintenance of cell-cell interaction and inhibits tumor cell growth [10]. Apo E decreases expression of canonical β-catenin-dependent Wnt signaling pathway [7], whose constitutive activation plays an important role in carcinogenesis [14]. It was demonstrated that apo E increases expression of mRNA of perlecan, the main proteoglycan of the heparan sulfate family, which mediates the antiproliferative effect of apoE in cultured smooth muscle cells from the aorta [11]. Apo E inhibits serum-stimulated proliferation of rat embryonic fibroblasts by blocking protein kinase activation [8]. It was also reported that apo E inhibits vascular hyperplasia during inflammation caused by denudation of the carotid arteries in mice [9].

Here we verified the hypothesis on competitive interactions between apo A and E during regulation of intracellular regeneration and proliferation.

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MATERIALS AND METHODS

Experiments were carried out on hepatocytes isolated from male Wistar rats weighing 180-200 g. Hepatocytes were isolated by the method of recirculatory enzymatic perfusion with 0.03% collagenase (ICN Biomedicals Inc.) and separated from nonparenchymal cells by differential centrifugation. Cell viability evaluated by trypan blue (Serva) exclusion was ≥90%. The cells were resuspended in RPMI-1640 medium (Biolot), pH 7.4, containing 20 mM HEPES (ICN Biomedicals Inc.), 10% FCS (Serva), 2 mM glutamine (Vektor), 100 U/ml penicillin, 50 μg/ml gentamicin, 5.6 mM glucose, and 10 nM insulin Serva). Incubation was performed in a Cole-Parmer CO_2-incubator (37°C, 5% CO_2 and 95% air) in 6-well collagen-coated plates (Orange Scientific). Cell density in the primary monolayer culture was 800 cell/mm^2.

Blood plasma lipoproteins were isolated by density centrifugation in KBr solutions in the presence of 3 mM EDTA-Na on an Optima L-90K ultracentrifuge (Beckman Coulter). The HDL and VLDL fractions were delipidated with ethanol—acetone 1:1 mixture and repeatedly washed with ether. Apo A-I and E were isolated by gel-filtration (1.6×100 cm column, Sepharose CL 6B (Amersham Biosciences), eluent: 0.01 M tris-HCl buffer, pH 8.6, containing 6 M urea, 0.01% sodium azide, and 1 mM phenylmethanesulfonyl fluoride. The purity of isolated apolipoproteins was verified by SDS-PAAG electrophoresis (Serva, Fig. 1, 2). Low-molecular-weight proteins phosphorylase (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carboanhydrase 30 kDa), and lactalbumin (14.4 kDa) were used as molecular weight markers. Protein bands were visualized with 0.1% Coomassie G-250 in methanol-10% acetic acid mixture (1:1).

Reduced steroid hormone tetrahydrocortisol (THC) was kindly provided by Yu. A. Pankov, Academician of the Russian Academy of Medical Sciences (Institute of Experimental Endocrinology). For preparing complexes of apo A-I with THC (apo A-I—THC), the components in 1:2 molar ratio were incubated in 0.05 M potassium-phosphate buffer (pH 7.4) containing 0.15 M NaCl for 5 min at room temperature. The concentrations of apo A-I and apo E in the incubation medium were 60 and 10 μg/ml, respectively, the concentration of THC was 5×10^{-6} M.

The rates of DNA, RNA, and protein biosynthesis in hepatocyte culture were evaluated by incorporation of ^{14}C-leucine, ^{3}H-thymidine, and ^{3}H-uridine, respectively (2 μCi/ml medium, Amersham). The reaction was stopped by adding 0.2 n NaOH. The content of each well was transferred onto cellulose filters (Whatman 3 MM); radioactivity was measured on a Mark-III scintillation counter and expressed in cpm/mg protein.

The data were processed statistically using Student's t test at $p<0.05$ significance level.

RESULTS

It was shown that apo A-I, cortisol, and its complexes with apo A-I did not change the rate of nucleic acid and protein biosynthesis in primary culture of rat hepatocytes; only apo A-I—THC complex exhibited biological activity [6]. In this study, the apo A-I—THC complex increased the rate of DNA, RNA, and protein biosynthesis in cultured hepatocytes by 1.5, 2, and 1.8 times, respectively (Table 1). Thus, the reduced Δ^4-3-keto group of A-ring in the molecule of steroid hormone plays an important role in activation of biosynthetic processes. The hydroxyl group in position 3 directly participates in breaking of hydrogen bonds in sites of complex binding with DNA, *i.e.* hydrogen bonds in GC pairs break and close on the hydroxyl group of the hormone [12].

