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# The role of lipoproteins in transport of steroid hormones and hormonal regulation of gene expression

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## ABSTRACT

The interaction of steroid hormones with individual classes of lipoproteins and apoproteins was analysed. Plasma lipoproteins bind steroid hormones and can therefore play a role in their active transport in the body. High density lipoproteins demonstrate the highest affinity for steroids. The lipoprotein-steroid complex formation involves the protein components of lipoproteins with the apolipoprotein A-I as one of the protein components responsible for the binding of steroid hormones. The constants of the complex formation between lipoproteins and steroid hormones suggest specificity of this binding. The reviewed data suggest a real possibility of penetration of steroid hormones into cells by a receptor-mediated endocytosis using the apolipoprotein complexes as a vehicle. 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 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. ApoA-I, apoB and apoE may be involved in the regulation of transcriptional activity of chromatin. A novel mechanism of protein biosynthesis regulation in liver under the action of reduced forms of steroid hormones (tetrahydrocompounds) and apolipoprotein A-I is discussed.

**KEYWORDS:** liver, lipoproteins, steroid hormones, apolipoprotein A-I, protein biosynthesis, nucleolus, gene expression

## 1. INTRODUCTION

Blood lipoproteins are very significant for structural and functional organization of eukaryotic chromatin [1]. Experiments with $^{125}$I-labeled high (HDL), low (LDL), and very low (VLDL) density lipoproteins demonstrated that the protein component of lipoprotein could be detected in the liver nuclei 30 min after intravenous administration [2]. Apolipoproteins can transport 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 [3]. Also, tocopherol can enchance the activity of the nuclear RNA-polymerase [4]. Tocopherol contents are higher in transcriptionally active chromatin and nuclear matrix than in transcriptionally inactive chromatin [5]. Recently, considerable efforts have been focused on the influence of HDL on gene expression.

The stimulated effect of HDL was demonstrated for the proliferation of lymphocytes [6], epithelial

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[7], endothelial [8], smooth muscle [9], and tumor [10] cells. Desanctis *et al.* [6] have shown that all classes of lipoproteins enhanced proliferation of T cells and natural killer cells. However, HDL (unlike all other classes of lipoproteins) has 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 [11]. HDL inhibited cytokine-induced (tumor necrosis factor-α; TNF-α) expression of adhesion factor in human umbilical vein endothelial cells. Interestingly, $HDL_3$ fraction was more potent than $HDL_2$ fraction [12].

Previously it was shown that glucocorticoids and HDL exert a cooperative effect enhancing the RNA and protein biosynthesis in white rat liver [13]. The active form of hormone operating in nucleous together with apolipoprotein A-I (apoA-I) is reduced form, tetrahydrocortisol (THC) [14], which was previously considered to be an inert metabolite [15]. Fluorescent probing of the native DNA from rat liver using acridine orange showed that the cortisol-apoA-I complex had virtually no effect on the secondary structure of DNA, while the tetrahydrocortisol-apoA-I (THC-apoA-I) complex increased the number of single-stranded regions [16]. Small-angle X-ray scattering (SAXS) verified the effect of THC-apoA-I complex on the DNA structure and allowed determination of the stoichiometry of this interaction [17]. Fermentative analysis of DNA associated with THC-apoA-I complex allowed estimation of the number of newly arising nuclease-sensitive regions (1-2 per 100 t.p.n.) and their distribution in the rat genome [17].

The aims of this work were:

1. to investigate being capacity of plasma lipoproteins towards different steroid hormones;

2. to study the affinity of apoA-I to DNA in comparison to some other serum proteins and estimate the interaction constants of the protein-steroid hormone complexes with various synthetic oligonucleotides and nucleotide specificity of interaction between DNA and tetrahydrocortisol (dehydroepiandrosterone)-apoA-I;

3. to show influence of tetrahydrocortisol (dehydroepiandrosterone)-apoA-I complexes on rate of protein biosynthesis (gene expression).

## 2. MATERIALS AND METHODS

### Isolation of lipoproteins and apolipoprotein A-I

Wistar rats (180-200 g) were used in the study. Lipoproteins were isolated from the serum by ultracentrifugation after removal of chylomicrons [18]. 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 an Optima L-90K centrifuge (Beckman-Coulter, USA) 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 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 phenylmethyl-sulfonyl 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 (PAAG) electrophoresis in the presence of SDS [19]. 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).

**Determination of the equilibrium association constants**

The interaction of steroid hormones with lipoproteins and apoproteins was studied by ultracentrifugation, gel-filtration, equilibrium dialysis, and fluorescence spectroscopy. The equilibrium association constant ($K_a$) for cortisol, corticosterone and progesterone was determined by [20]. The distribution of corticosteroids among individual Lp fractions was estimated upon addition of the [$^3$H]-labeled hormones to rats blood serum. Serum was incubated with cortisol, corticosterone, pregnenolone and desoxycorticosterone (DOC) for 30 min at 37°C. This was followed by preparative isolation of Lp by "Optima-90K" Ultracentrifuge. The obtained VLDL, LDL, HDL and infranatant were assessed for radioactivity content using β-counter Mark-III (USA).

**Preparation of polyclonal antibodies**

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 repelleted 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 (Toyosoda, Japan). The antibodies were stored at -70°C. They were identified by double radial immunodiffusion [21] or immuno-blotting [22]. ApoA-I was quantified by ELISA [23]. ApoA-I, apoB, and apoE were blotted by a semi-dry method [22]. Goat anti-rabbit IgG conjugated with horseradish peroxidase were used as secondary antibody (Sigma, USA).

**Isolation of hepatocytes, nuclei and chromatin**

Hepatocytes were isolated by the collagenase method [24]. 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 [25] 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 [25]. Proteins of the nuclear fractions were electrophoresed through 12.5% PAAG in the presence of SDS [19]. DNA contents in the fractions were determined as described [26].

**Isolation of DNA and determination of association constants**

DNA was isolated from rat liver and salmon milt by the techniques described in [27]. Denatured DNAs were bonded to AE-cellulose (Pharmacia,

Sweden) by the method described in [28]. ApoA-I was transferred into 0.05 M buffer of tris-HCl pH 7.5, 0.8% NaCl, by dialysis and spread over the column (V = 1 ml) with affine sorbent. The same buffer was used as an eluent up to the fading of optical absorbance at the wavelength of 280 nm. The protein bonded to DNA was eluted by 1 M NaCl in the buffer mentioned above. Optical density was recorded with a Milichrom ultramicro-spectrophotometer (Novosibirsk). Immobilized DNA was bonded to BSA and IgG in a similar way. Bovine serum albumin (BSA) and immuno-globulines (IgG) were of Sigma production (USA).

Desoxyribooligonucleotides 5`-pATCTTTAACT GATGAACTTCT (21N), 5`-pCCTGGGCAGAT TGGTATCAAGGTTACAA (28N), 5`-pTGCCTG GAGCTGCTTGATGC (20N) and $p(T)_{19}$ in the form of sodium salts were synthesized at the SRC Vector (Novosibirsk). $CC(GCC)_n$ triethylammonium salts with n = 3, 5 were synthesized at the Novosibirsk Institute of Biochemistry. 21N, 28N and 20N oligonucleotides, along with $CC(GCC)_n$ were marked at the 5`-end of $\gamma$-$^{32}$P-ATP (Isotope, St.-Petersburg, 1μcurie/mmol) by polynucleo-tidekinase of T4 bacteriophage (Sibenzyme, Novosibirsk). $^{32}$P-marked oligonucleotides were separated from the excess of [$^{32}$P]-ATP by gel-filtration at a 1 ml column with sefadex G-25 fine or by electrophoresis in 20% PAAG under denaturing conditions followed by electroelution of the marked oligonucleotide. Oligonucleotides were precipitated with 2% lithium perchlorate in acetone. Specific radioactivity was $1.4 \cdot 10^{10}$ cpm/μmol for 21N, and $2 \cdot 10^{10}$ cpm/μmol for 20N. Alkylating derivatives 4-(N-2-chloroethyl-N-methylamino) benzyl-5`-$^{32}$P-phosphamides of oligonucleotides were synthesized according to [29]. Affine modification of apoA-I by alkylating derivative of oligonucleotide was performed in 0.05 M buffer of tris-HCl pH 7.5, containing 0.8% NaCl, at 37°C for an hour, which proved to be a sufficient time for virtually complete hydrolysis of C-Cl in the reagent [30]. The reaction mixture comprised $5 \cdot 10^6$ M of apoA-I and 2, 4, 6, 8, 10, and 20 molar excess of the reagent. To suppress the affine modification, a free oligonucleotide was added to apoA-I solution in 10, 20, 50, 100, and 200-fold molar excess with respect to the reagent (oligonucleotide of the same composition with a sewed alkylating group). It was added to the reaction mixture after its incubation with the free oligonucleotide, inhibiting the affine modification. Concentration of the reagent was $5 \cdot 10^{-5}$ M. The reaction mixture was kept under the alkylation conditions as described above. To determine the degree of protein modification by the reagent, after the reaction completion, the mixture was spread over PAAG in 0.1% SDS, and electro-phoresis was conducted according to Laemmli [19]. Then autoradiography of the gel was performed, the strips of radioactive protein were removed from the gel and calculated with a standard.

