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Journal of Steroid Biochemistry & Molecular Biology 87 (2003) 309–318
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Tetrahydrocortisol–apolipoprotein A-I complex specifically interacts with eukaryotic DNA and GCC elements of genes

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Received 6 December 2002; accepted 9 September 2003

Abstract

Tetrahydrocortisol stimulates DNA and protein biosynthesis in hepatocytes only when it enters the complex with apolipoprotein A-I. Tetrahydrocortisol–apolipoprotein A-I (THC–apoA-I) complex specifically interacts with eukaryotic DNA isolated from rat liver. In the process of interaction, rupture of hydrogen bonds between the pairs of nitrous bases occurs with the formation of single-stranded DNA structures. In such state DNA forms complexes with DNA-dependent RNA-polymerase. The most probable site of binding the tetrahydrocortisol–apolipoprotein A-I complex with DNA is the sequence of $CC(GCC)_n$ type entering the structure of many genes, among them the structure of human apolipoprotein A-I gene. Oligonucleotide of this type has been synthesized. Association constant (K_{ass}) of it with tetrahydrocortisol–apolipoprotein A-I complex was shown to be $1.66 \times 10^6\,M^{-1}$. Substitution of tetrahydrocortisol for cortisol in the complex results in a considerable decrease of K_{ass}. It was assumed that in the GC-pairs of the given sequence tetrahydrocortisol itself participates in the formation of hydrogen bonds with cytosine, favoring their rupture with complementary base—guanine.

Keywords: Tetrahydrocortisol; Apolipoprotein A-I; DNA; Small-angle X-ray scattering

1. Introduction

Participation of lipoproteins in regulation of cell metabolism attracts considerable interest of many researchers. Ranganathan and Kottke [1] revealed the stimulating effect of HDL on apoA-I secretion in HepG2 cells. This effect was absent at the cells incubated with LDL, apoA-I, fibroblast growth factor (FGF), epidermic growth factor (EGF), and transformation growth factor (TGF). Physiological concentrations of HDL inhibited the synthesis of platelet-activating factor in endothelial cells [2]. In natural killer (NK) cells, chylomicrones and VLDL stimulated the secretion of IL-8, TNF-α, IL-1, IL-2, IF-γ, but inhibited the secretion of colony-stimulating factor in granulocytes and macrophages (G-CSF). Handwerger et al. [3] showed that even within 6 h after the incubation of human trophoblast cells with apoA-I the placental lactogen expression increased, while the presence of cyclohexymide and actinomycin D completely inhibited the lactogen synthesis.

Formerly we have shown that the cooperative effect of HDL and glucocorticoids accelerates the rate of ^{14}C-leucine incorporation to protein in the co-culture containing non-parenchymal liver cells and hepatocytes [4]. The resident macrophages of liver (the Kupffer cells) turned to play the key role in this cell system. Their stimulation by lypopolysaccharides (LPS) markedly activated the rate of protein synthesis [5]. This mechanism did not take place in the hepatocyte culture. In our following studies THC–apoA-I complex was shown to increase gene expression and the rate of protein biosynthesis in hepatocytes [6,7]. The complex was formed in resident liver macrophages–Kupffer cells, which, capturing the products of cell's degradation, cooperatively incept HDL_3 and steroid hormones. In the secondary lysosomes of KC, HDL_3 are disintegrated with the formation of apoA-I, and the Δ^4-3-ketogroup of glucocorticoid A ring is reduced. Due to exocytosis, the formed complex enters intercellular space, it is captured by parenchymal cells (hepatocytes) and transferred to nuclei, which results in an increased gene expression. Hepatocytes' chromatin activation under the effect of the complex THC–apoA-I was estimated by fluorescent probe binding and by electronic microscopy [8,9]. This mechanism starts when acceleration of regeneration

Abbreviations: apoA-I, apolipoprotein A-I; HDL, high-density lipoproteins; K_{ass}, association constant; KC, Kupffer cells; LP, lipoproteins; Mph, macrophages; NPC, non-parenchymal hepatic cells; RNA-PM, DNA-dependent RNA-polymerase; SAXS, small-angle X-ray scattering; THC, tetrahydrocortisol

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doi:10.1016/j.jsbmb.2003.09.004

processes is needed for the cells of parenchymal organs and tissues [10]. This phenomenon can be observed at the restoration period after intense physical load, at the regeneration period after partial liver resection, and in some other states [9]. The mechanism of molecular interaction between THC–apoA-I complex and DNA is still unknown. Structure of the sites binding this complex to DNA is also not clear.