We hypothesized that apo E can participate in the regulation of protein and nucleic acid biosynthesis by the negative feedback mechanism. In the absence of apo A-I—THC complex in the incubation medium, apo E had no effect on protein and RNA biosynthesis, but 1.6-fold reduced the rate of labeled thymidine incorporation into DNA compared to the control. At the same time, apo E completely abolished the increase in DNA, RNA, and

TABLE 1. Changes in Rates of DNA, RNA, and Protein Biosynthesis in Rat Hepatocytes (cpm/mg protein)

Experimental conditions	DNA	RNA	Protein
Control	1343±74	1155±52	18,016±554
Apo A-I—THC	1985±104*	2275±173*	32,802±1175*
Apo E	862±70*+	1134±131+	19 900±2456+
Apo A-I—THC+apo E	803±48*+	1132±191+	21,823±2290+

Note. $p<0.05$ compared to: *control; +apo A-I—THC complex.

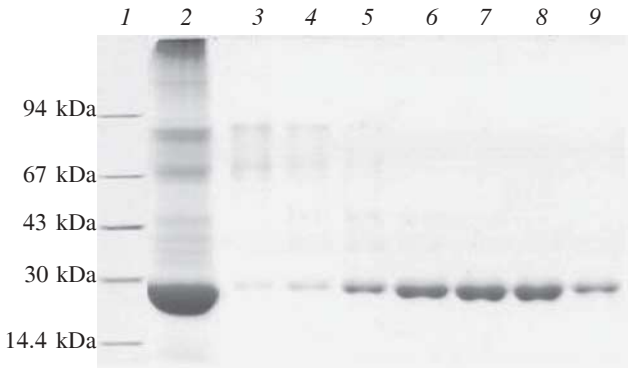

Fig. 1. Electrophoregram of chromatographic purification of apo A-I. 1: low-molecular-weight standard proteins; 2: total HDL proteins; 3-9: stages of apo A-I purification.

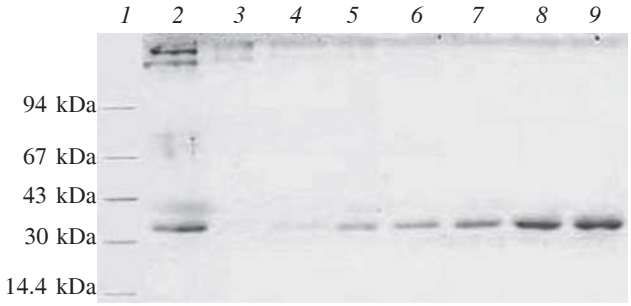

Fig. 2. Electrophoregram of chromatographic purification of apo E. 1: low-molecular-weight standard proteins; 2: total VLDL proteins; 3-9: stages of apo E purification.

protein biosynthesis induced by apo A-I—THC complex (Table 1).

Thus, a competitive relationship exists between apo A-I—THC complex and apo E: apo A-I—THC complex stimulates DNA, RNA, and protein synthesis in hepatocytes, while apo E completely abolished this effect. This interaction between these two proteins is probably realized at the level of their interaction with $(GCC)_n$-type DNA sequences. These sites probably exhibit higher affinity to apo E, which prevents their interaction with apo A-I—THC complex and activation of biosynthetic processes.

We believe that this mechanism can be involved into regulation of not only intracellular regeneration, but also cell proliferation. The key role in this mechanism is played by resident macrophages (Kupffer cells in the liver). These cells phagocytize products of cell degradation and cooperatively via receptor-mediated endocytosis engulf HDL_3 particles and steroid hormones [13]. In secondary lysosomes, HDL_3 undergo disintegration with the formation of free apo A-I, while steroid hormones are reduced to tetrahydro compounds in the reactions catalyzed by α- and β-reductases. The products form bioactive complex are transported into interstitial space and then into hepatocyte nuclei, where they enhance gene expression. We previously demonstrated that macrophages activated via this route started to express apo E [4], which in turn suppressed DNA, RNA, and protein synthesis by the feedback mechanism.

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