The ability of GC-rich oligonucleotide (ON1) to bind apoA-I and its hormone complex was studied using the gel-retardation method. For this purpose, THC-apoA-I complex or apoA-I protein ($10^{-4}$-$10^{-6}$ M) was incubated with ON1 at room temperature for 5 min in TE buffer, then a solution was added for spreading it over the gel (10% glycerol, 0.025% bromphenol blue), or the same solution with 6 M urea. The resulting complexes ON1-THC-apoA-I or ON1-apoA-I were separated from the remaining free oligonucleotide by electrophoresis in 15% or 20% PAAG in the presence or in the absence of 6 M urea. PAAG was dyed with bromic ethidium, Cumassy R-250 or Stains-all. The gel dyed with bromic ethidium was photographed in UV light on the Micrat-isopan film. The gels dyed with Cumassy R-250 or Stains-all were photographed in visible light on the same film.

The specificity of binding the THC-apoA-I complex with DNA was studied in the reaction of competitive inhibition with GC-rich (ON1) and AT-rich (ON2) oligonucleotides. In one case, the corresponding oligonucleotide was added in raising concentration (6.1-610 μg/ml of the reaction mixture) after binding of 0.5 μg DNA to $1.6 \cdot 10^{-7}$ μmol of THC-apoA-I complex, as described above; in the other case, at first oligonucleotide was added to THC-apoA-I complex, and then DNA. Each reaction mixture was subjected to fermentative hydrolysis by S1 nuclease. DNA was analyzed by electrophoresis in 0.5%, 1% and 1.5% agarous gels. The gels were dyed with bromic ethidium and photographed in UV light.

To determine association constants of apoA-I and apoA-I hormone complexes with oligonucleotides $CC(GCC)_5$, ATCTTTAACTGATGAACTTCT and $(T)_{19}$, the small-angle X-ray scattering spectra of their equilibrium mixtures were taken with a Siemens diffractometer (Germany) as described in [14].

**Infra red spectroscopy**

Samples for IR spectra recording were prepared as films on a fluorite support. Size of the optical aperture 8 mm in diameter was bounded by a fluoroplastic plate. Lipoprotein samples were deposited uniformly on the support over the whole aperture section in a 50-μL volume. Both of these samples (control lipoprotein and test lipoprotein + cortisol) were simultaneously put into the vacuum case with the initial temperature 4 ± 1°C. Vacuum was brought to 0.1-0.2 atm (104 n/m$^2$) with a vacuum pump and then dried at this pressure for 80 min. In a deeper vacuum, lipoproteins are partially entrapped by water vapor, and sublimation occurs. After drying, the homogeneous film was obtained over the whole aperture section. Spectra were recorded with an IR Specord-M 80 spectrometer (Germany). Spectrometer with the samples was blown with dry air for 30 min, then the scanning regime was switched on, and spectra of the samples were recorded with the dry air feed not turned off. Repeated recording of differential spectra of the samples (control-control) showed the zero absorption. In our opinion, drying of lipoproteins, i.e., the lipid-containing structures, slightly changes the protein conformation, since only the free water is separated, and the molecules of bonded $H_2O$ do remain and determine the conformation of proteins and lipids. In our samples, the protein structure is stabilized largely through the interaction with lipids [17]. According to IR spectra we obtained, addition of dehydrating agents (methanol, ethanol) to the samples even in low amounts ($10^{-6}$ - $10^{-7}$ M) significantly affected the disordering of the protein secondary structure. This fact was taken into account in our study, and in the test we added cortisol in methanol ($C_0$ = 0.01 M) preliminarily diluted with $K^+$, $Na^+$-phosphate buffer by 3 and 7 orders of magnitude ($4.15 \times 10^{-6}$; $1.6 \times 10^{-9}$), and in the control added an equivalent methanol amount into the mixture with the same buffer. The Merck reagents were used in the study.

**Rate of protein biosynthesis**

Rate of protein biosynthesis was found from incorporation of [$C^{14}$]-leucine, which was added to the medium in the amount of 37 Bk/ml 2 hours before completion of the incubation. Reaction was arrested by introducing a 0.2 M solution of NaOH. 100 μl of the well content was transferred on each of FN16 filters pretreated with a 0.1 M leucine solution in 10% TCA. The filters were sequentially washed with TCA solution and alcohol-ether mixture. Radioactivity was measured using a Mark II counter (USA).

## 3. RESULTS

**Analysis of the interaction between lipoproteins and steroid hormones**

It was shown that the blood serum lipoproteins bind steroid hormones and can serve as active carriers. As shown by intraperitoneal injection of tritium-labeled glucocrticoids in the rats, 15% of corticosterone and 18% of cortisol became bound to blood serum Lp within 30 min. *In vitro* experiments gave even a higher percentage of steroids bound to Lp fractions. Main part of the label was present in the protein fraction of infranatant (Table 1).

In rats corticosterone is the main glucocorticoid hormone, their blood cortisol content being less than 1 μg/dL. In this connection, it was interesting to examine the cortisol distribution among rat blood Lp. Distribution of the tritium-labeled hormone after its intraperitoneal injection in rats was similar to that of corticosterone. The only distinction was a higher content of label in the VLDL fraction.

It is commonly accepted that a major part of glucocorticoids (up to 90%) in blood serum are bound to proteins, mainly to corticosteroid-binding globulin (transcortin); the other part of hormones remains free and is regarded as ‘biologically active’. Transcortin is a glycoprotein with molecular weight of 52 kDa. The protein is synthesized on hepatocyte ribosomes, which is followed by its glycosylation.

Since a large part of apolipoproteins is represented by glycoproteins, it was natural to suggest that steroids are bound to Lp via glucosaminoglycan-containing apolipoproteins. On the other hand,

**Table 1.** Distribution of labeled corticosteroids between blood serum Lp during *in vivo* and *in vitro* testing (% of the total radioactivity).

| Steroids | VLDL | LDL | HDL | Infranatant |
|---|---|---|---|---|
| *In vitro* | | | | |
| Pregnenolone | 18 ± 1.1 | 12 ± 0.5 | 8 ± 0.2 | 61 ± 2 |
| Desoxycorticosterone | 14 ± 0.9 | 8 ± 0.3 | 5 ± 0.2 | 73 ± 7 |
| Cortisol | 18 ± 1.1 | 14 ± 2.5 | 9 ± 0.2 | 58 ± 5 |
| Corticosterone | 16 ± 0.4 | 11 ± 2.5 | 7 ± 0.2 | 66 ± 4 |
| *In vivo* | | | | |
| Corticosterone | 7 ± 0.6 | 4 ± 0.6 | 4 ± 0.1 | 85 ± 6 |
| Cortisol | 12 ± 0.2 | 3 ± 0.6 | 3 ± 0.1 | 82 ± 5 |

Note: In each group, 4 experiments were performed.

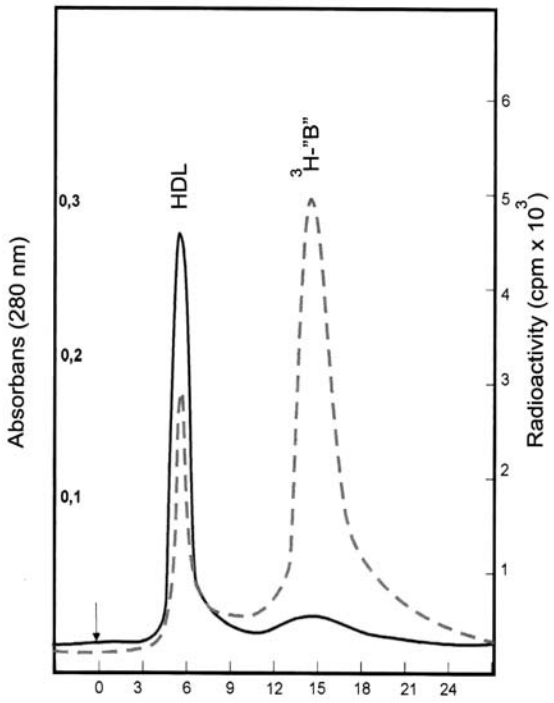


**Figure 1.** Chromatographic elution profile of HDL and $^3$H-corticosterone. Protein absorption - solid line. Distribution of radioactivity - dotted line. Column with Sephadex G-50 (1 x 20 cm). Eluent: 5 mM Tris/acetate, pH 7.4, 0.15 M NaCl.

a possible binding of steroids by the lipid phase of Lp could not be ruled out. Besides, we took into account that infranatant contains a substantial part of non-structural apolipoproteins. This is the so-called 'free pool' of apoLp, which can also bind steroid hormones.

The possibility of steroid transport by Lp was verified chromatographically. Figure 1 shows the elution profile of HDL fraction and radioactivity. The equal yields of labeled corticosterone and HDL indicate a possible formation of the hormone-Lp particle complex.

To compare different classes of Lp with respect to their binding ability toward steroid hormones, we used also the equilibrium dialysis, which is regarded as the most robust method for studying the processes of complex formation in a receptor-ligand system under thermodynamic equilibrium.

For equilibrium dialysis 0.5 ml of Lp (0.1-0.3 mg protein/ml) was placed in each dialysis tubing (8/32, Serva, Germany). Dialysis was performed for 48-72 h at 4°C against a 5 mM Tris/acetate buffer (pH 7.4), 0.15 M NaCl, containing tritium-labeled cortisol or corticosterone and the growing concentrations of a non-labeled ligand. Non-specific sorption of dialysis membrane measured in the control experiment was 11%.