The aims of the present work were: (1) to show that tetrahydrocortisol stimulates synthesis of DNA and protein in hepatocytes only when it composes the complex with apoA-I; (2) to study interaction of THC–apoA-I complex with eukaryotic DNA isolated from rat liver; (3) to perform data base search (GenBank, EMBL DNA library) for the supposed DNA fragment interacting with the complex; (4) to synthesize the same oligonucleotides and to show their interaction with the THC–apoA-I complex.

2. Materials and methods

2.1. Isolation of lipoproteins, production of apolipoprotein A-I

The study was carried out with Wistar rats weighing 180–200 g. Lipoproteins of blood serum were isolated by ultracentrifugation after chylomicrones removal from the blood serum [11]. Four main fractions of lipoproteins were obtained: VLDL ($0.94 < d < 1.006$ g/ml), LDL ($1.006 < d < 1.063$ g/ml), HDL_2 ($1.063 < d < 1.125$ g/ml), and HDL_3 ($1.125 < d < 1.21$ g/ml). Further the HDL_3 fraction was used. Delipidation of HDL was conducted with a cooled chloroform–methanol mixture (1:1), which was followed by multiple ether washing. An apo–HDL mixture was deposited over the column (1.6 cm × 100 cm) with 6B-CL Sepharose (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). Elution profile was recorded from a UV detector 2151 LKB (Sweden). The purity of apoA-I was tested by electrophoresis in polyacrylamide gel (PAG) with sodium dodecylsulphate (SDS) [12]. Apolipoprotein A-I formed a single band as a homogeneous 28 kDa protein. Protein bands were visualized with 0.1% Coomassie G-250 solution in the mixture of methanol and 10% acetic acid (1:1). Protein concentration in the samples was measured according to Lowry et al. [13].

2.2. Isolation of hepatocytes, DNA and protein synthesis in hepatocytes

Liver cells were isolated after recirculation perfusion of the organ with a 0.03% solution of collagenase-I (Boehringer Mannheim, Germany), as described earlier [7]. Hepatocytes were separated from nonparenchymal cells (NPC) by differential centrifugation with a centrifuge J2-21 (Beckman, USA). $(38 \pm 3) \times 10^6$ hepatocytes and $(40 \pm 3) \times 10^6$ NPC were isolated from 1 g of rat liver. Cell viability was evaluated by Tripan Blue exclusion test. It was shown that more than 90% hepatocytes and 95% NPC to be viable. The obtained hepatocytes were resuspended in 5 ml Krebs-Ringer phosphate buffer to give 2–3 mg cell protein per 1 ml buffer. The cells were incubated over 24-hole Linbro plane-tables (Germany) with collagen-I support for 24 h. THC was added into incubation medium with concentration 10^{-6} M, apoA-I and its complex with THC—with concentration 70 mkg/ml. The complex was obtained via incubation of apoA-I and THC in 10 mM Tris–HCl buffer, pH 8.6, with the molar ratio 1:2, for 5 min at room temperature. The rate of DNA synthesis was determined by insertion of ^{3}H-thymidine, while the rate of protein biosynthesis—by insertion of ^{14}C-leucine. In both cases, concentration of tracer in the culture medium was 37 kBk/ml. To measure the DNA radioactivity the cells were transferred to a GF/C filter and washed out successively with 10% trichloracetic acid (TCA) and 95% ethanol [14]. To measure the protein radioactivity the cells were transferred to a FN-16 filter pretreated with 0.1 M leucine solution in 10% TCA solution. Then the filters were washed out successively with TCA solution and ethanol–ether mixture [15]. The samples radioactivity was measured with a fluid-scintillation counter Mark-III (USA). Radioactivity was calculated in impulses per minute per a hole.