The corticosterone saturation curve for the rat HDL fraction list the complex-forming process as a function of concentration of excessive non-labeled ligand introduced in the external buffer solution. This is a saturating curve that points to an increase in the number of HDL-corticosterone complexes during the saturation. Based on the calculation of binding parameters in Scatchard coordinates Table 2 lists the association constant ($K_a$) for different Lp classes obtained by equilibrium dialysis.

**Table 2.** Equilibrium association constant ($K_a$) for lipoproteins interaction with corticosterone and cortisol in Scatchard coordinates.

| Lp fractions | Corticosterone | Cortisol |
|---|---|---|
| VLDL | $(8 \pm 0.1) \cdot 10^6$ $M^{-1}$ | $(5 \pm 0.1) \cdot 10^6$ $M^{-1}$ |
| LDL | $(1 \pm 0.04) \cdot 10^6$ $M^{-1}$ | $(4 \pm 0.1) \cdot 10^6$ $M^{-1}$ |
| HDL | $(9 \pm 0.2) \cdot 10^6$ $M^{-1}$ | $(8 \pm 0.1) \cdot 10^6$ $M^{-1}$ |

Note. The values are averaged over 5 measurements. The corticosterone experiments were performed with rat serum Lp; the cortisol experiments, with human serum Lp.

High specificity and sensitivity of fluorescence methods underlie their wide application for analysis of the protein-ligand interaction. It is known that of three fluorescent amino acids (tryptophan, tyrosine, phenylalanine) only the tryptophan provides ample information on the structure of macromolecule. Tryptophan is regarded as a natural 'reporter label'; so parameters of its fluorescence give an insight into its microenvironment and transformations caused by certain conditions, in particular, by the formation of transcortin-steroid complexes. Besides, the tyrosine fluorescence (with excitation wavelength of 280 nm) is suppressed by polar groups (CO, $NH_2$) and adjacent peptide bonds; phenylalanine gives a very low quantum yield.

Examination of fluorescence spectra obtained from different Lp classes showed that they differ both in the form and in characteristic wavelengths of the maxima. HDL had a maximum at 333 nm, the fluorescence maximum of VLDL being in the region of 338 nm. Interestingly, the fluorescence of tryptophan in an aqueous solution reached its maximum at 354 nm. The characteristic short-wave shift of Lp tryptophanyl fluorescence can be attributed to specific features of the micro-environment: polar (aqueous) for tryptophan solution, and nonpolar (lipid) for the protein component of Lp.

The interaction of corticosterone with HDL was accompanied by tryptophan fluorescence quenching (Figure 2). A decrease in fluorescence intensity was most pronounced for HDL (55-65%) as compared to VLDL (30-35%) and LDL (15-20%). Shapes of the spectra and their half-width remained virtually unchanged. In all cases, a 'blue' 1-3 nm shift was observed, which is attributed to local conformational rearrangements

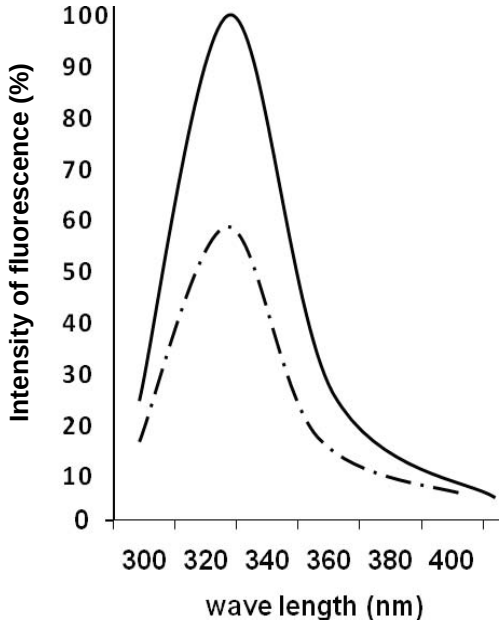


**Figure 2.** Tryptophan fluorescence quenching of HDL upon its interaction with the corticosterone: — HDL; — · — HDL + hormone.

of the protein component of Lp upon its interaction with the hormone.

Titration was performed by adding 1 μl aliquots of the hormone to 2 ml of Lp solution in a thermostated cuvette. The concentration of ethanol at the end of experiment was not higher than 0.5%. Fluorescence quenching caused by ethanol did not exceed 8-10% of the total fluorescence decay. The quenching had a saturable nature and attained its maximum only at a certain molar ratio of steroid and protein.

Our study of the time dependence of fluorescence quenching at the introduction of saturating amounts of corticosterone and cortisol gave nearly

the same curves for the two hormones. Total saturation of the binding regions of Lp particles with the hormones was observed within 20-30 minutes after the onset of experiment. The fluorescence decay and the recorded short-wave shift of the fluorescence maximum upon formation of steroid-Lp complex may be caused by quenching of tryptophan luminophores and by a relative increase in the contribution of total protein fluorescence due to tyrosine residues.

In the experiments with varying pH, a maximum value of tryptophan fluorescence of apoA-I (0.05 mg/ml) was observed at physiological pH. The addition of corticosterone to apoA-I did not change the spectrum shape, although a decay in tryptophan fluorescence made up nearly 40% of its initial value.

The fluorescence polarization method is highly convenient for the study of Lp binding to steroid hormones. Changes in fluorescence polarization are caused by altering mobility of the entire molecule or its part that contains fluorophores. Natural fluorophores (tyrosine, tryptophan) are present virtually in all proteins. The indole rings of tryptophan residues are remarkably sensitive and complex fluorophores. For some transport proteins, it is known that binding to a ligand increases the fluorescence polarization due to a decrease in fluorophore mobility. Thus, this effect is a sensitive indicator of the complex-forming processes.

In our studies, the extent of fluorescence polarization in HDL fractions was found to increase upon interaction with corticosterone or cortisol (Figure 3). A segment of fluorescence polarization steadily rising within the first 10 minutes appeared on the recorded curve.

Analysis of the Lp interaction with steroid hormones demonstrated that the most pronounced binding occurs with HDL, where the main structural and functional protein is represented by apoA-I. In this connection, the possibility of corticosterone binding by isolated apoA-I was investigated.

The obtained fluorescence quenching curves were used to calculate the values of equilibrium association constant ($K_a$) by the method of Attallah and Lata (Table 3). Molecular weights of apoA-I, HDL, LDL and VLDL were taken equal to $2.8 \cdot 10^4$, $3 \cdot 10^5$, $1 \times 10^6$ and $5 \cdot 10^6$ Da, respectively. Equilibrium association constant for the hormones with isolated apoA-I happened to be lower than

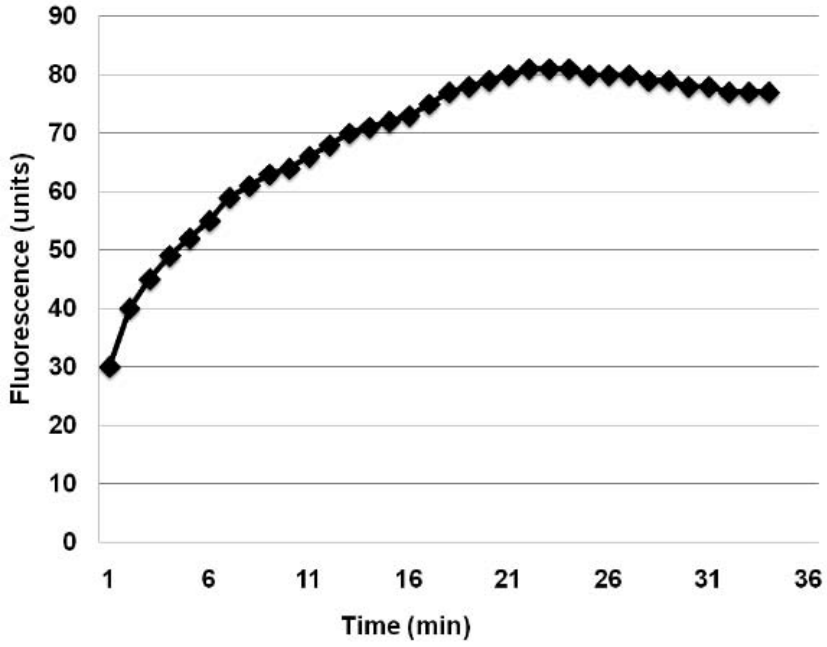


**Figure 3.** Changes in fluorescence polarization of rat HDL upon its interaction with the corticosterone.

**Table 3.** Equilibrium association constant (*K*a) for lipoprotein fractions upon interaction with corticosteroids as calculated from fluorescence quenching.

| Steroids | VLDL | LDL | HDL | ApoA-I |
|---|---|---|---|---|
| Corticosterone | $0.6 \cdot 10^6$ $M^{-1}$ | $0.67 \cdot 10^6$ $M^{-1}$ | $3.6 \cdot 10^6$ $M^{-1}$ | $0.8 \cdot 10^6$ $M^{-1}$ |
| Cortisol | $1.02 \cdot 10^6$ $M^{-1}$ | $0.14 \cdot 10^6$ $M^{-1}$ | $4.0 \cdot 10^6$ $M^{-1}$ | $0.3 \cdot 10^6$ $M^{-1}$ |
| Tetrahydrocortisol | $0.92 \cdot 10^6$ $M^{-1}$ | $0.28 \cdot 10^6$ $M^{-1}$ | $3.66 \cdot 10^6$ $M^{-1}$ | $0.56 \cdot 10^6$ $M^{-1}$ |
| Testosterone | $1.2 \cdot 10^6$ $M^{-1}$ | $0.27 \cdot 10^6$ $M^{-1}$ | $4.8 \cdot 10^6$ $M^{-1}$ | $0.7 \cdot 10^6$ $M^{-1}$ |
| Progesterone | $0.6 \cdot 10^6$ $M^{-1}$ | $0.64 \cdot 10^6$ $M^{-1}$ | $4.4 \cdot 10^6$ $M^{-1}$ | $0.5 \cdot 10^6$ $M^{-1}$ |
| Pregnenolon | $0.74 \cdot 10^6$ $M^{-1}$ | $0.53 \cdot 10^6$ $M^{-1}$ | $3.82 \cdot 10^6$ $M^{-1}$ | $0.45 \cdot 10^6$ $M^{-1}$ |

Note. The values are averaged over 5 measurements.

those for native HDL. This is caused most likely by removal of lipids, which are necessary for stabilization of the protein structure and maintaining a certain conformational interrelations that impart surfactant features to the protein.

Therefore, ultracentrifugation, chromatography, tryptophan fluorescence quenching and equilibrium dialysis allowed us to demonstrate that the blood serum lipoproteins bind steroid hormones and can serve as their active carriers in the organism. Affinity for steroids was most pronounced in HDL particles. Protein components of Lp were shown to be involved in the formation of Lp-steroid complexes, steroids being adsorbed most likely on the surface of Lp particles. ApoA-I is among the main apolipoproteins that can bind steroid hormones. More fine structural rearrangements of apoLp upon their interaction with steroid hormones were studied by means of IR spectroscopy.

**Changes of IR-spectra**

According to IR spectra, interaction between human HDL and cortisol results in a considerable increase in the intensity of absorption bands at 1654 $cm^{-1}$ (amide-I), 1546, 1520 $cm^{-1}$ (amide-II), 3292 $cm^{-1}$ (amide A), and produces a decrease in their half width, which indicates the growing moiety of $\alpha$-helixes (the tangle $\leftrightarrow$ $\alpha$-helix transition). The absorption bands at 1696 and 1630 $cm^{-1}$ slightly increased in intensity, which reflected the growth of $\beta$-structure moiety (the tangle $\leftrightarrow$ $\beta$ -structure transition) (Figure 4).

An increase in the integral intensity of amide I band comprised about ¼ ÷ ⅓ of the control sample. Upon resolving the amide I band of differential spectrum (curve 3) into components, the $\beta$-structure moiety exhibited a 10-12% increment, whereas $\alpha$-helixes accounted for 18-20%. Interestingly, a new absorption band appeared at 3242 $cm^{-1}$, probably the $\beta$-structure amide A [31]. In HDL, upon their interaction with cortisol, the change in the secondary structure was more pronounced than in apoA-I. A considerable increase was noted in the absorption band intensity at 1470 $cm^{-1}$, corresponding to the deformation oscillations of CH bonds; the splitting of this band also became more pronounced (1468 and 1456 $cm^{-1}$), which indicated the strengthening of interaction between hydrocarbon chains due to their increasing ordering [32].

The absorption band intensity of the ester lipid group CO bond rose markedly, which also indicated the increased ordering of hydrocarbon chains [33].

In addition, an about 5 $cm^{-1}$ frequency shift of the absorption band of the mentioned bond to the long wave region was observed, which was reflected on the differential curve by the appearance of asymmetry and a weak maximum at 1733 $cm^{-1}$. This can be explained by an increase in the number of hydrogen bonds that are likely to form between unesterified cholesterol and human apoA-I in native HDL.

The intensity of absorption bands at 1244 (P=O bond), 1088 and 1172 $cm^{-1}$ (P—O—C and C—O—C bonds, respectively) increased, as well as the valent oscillations of CH bonds at 2928 and 2852 $cm^{-1}$. As seen from the spectra, the HDL structure undergoes significant transformations

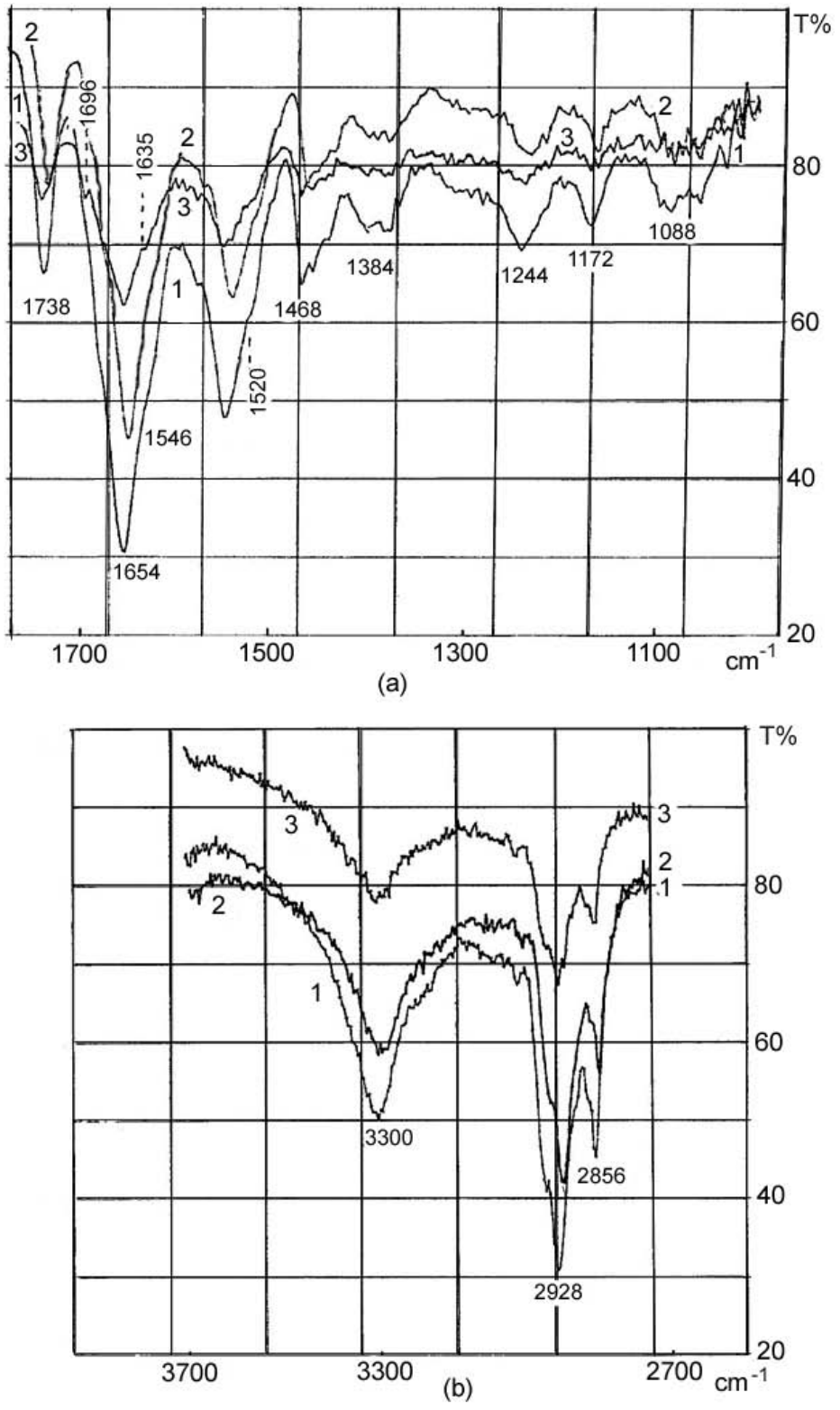


**Figure 4.** IR absorption spectra of human HDL preincubated with cortisol ($C_{cortisol}$ = 4.15·10$^{-5}$ M, $C_{buffer}$ = 4 mM, pH = 7.24): (a) $\nu$ = 1000-1800 cm$^{-1}$, (b) $\nu$ = 2700-3700 cm$^{-1}$; (1) HDL + cortisol, (2) HDL (control), (3) differential spectrum.

upon interaction with cortisol, with these transformations occurring in both protein and lipid moieties.

Analysis of IR spectra of rat HDL after their preincubation with cortisol ($1.6 \cdot 10^{-9}$ M) revealed some peculiarities. For example, intensity of the absorption bands at 1654, 1548, and 3296 $cm^{-1}$ decreased, and resolution of the absorption bands at 1696, 1684, 1635, 1624, and 1520 $cm^{-1}$ increased. This gave grounds to suggest a 5-7% decrease in the moiety of $\alpha$-helix and disordered structure (tangle), since the absorption band at 1654 $cm^{-1}$ is a derivative of the two structures: $\alpha$-helix and disordered structure [34]. One may assume also some increase in the $\beta$-structure moiety. In our opinion, the $\alpha$-helix $\rightarrow$ $\beta$-structure and tangle $\rightarrow$ $\beta$-structure transitions occur under the action of cortisol in HDL apoproteins, primarily in apoA-I. In addition, a considerable intensity increase was noted in the absorption band at 1744 $cm^{-1}$ corresponding to free oscillations of CO-ester bond in lipids, which indicated the increasing lipid ordering in deeper layers of lipoprotein particle, where contact with proteins is weaker (Figure 5).