2.3. Choice of interaction sites between THC–apoA-I complex and DNA

The mechanism of activation of protein synthesis with participation of THC–apoA-I complex defines the processes of cell regeneration [9,10]. It means that this mechanism is associated with the concurrent expression of many genes. It follows that the DNA regulatory regions, sensitive to the action of the complex, must be widespread in the cell genome. The potential *cis*-element of the GCC-type meets these requirements. Computer search over the data base of mammalian nucleotide sequences (GenBank, EMBL DNA library) revealed the presence of a sequence of $CC(GCC)_3$ type at the 3′-end nontranslated section of the human apoA-I gene (Fig. 1). The same sequence (only with a greater number of iterations) was found in the regulatory region of many mammalian and human genes. The 5′-flanking sequence of Homo sapiens multidrug resistance protein gene is an example [16]. In our study, a similar sequence was chosen for specificity analysis of interaction with tetrahydrocortisol–apoA-I complex. For comparison, a fragment of kDNA-regulatory region of cytochrome P-450 gene and oligonucleotide T19 were used. Primary structures of oligonucleotides were as follows:

1. 5′-CCGCCGCCGCCGCCGCC-3′
2. 5′-ATCTTTAACTGATGAACTTCT-3′
3. 5′-TTTTTTTTTTTTTTTTTTT-3′

Synthesis of all three oligonucleotides was conducted at the Federal Research Center of virology and biotechnology

TCCCCCACGG CCCTTCAGGA TGAAAGCTGC GGTGCTGACC TTGGCCGTGC TCTTCCTGAC
GGGGAGCCAG GCTCGGCATT TCTGGCAGCA AGATGAACCC CCCCAGAGCC CCTGGGATCG
AGTGAAGGAC CTGGCCACTG TGTACGTGGA TGTGCTCAAA GACAGCGGCA GAGACTATGT
GTCCCAGTTT GAAGGCTCCG CCTTGGGAAA ACAGCTAAAC CTAAAGCTCC TTGACAACTG
GGACAGCGTG ACCTCCACCT TCAGCAAGCT GCGCGAACAG CTCGGCCCTG TGACCCAGGA
GTTCTGGGAT AACCTGGAAA AGGAGACAGA GGGCCTGAGG CAGGAGATGA GCAAGGATCT
GGAGGAGGTG AAGGCCAAGG TGCAGCCCTA CCTGGACGAC TTCCAGAAGA AGTGGCAGGA
GGAGATGGAG CTCTACCGCC AGAAGGTGGA GCCGCTGCGC GCAGAGCTCC AAGAGGGCGC
GCGCCAGAAG CTGCACGAGC TGCAAGAGAA GCTGAGCCCA CTGGGCGAGG AGATGCGCGA
CCGCGCGCGC GCCCATGTGG ACGCGCTGCG CACGCATCTG GCCCCCTACA GCGACGAGCT
GCGCCAGCGT TTGGCCGCGC GCCTTGAGGC TCTCAAGGAG AACGGCGGCG CCAGACTGGC
CGAGTACCAC GCCAAGGCCA CCGAGCATCT GAGCACGCTC AGCGAGAAGG CCAAGCCCGC
GCTCGAGGAC CTCCGCCAAG GCCTGCTGCT CGTGCTGGAG AGCTTCAAGG TCAGCTTCCT
GAGCGCTCTC GAGGAGTACA CTAAGAAGCT CAACACCCAG TGAGGCGCCC GCCGCCGCCC
CCCTCCCCGG TGCTCAGAAT AAACGTTTCC AAAGTGGG

Fig. 1. Human apolipoprotein A-I gene structure. The $CC(GCC)_3$ sequence is the potential *cis*-element and locates the position of (+6)–(+16) from the translational stop-codon, its coordinates are (+896)–(+906) before the signal of polyadenylation.