Comparison of IR spectra in human and rat Lp after their preincubation with cortisol allowed to reveal the essential distinctions. In human HDL, upon interaction with cortisol the $\alpha$-helix and $\beta$-structure moiety increases against the background of decreasing moiety of disordered structure, which indicates the occurrence of the tangle $\rightarrow$ $\alpha$-helix and tangle $\rightarrow$ $\beta$-structure transitions. At the same time, in rat lipoproteins it was noted the process of despiralization and decreasing of the tangle against the background of increasing $\beta$-structure moiety, i.e., the $\alpha$-helix $\rightarrow$ $\beta$-structure and tangle $\rightarrow$ $\beta$-structure transitions occurred. In addition, the lipid ordering in human proceeded through the formation of new hydrogen bonds, probably of phospholipids with proteins and cholesterol or of cholesterol molecules with apoproteins [35]. At the same time, in rats the lipid ordering was not attended by changing the number of hydrogen bonds, which is likely to be caused by the lipid ordering in deeper layers of the lipoprotein particle, where contact with proteins is weaker or absent.

The features of human and rat HDL interaction with cortisol seem to be related to the degree of lipoprotein ordering. Analysis of IR spectra of the cortisol free human and rat HDL showed that the ordering of the HDL is higher in both protein and lipid components, which manifests itself in (i) a decrease in half-width of absorption bands in lipid (1744 $cm^{-1}$) and protein (1654 $cm^{-1}$) spectral regions and (ii) a resolution increase in many of them (1696, 1684, 1520 $cm^{-1}$). Decreasing half-width of absorption band with the maxima at 2928 and 2852 $cm^{-1}$, corresponding to the valent oscillations of CH bonds, along with a higher resolution of the bands at 1464, 1456, and 1436 $cm^{-1}$ (deformation oscillations of $CH_2$ groups) additionally indicate a higher ordering of HDL structure in rats than that in human (Figure 5) [34].

Transport forms of steroid hormones can penetrate into the cell nucleus and participate in the regulation of gene expression.

**Detection of apolipoprotein A-I, B, and E in the nuclei of various rat tissue cells**

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 (Figure 6). The term ‘immuno-reactivity’ is used because not only intact apolipo-proteins 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 (Figure 7). 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

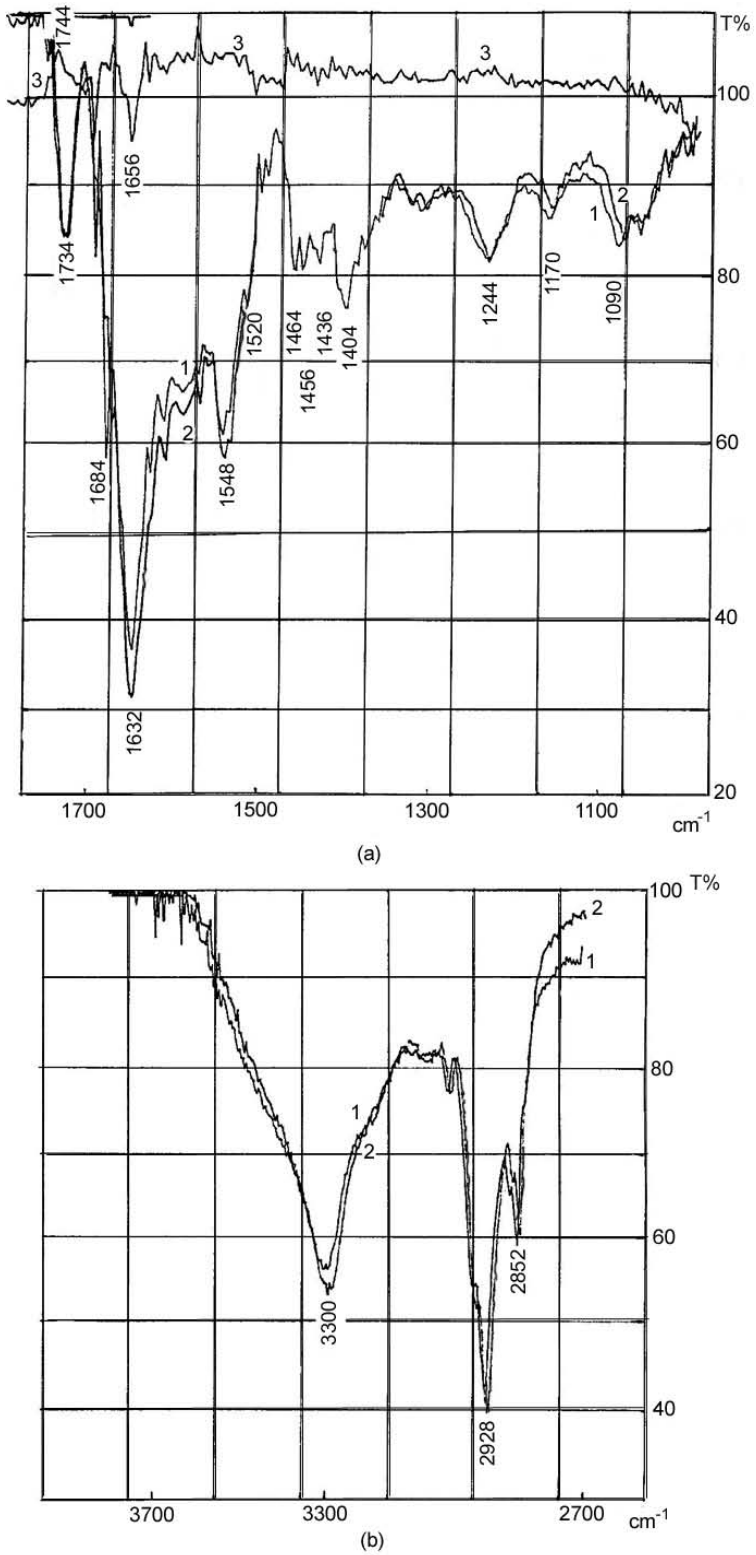


**Figure 5.** IR absorption spectra of rat HDL preincubated with cortisol ($C_{cortisol}$ = 4.15·10$^{-5}$ M, $C_{buffer}$ = 4 mM, pH = 7.24): (a) $\nu$ = 1000-1800 cm$^{-1}$, (b) $\nu$ = 2700-3700 cm$^{-1}$; (1) - HDL + cortisol, (2) - HDL (control), (3) - differential spectrum.

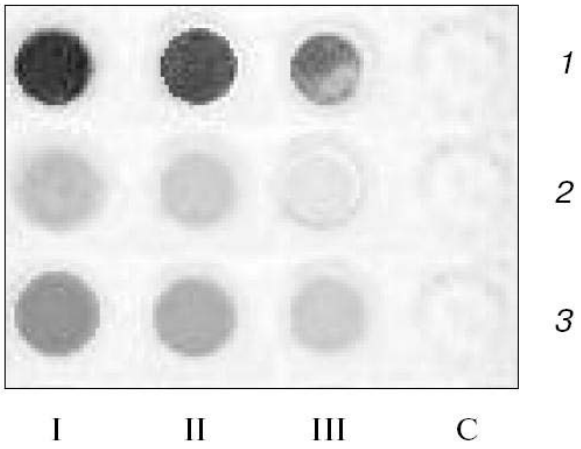


**Figure 6.** Dot immunoanalysis of apoA-I, apoB, and apoE immunoreactivity in liver nuclei in the fraction of acidic non-histone proteins. I, II, and III) dilutions 1:1, 1:2, and 1:10; C) control; 1) apoA-I immunoreactivity; 2) apoB immunoreactivity; 3) apoE immunoreactivity.

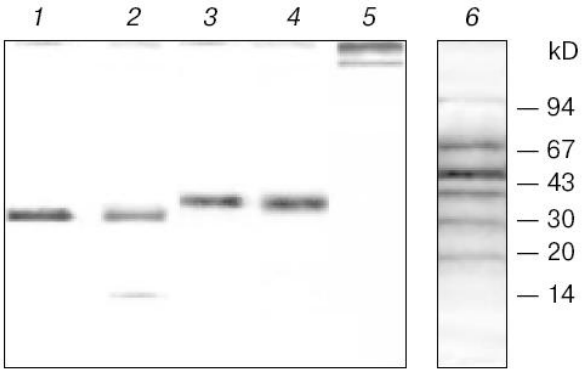


**Figure 7.** Immunoblot of proteins with apoA-I, apoB, and apoE immunoreactivity in rat liver nuclei: 1) apoA-I standard; 2) apoA-I immunoreactivity in rat liver nuclei; 3) apoE standard; 4) apoE immunoreactivity in rat liver nuclei; 5) apoB standard; 6) apoB immunoreactivity in rat liver nuclei.

weights varied significantly. The fraction with the highest molecular weight was about 100 kD, and the fraction with the lowest molecular weight was about 15 kD. The 60-kD fraction (molecular weight slightly lower than that of the serum albumin) had the highest intensity.