"Vector", Ministry of Health, Russian Federation. Cortisol (Serva, Germany) was used in the study, and its reduced form, tetrahydrocortisol, was kindly gifted by Academician Yu.A. Pankov (Russian Academy of Medical Sciences, Institute of experimental endocrinology, Moscow). Complexes of glucocorticoids with apoA-I were obtained by keeping their 2:1 molar ratio mixture in 0.05 M K-phosphate buffer, pH 7.4, for 5 min at room temperature. Its ability to form the complexes was demonstrated previously [17]. The formation of the complex occurs very fast because the steroid hormones readily interact with the hydrophobic surface of the amphipatic regions of apolipoprotein A-I. Its K_{ass} is equal to $(0.4 \pm 0.1) \times 10^6\,M^{-1}$ and is significantly higher for HDL—$(2.0 \pm 0.2) \times 10^6\,M^{-1}$, that is probably related with the influence of lipids on the protein conformation. The preparation of DNA-dependent RNA-polymerase of T7 phage was isolated from *E. coli* strain containing a cloned gene of RNA-polymerase from bacteriophage T7. Its purity was no less than 90% according to the data of electrophoresis under denaturating conditions.

2.4. Small-angle X-ray scattering

SAXS is very sensitive and precise physical method for analyzing the structural changes in macromolecules and examining their interactions in the solution and the kinetics of these processes. The potentialities of this method in studying the structural changes in biological macromolecules still remain far from being exhausted. The SAXS method was used to estimate the effects of interaction of eukaryotic DNA and three synthetic single-chain oligonucleotides with the THC–apoA-I complex. Small-angle X-ray patterns were measured with a Siemens diffractometer (Germany). Wave length of X-ray radiation $\lambda = 0.154$ nm (Cu Kα). Homogeneous preparations of apoA-I, RNA-PM, DNA and oligonucleotides with initial concentrations of 0.40, 1.52, 3.12, and 4.16 mg/ml, respectively, were used. To study the interaction effects of the native DNA with RNA-PM in the presence of apoA-I, mixtures of solutions of DNA, RNA-PM, apoA-I and complexes of apoA-I with glucocorticoids (cortisol and THC) of various concentrations were prepared preliminarily. Upon measuring the SAXS patterns, temperature of the initial preparations was 20 °C, while that of DNA and RNA-PM mixtures was 37 °C. To examine the interaction of oligonucleotides with apoA-I, mixtures of solutions of oligonucleotides with apoA-I and its complexes with glucocorticoids of various concentrations were prepared. Small-angle X-ray patterns were measured over the angle range $0.0245 \leq h \leq 3.423\,nm^{-1}$, where $h = 4\pi \sin(\theta)/\lambda$, 2θ is the scattering angle. In the SAXS experimental data corrections were made for background scattering and collimation, the X-ray plots were smoothed.

3. Results

It was shown for the first time that the reduced form of cortisol, tetrahydrocortisol, is the biologically active form of hormone. It contributes to the stimulation of both the protein and DNA biosynthesis in liver cells (hepatocytes). However, this effect appears only when the hormone forms a complex with apolipoprotein A-I (Table 1). Probably, apoA-I acts as a targeted carrier of hormone to the cells nuclei for its further interaction with certain sites of deproteinized DNA regions. We verified this hypothesis using the small-angle X-ray scattering technique.

Previously, the SAXS method was successfully used in our study on interaction of specific tRNA with tRNA-synthetases [18], of oligonucleotides with methyltransferases [19], in evaluating the structural changes in HDL

Table 1
Effect of THC–apoA-I complex on the rate of DNA and protein biosynthesis in hepatocytes

Incubation conditions	Insertion of ^{3}H-thymidine into DNA (imp/min per hole)		Insertion of ^{14}C-leucine into protein (imp/min per hole)	
	Without apoA-I	With apoA-I	Without apoA-I	With apoA-I
Control	4459 ± 157	3320 ± 511	760 ± 34.7	386 ± 21.1
Tetrahydrocortisol	3393 ± 333[a]	4928 ± 115[a,+]	692 ± 34.7	1098 ± 107.4[a,+]

[a] Reliable distinctions compared to the corresponding control.
[+] Compared to the parameters "without apoA-I", $P < 0.05$.

[20], and revealing the fractional composition of lipoproteins in plasma and blood serum [21]. SAXS was proven useful in studying the mechanisms of molecular interaction, in particular, allowing one to analyze stoichiometry and equilibrium constants of macromolecular complexes, structural and weight properties of macromolecules.

Fig. 2a shows the SAXS plots obtained for RNA-PM preparations, DNA (Fig. 2b), and apoA-I (Fig. 2c), and their mixtures with apoA-I and glucocorticoids (Fig. 3a). Radius of gyration (R_g) value for RNA-PM of T7 phage ($R_g = 6.83 \pm 0.15$ nm) obtained from the SAXS data is close to the value ($R_g = 6.6 \pm 0.3$ nm) reported for β′-subunit of RNA-PM from *E. coli* by other authors [22]. DNA and apoA-I structures were described in our work previously [7]. Free DNA in a solution is known to take the shape of Gaussian ball [23] with cavities filled by a solvent. Thus, the value of radius of gyration $R_g = 39.2 \pm 0.5$ nm, obtained by SAXS for native DNA isolated from rat liver, reflects a full volume filled with a knot of DNA double chain with inflections, and molecular mass estimated at 26×10^6 kDa agrees well with the literature data [24]. The DNA structure used in this work is presented in Table 2.

To assess the effects of RNA-PM and apoA-I interaction with DNA by SAXS, equilibrium mixtures of RNA-PM and DNA with apoA-I and its complexes with tetrahydrocortisol were used. Fig. 3 and Tables 3 and 4 present the SAXS plots and list the values of structural characteristics of scattering particles obtained from DNA preparation and DNA mixtures with RNA-PM and apoA-I in the presence and in the absence of THC. Values of R_g and $I(0)$ for various mixtures of apoA-I with DNA and RNA-PM were calculated from experimental data. One can see from Fig. 3 and Tables 3 and 4 that a marked interaction of DNA with apoA-I and RNA-PM was observed only in the THC presence. In the absence of THC small interaction of DNA with apoA-I and RNA-PM was observed. The size of P*n*S complexes (here P stands for RNA-PM; S denotes DNA aggregates with THC–apoA-I; *n* is the number of P molecules in the complex) within the accuracy of measurements corresponded to the size of DNA molecules in a free state ($R_g = 39.2 \pm 0.6$ nm), while reliably changed only the values of $I(0)$ related to concentration and molecular mass of P*n*S complexes [18,19]:

$$I(0) \sim N \times M^2, \quad (1)$$

where N, M are, respectively, the number and the mass of P*n*S complexes involved into scattering.

It is known that the integrity of secondary DNA structure can be judged from the occurrence of diffraction maximum in the angle range of $h \sim 0.266\,\mathrm{nm}^{-1}$ [24] at the SAXS plot corresponding to the diameter of DNA double chain (~2.2 nm). One can see in Fig. 3b that at $h \approx 0.27 \pm 0.02\,\mathrm{nm}^{-1}$ the diffraction maximum is clearly observed at SAXS plots from preparations of the native DNA and DNA mixtures with apoA-I without hormones, while with similar mixtures in the presence of THC the peak value decreases reliably. This indicates the rupture of secondary DNA structure in the process of DNA interaction with THC–apoA-I complex. Such interaction resembles the enzyme–substrate interaction, in which hormone plays the role of a cofactor. The interaction results in the rupture of hydrogen bonds between the pairs of DNA nitrous bases and the formation of single-stranded structures. To evaluate the stoichiometry of P*n*S complex formed due to RNA-PM (P) interaction with DNA-apoA-I-THC complex (S) we used a simplified scheme [7]:

$$n \times \mathrm{P} + \mathrm{S} \Leftrightarrow \mathrm{P}n\mathrm{S}, \quad (2)$$

where n is the number of protein molecules bound into P*n*S complexes. From estimates obtained in our SAXS experiments (Table 4) and from the results of studies reported in [18,19] one can assume that in these interactions the equilibrium is shifted essentially to the formation of complexes, i.e., it is highly cooperative. Then, upon formation of P*n*S complexes in the mixtures, parameter n in scheme (2) will grow up to some maximum value with increasing protein concentration (C_p) in the mixtures. Using (1) for estimation