According to dot blot, apolipoprotein A-I is nonuniformly distributed between chromatin fractions (Table 4). 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.

**Table 4.** Assay of apoA-I immunoreactivity in chromatin fractions of rat liver nuclei.

| Chromatin fraction | ApoA-I content, ng/mg protein |
|---|---|
| Total chromatin | 60 ± 4.5 |
| Transcriptionally inactive chromatin | 52 ± 3.8 |
| Transcriptionally active chromatin | 100 ± 5.0 |
| Nuclear fraction | 110 ± 5.2 |

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 (Figure 8). 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 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

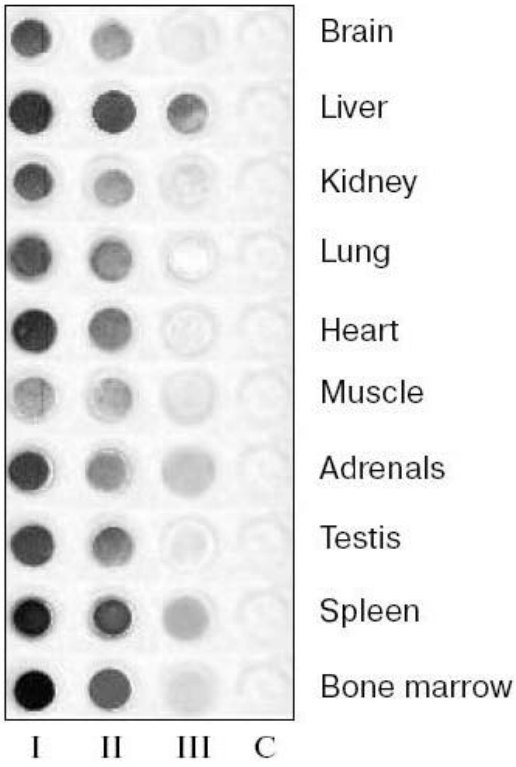


**Figure 8.** Dot immunoassay of apoA-I immunoreactivity in the acidic nuclear non-histone protein fraction of various tissues. I, II, and III - dilutions 1:1, 1:2, and 1:10; C - control.

**Table 5.** The degree of binding serum proteins to DNA immobilized on AE-cellulose.

| Immobilized DNA | Amount of bound protein (%) | | |
|---|---|---|---|
| | ApoA-I | BSA | IgG |
| Rat DNA | 40 ± 10 | 20 ± 5 | 9 ± 4 |
| Salmon milt DNA | 25 ± 5 | 18 ± 5 | 10 ± 4 |

of 100 kD. ApoE immunoreactivity includes a single band corresponding to the plasma apoE. A regulatory role of apoA-I was demonstrated previously [13, 14, 17]. Regulatory roles of the apoB fragments and apoE are now being studied.

**Sites of interaction of eukaryotic DNA with steroid hormone-apolipoprotein A-I complexes**

According to the literature data, some serum proteins, e.g., albumins and immunoglobulines, have high affinity to nucleic acids [36]. The results of affine chromatography listed in Table 5 show that apoA-I has even higher affinity to DNA as compared to BSA or immunoglobulines.

The concentration dependence of apoA-I modification by alkylating reagent 21N-RC1, based on the results of electrophoresis, is presented in Figure 9. One may see that even a small excess of oligonucleotide derivative results in a plateau, with the maximum degree of protein modification being rather low. In our opinion this may be explained by a small number of nucleophilic sites apt to alkylation in the vicinity of the sites of substrate binding and/or by their conformation inaccessibility under the conditions of modification reaction. The nature of concentration dependences for the other two reagents, 20N-RC1 and 28N-RC1, is similar to that presented in Figure 9. This implies that AT-(21N) or GC-rich (20N) sequences have no preferable affinity to apoA-I at least within the lengths of oligonucleotides we used, i.e., from 20 to 28. Figure 10 shows the concentration dependence of apoA-I protein modification by 21N-RC1 reagent in the presence of free oligonucleotide 21N. The maximum inhibition of protein modification occurs at the 50-fold molar excess of the inhibitor with respect to alkylating reagent. About one third of the initial modification is retained in this case, which indicates the competitive nature of inhibition, and hence the specificity of apoA-I modification. The error was calculated for the data of modification inhibition after their representation in Sketchard coordinates. It appeared to be rather high, ± 45%. This error is due to a low degree of protein modification, therefore, we estimated only the order of magnitude of the constant of protein association with oligonucleotide, which comprises $10^6$ $M^{-1}$.

Thus, for the first time the affinity of apolipoprotein A-I to eukaryotic DNA was demonstrated. The constant of such interaction was estimated on model synthetic heterodesoxy-ribooligonucleotides. The order of magnitude of this constant is comparable to that of immuno-globulines associated with homogeneous oligo-nucleotide $p(dT)_{16}$ [36]. This may indicate the general nature of interaction of serum proteins with the products of partial degradation of nucleic acids got into the blood channel.

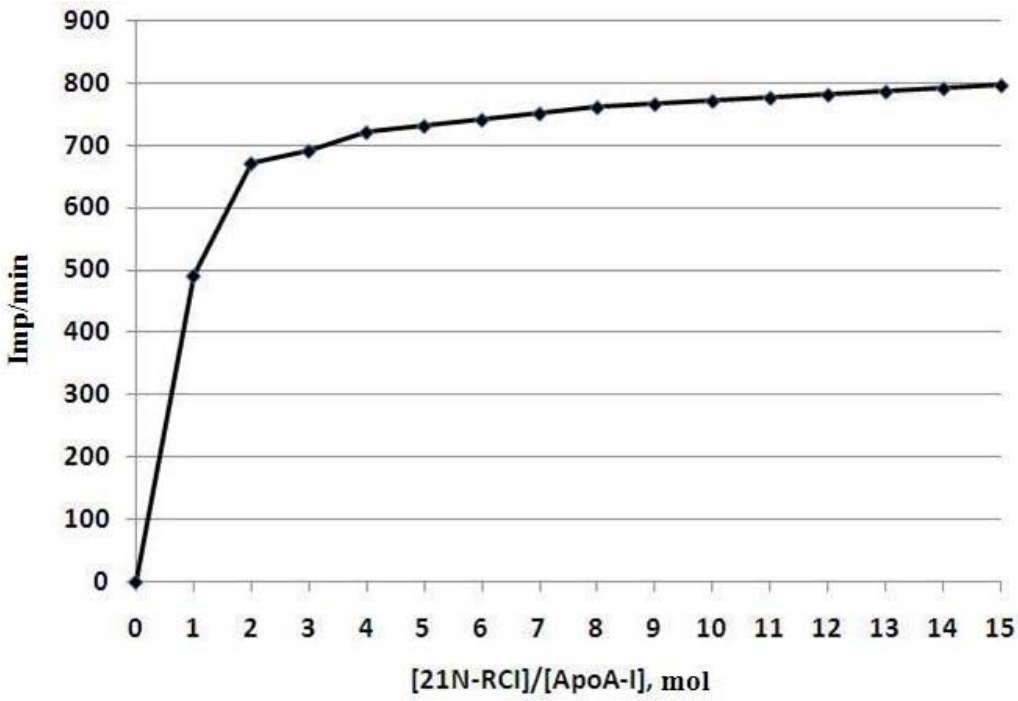


**Figure 9.** Dependence of apoA-I affine modification on alkylating reagent 21N-RC1 concentration. Abscissa – the inclusion of label $P^{32}$ in protein.

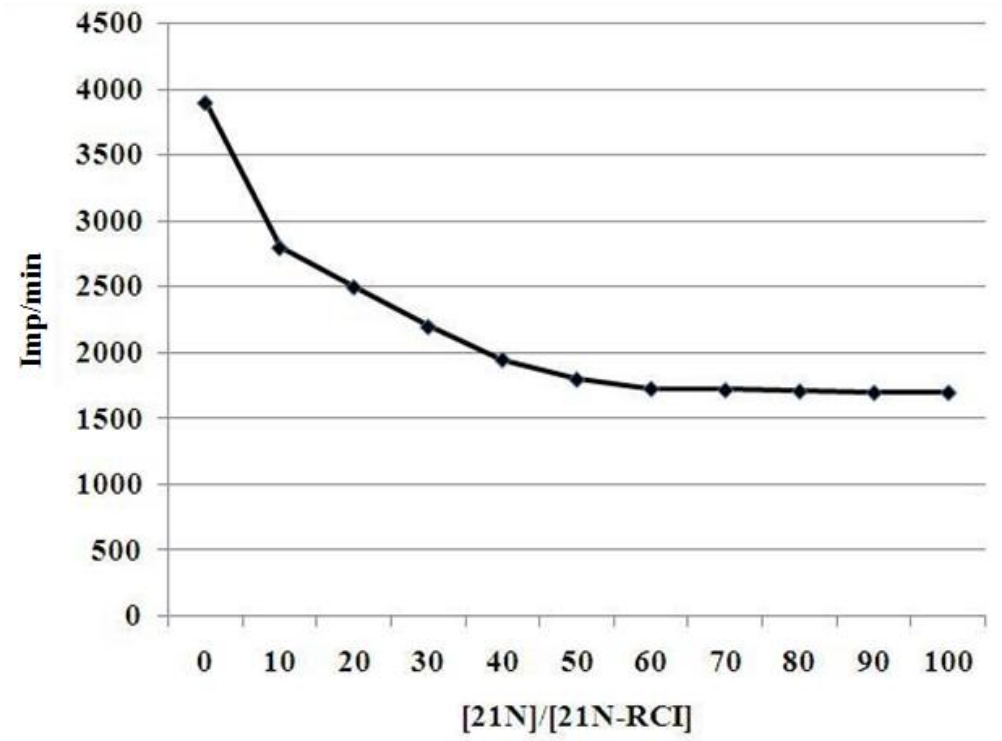


**Figure 10.** Dependence of apoA-I modification by alkylating reagent 21N-RC1 inhibition on the excess of free oligonucleotide 21N. Abscissa – the inclusion of label $P^{32}$ in protein.

To verify the formation of oligonucleotide-apoA-I associates in the presence of steroid hormone, their mixtures were analyzed by the gel-retardation method. One may see from Figure 11 that both apoA-I and its complex with THC form adducts with GC- (ON1) and AT-rich (ON2) oligonucleotides. Since ON1 and ON2 have a low molecular weight but bear a rather high negative charge as compared to those for apoA-I, adducts in gel are promoted rather than inhibited with respect to the initial protein (Figure 11, tracks 3-7). To reveal the nature of bonds in the oligonucleotide-THC-apoA-I adduct, a dissociating agent, 6 M urea, was added to the adduct before spreading the mixture over the gel (Figure 11, track 7). One may see that the adduct dissociated to a great extent though not completely. In our opinion this indicates the predominance of hydrophobic interactions and does not exclude the participation of electrostatic interactions in the formation of complex oligonucleotide-protein-steroid adducts.

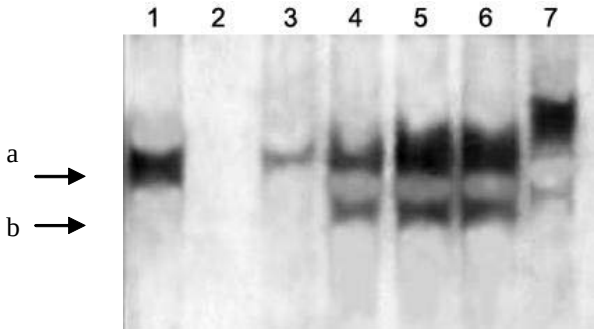


**Figure 11.** Electrophoregram separation of nucleic acids in native conditions in 15% PAAG after binding of oligonucleotides with ApoA-1 (in the absence or presence of THC): a - apoA-I; b - adduct apoA-I-THC-oligonucleotide. The gel is colored with Coomassie R-250. The amount of apoA-I: tracks 1, 3 - 3 mcg; track 4 - 6 mcg; tracks 5-7 - 9 mcg. Tracks: 1 - apoA-I, control; 2 - $CC(GCC)_3$; 3-5 - apoA-I + $CC(GCC)_3$; 6 - apoA-I-THC + $CC(GCC)_3$; 7 - apoA-I-THC + $CC(GCC)_3$, in 6 M urea.

The choice of nucleotide sequence of $CC(GCC)_n$ type as possible sites of binding the THC-apoA-I complex with rat DNA is not casual. The literature data are available on the sequences of this type found in the regulator regions of genes, e.g., in human apoA-I gene [14], in the gene of multiple drug resistance *mdr 1* [37], etc.

Direct localization of numerous single-stranded regions forming in DNA under the action of THC-apoA-I complex seems to be a difficult task. Therefore, we experimentally tested several synthetic oligonucleotides for the specificity of their interaction with steroid hormone – apoA-I complexes. Previously [38], association constants of apoA-I with some oligonucleotides were determined by SAXS in the absence and in the presence of cortisol and THC (Table 6).

One may see from Table 6 that association constant of GC-containing oligonucleotide increases 4.5-fold after replacement of cortisol in steroid hormone - apoA-I complex by its reduced form, tetrahydrocortisol. In the presence of THC, association constant increases 3-fold as compared to the protein-oligonucleotide association constant, while in the presence of cortisol it slightly decreases. Besides, note that the order of magnitude for the protein-oligonucleotide association constant determined in the experiments on affine modification agrees with the SAXS data within the experimental error. Based on these results, we selected two sequences for the study on competitive inhibition of THC-apoA-I interaction with rat DNA followed by splitting by S1 nuclease: $CC(GCC)_3$ and 5`-pATCTTTAACTGA TGAACTTCT (21N). One may see from Figure 12

**Table 6.** Constants of apoA-I association with oligonucleotides in the presence and in the absence of steroid hormones according to the SAXS data.

| Protein / oligonucleotide | $CC(GCC)_5$ | AT-rich | $T_{19}$ |
|---|---|---|---|
| apoA-I | $5.37 \cdot 10^5$ $M^{-1}$ | - | - |
| cortisol-apoA-I | $3.65 \cdot 10^5$ $M^{-1}$ | - | - |
| THC-apoA-I | $1.66 \cdot 10^6$ $M^{-1}$ | $3.11 \cdot 10^5$ $M^{-1}$ | $2.32 \cdot 10^5$ $M^{-1}$ |

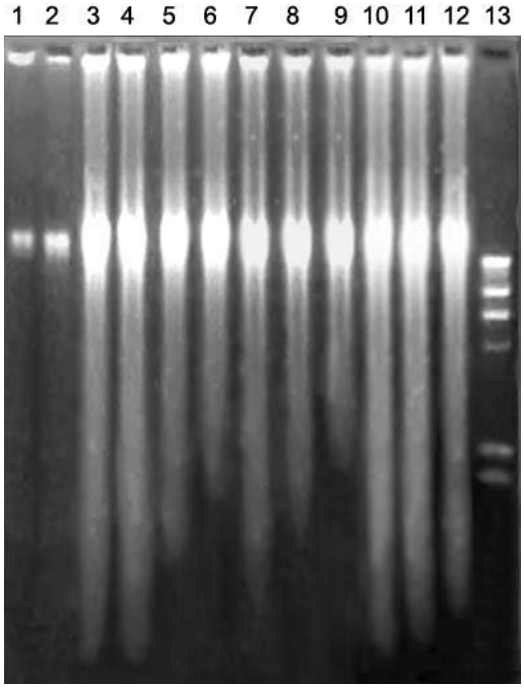


**Figure 12.** Competitive inhibition of DNA splitting by nuclease S1 at the interaction with THC-apoA-I complex in the presence of AT- and GC-rich oligonucleotides. Tracks: 1 – native DNA, control; 2 – DNA + nuclease S1; 3 - DNA + [apoA-I-THC]) + S1; 4-6 – (DNA + [apoA-I-THC]) + 0.12; 1.2; 12.0 ng ON1 + S1; 7-9 – DNA + ([apoA-I-THC] + 0.12; 1.2; 12.0 ng ON1) + S1; 10-12 - DNA + ([apoA-I-THC] + 0.12; 1.2; 12.0 ng ON2) + S1; 13 – DNA λ/Hind III.

(tracks 10-12) that AT-rich oligonucleotide actually does not affect the DNA splitting by S1 nuclease after its interaction with THC-apoA-I complex. On the contrary, $CC(GCC)_3$ inhibits this interaction, the strength of inhibition increasing with its concentration in the reaction mixture (Figure 12, tracks 3-5 and 6-8). The order of nucleotide addition does not matter, i.e., it competes successfully with DNA at binding with THC-apoA-I complex, preventing the emergence of single-stranded regions. Thus, it was shown that S1-nuclease-sensitive regions form at DNA interaction with THC-apoA-I complex in the sites of CCGCC type.

### The action of steroid hormone-ApoA-I complexes on the protein biosynthesis rate in cells

The studies made with hepatocyte culture showed that DHEA, DHEAS and THC in complexes with apoA-I strongly increase the rate of protein biosynthesis in cells (Figure 13).

Most pronounced was the action of DHEAS-apoA-I complex. Individually, apoA-I or each single hormone did not have a stimulating effect on protein biosynthesis in hepatocyte culture. Earlier it was shown that cortisol in a complex with apoA-I also did not increase the protein biosynthesis rate [13]. Apparently, it is exactly the 3-hydroxy group in reduced A-ring of steroids that is the key element of the mechanism. This leads also to a conclusion that hormones of the reticular and fascicular zones of adrenal cortex have synergism of action related with the enhancement of protein biosynthesis in cells. In glucocorticoid hormone, this effect appears only after the reduction of 3-keto group and double bond in the $4^{th}$ position of A-ring followed by the formation of biologically active complex with apoA-I. Both events are related with the activity of resident macrophages [17]. The complexes DHEAS (DHEA, THC)-apoA-I formation may also proceed due to macrophages, which capture the hormones in high density lipoproteins. A part of DHEAS (DHEA)-apoA-I complexes may form directly in blood due to the interaction of hormones with a free pool of apoA-I.