Table 2
Structural and mass parameters of native DNA from rat liver estimated using SAXS

R_g (nm)	R (nm)	$V\cdot(\times10^{-3})$ (nm^3)	$M_s\cdot(\times10^{-6})$ (Da)[a]	$M_s\cdot(\times10^{-6})$ (Da)[a]	$M_s\cdot(\times10^{-6})$ (Da)
39.0 ± 0.5	50.3	533	482	26.0 ± 2.0	27.0 ± 5.0

V, R are the volume and radius of the sphere, respectively.
[a] $M_p = V\rho_0/1.66$ (Da), where $\rho_0 = 1.5$ g/cm^3.

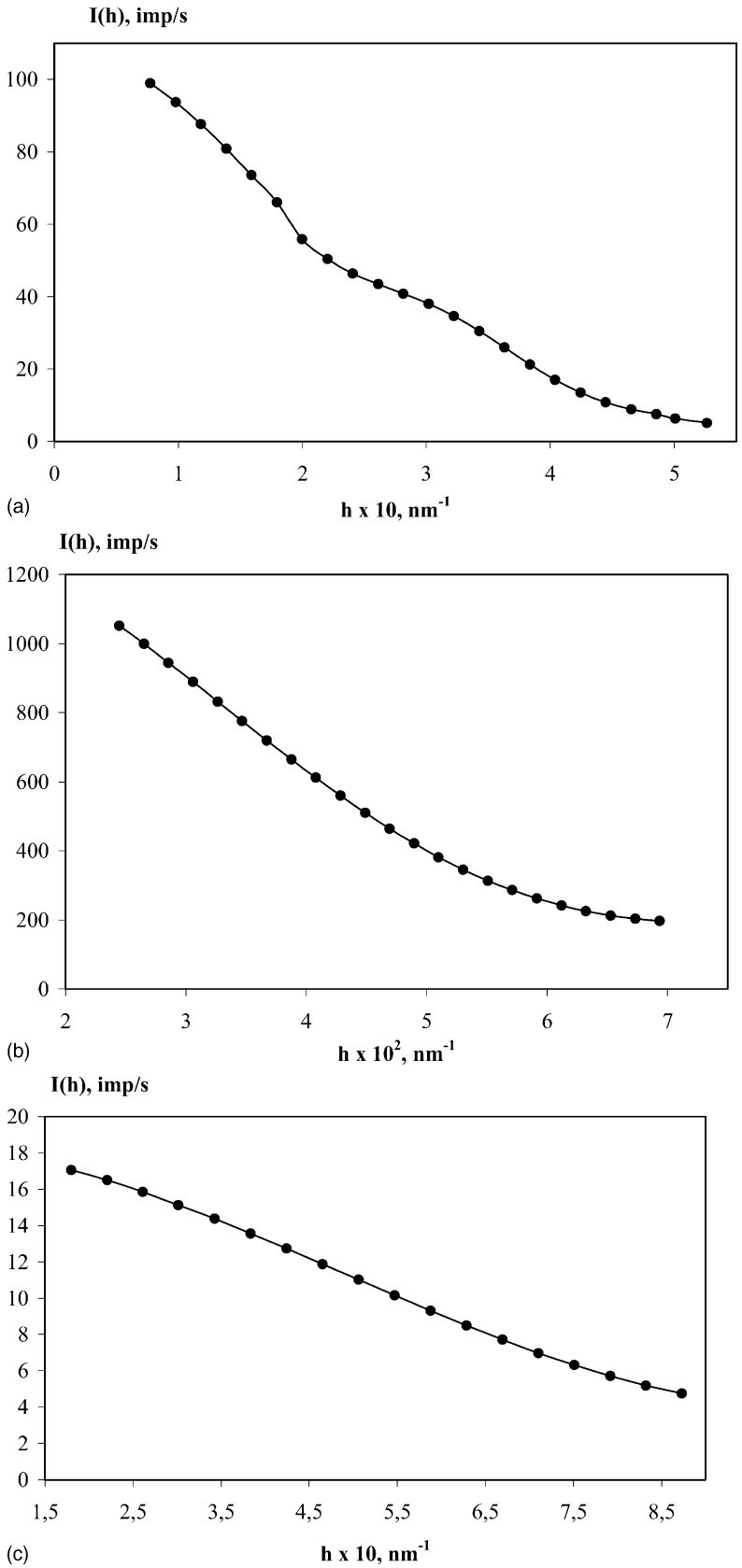