## DISCUSSION

Previously we have shown the effect *in vitro* of THC-apoA-I complex on the secondary structure of rat DNA [39], resulting in the emergence of single-stranded regions. It can be revealed by fluorescent probing, SAXS [16, 40] and fermentative analysis with splitting by S1 nuclease [41]. Formation of additional 1-2 S1-nuclease-sensitive regions was revealed at 100 t.p.n. of DNA. These regions being distributed mainly irregularly over the genome. However, among them some regions were found that produced fragments of size 5.5 and 6 kilobas after their splitting by S1 nuclease [17]. As shown in [42], the effect of THC-apoA-I complex on the DNA structure is dose-dependent and specific. Replacement of apoA-I by another protein of high-density lipoproteins, apoE, or replacement of THC by cortisol did not produce such an effect. Though apoA-I binds with DNA, it does not affect the DNA secondary structure [39]. At the same

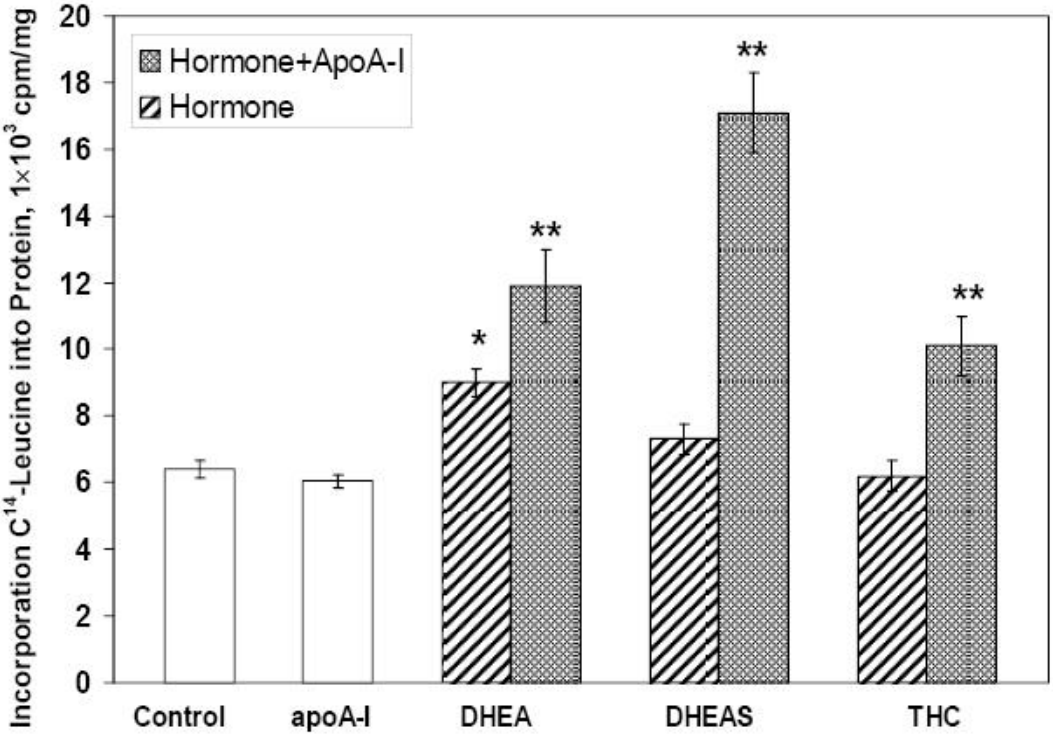


**Figure 13.** The effect of DHEA, DHEAS, THC and apoA-I on the protein biosynthesis rate in hepatocytes. *Reliable difference from control, $p < 0.05$. **Reliable difference from apoA-I, $p < 0.05$.

time, steroid hormones cortisol, THC, androsterone, and dehydroepiandrosterone form adducts with DNA and cause the emergence of some single-stranded regions, their amount being significantly lower than that of THC in complex with apoA-I [43]. It is macrophage-dependent promotion of protein synthesis by the cooperative action of high-density lipoproteins and cortisol [17]. Under such conditions, the rate of total protein synthesis increases much more significantly (2-2.5-fold) than the rate of gluconeogenesis ferments synthesis under the action of cortisol (1.1-1.2-fold) [44].

The interaction of hormone - apoA-I complex with GC-rich DNA regions results in a competitive rupture of hydrogen bonds in GC-pairs with participation of the active OH-group in the third position of the hormone A-ring (Figure 14). Local melting of DNA disturbs the stacking interaction between nitrous bases. ApoA-I, being the hormone carrier, contributes greatly to disordering of DNA secondary structure at the interaction sites. The action of hydrophobic forces between accessible nitrous bases and hydrophobic regions of apoA-I α-helices leads to further disordering of the DNA structure and its large-scale melting due to cooperative properties of the macromolecule. Such action of apoA-I manifests itself upon its excessive addition to the incubation medium and can be detected by IR spectroscopy. As was shown in our earlier works [41], this creates necessary conditions for the interaction of RNA-polymerase with DNA matrix and leads to enhancement of gene expression.

This regulation mechanism concerns the genes that have the (GCC/CGG)n sequences in their promoter regions. Among them are Human insulin-like growth factor binding protein 4 (IGFBP4) gene, Human multidrag resistance protein gene [37, 45], and many other genes. Promoter regions of the multidrag resistance and topoisomerase 2B genes containing GCC/CGG-sequences were analyzed using the TRANSFAC program. These regions were shown to include the binding sites of some transcription factors, such as Sp1, Adf-1, EGR2, and others. It is known that

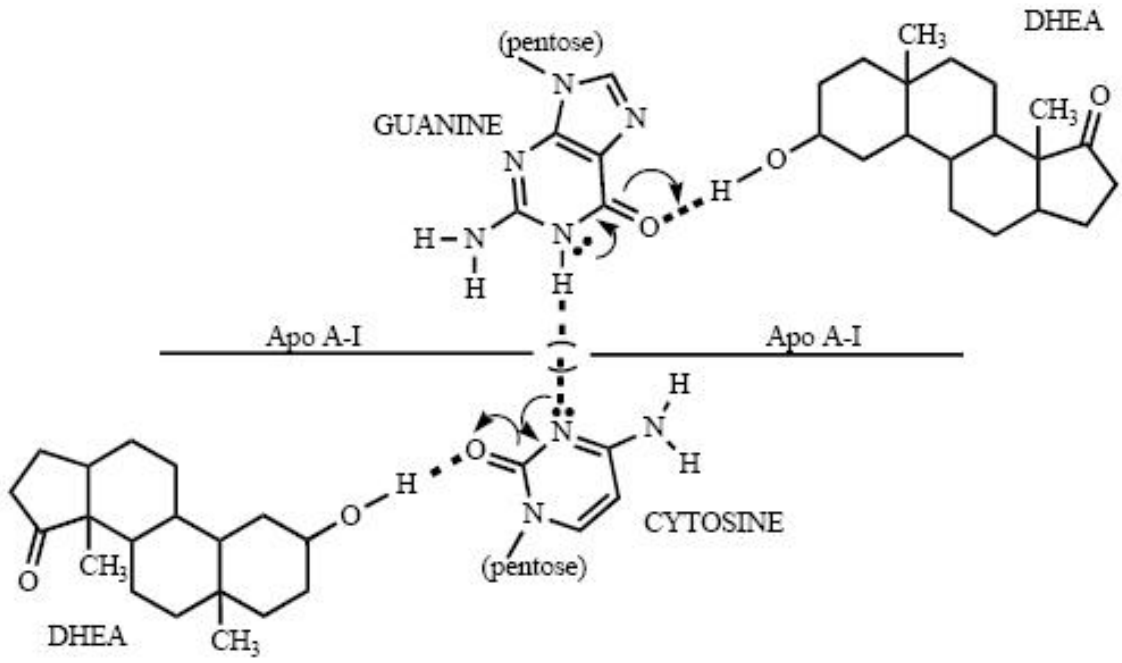


**Figure 14.** Schematic representation of the rupture mechanism of hydrogen bonds in GC-pairs of DNA under the action of DHEA –apoA-I complex.

TARI and MER22, the repetitive regulatory elements of genes, are GC-rich and also contain the (GCC/CGG)n sequences. TARI elements are associated with the telomeric regions. MER22 elements are present in the pericentric repetitive DNA of primates [46]. Sequences of (GCC/CGG)n type were found in the promoter region of mice ribosomal protein gene L32 and in the 3'-end region of human apoA-I gene [47]. All these data are in good agreement with our earlier results and evidence that apoA-I can be considered as a transcription factor.

## ABBREVIATIONS

Lp - lipoprotein
apoA-I - apolipoprotein A-I
apoLp - apolipoprotein
HDL - high density lipoproteins
LDL - low density lipoproteins
VLDL - very low density lipoproteins
THC - tetrahydrocortisol
THC - apoA-I complex - tetrahydro-cortisol-apoA-I complex
TNF-α - tumor necrosis factor-α-
SAXS - small-angle X-ray scattering
DHEA - dehydroepiandrosterone
DHEAS - dehydroepiandrosteronesulphate
PMSF - phenylmethylsulfonyl fluoride
$K_a$ - the equilibrium association constant
DOC - desoxycorticosterone
DTT - dithiothreitol
SDS - sodium dodecyl sulphate
DEAE - diethylaminoethyl
PMSF - phenylmethylsulfonyl fluoride
EDTA - ethylenediamine tetra-acetic acid
BSA - bovine serum albumin
IgG - immunoglobulines
PAAG - polyacrylamide gel
AE - cellulose
ON1 - CG-rich oligonucleotide
ON2 - AT-rich oligonucleotides
IR spectroscopy - infrared spectroscopy
IGFBP4 - insulin-like growth factor binding protein 4 gene

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