Fig. 2. SAXS plots of RNA-PM (a), DNA (b), and apoA-I (c) solutions in coordinates $I(h)$, h. Concentrations of RNA-PM, apoA-I and DNA in solutions: 4.16, 0.3 and 3.12 mg/ml, respectively. With smoothing and corrections for background scattering and collimation of X-ray beam.

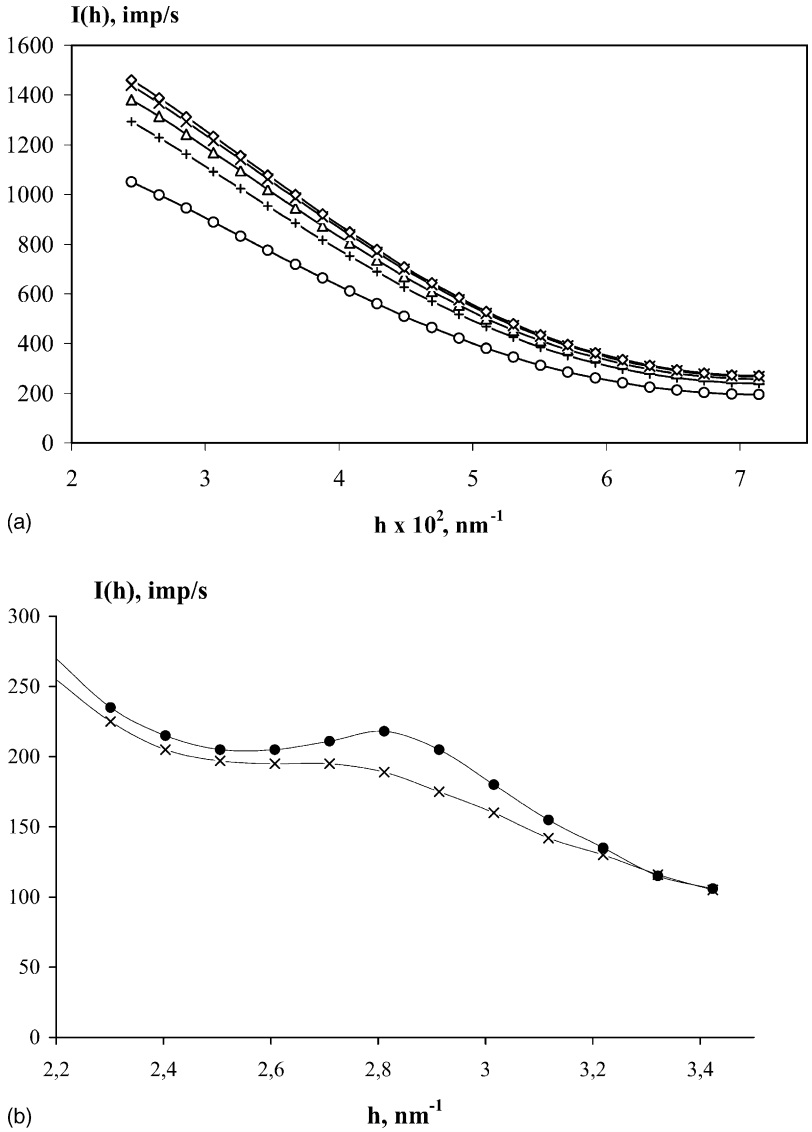

Fig. 3. SAXS plots of solutions of free DNA (○) and four DNA mixtures with apoA-I and RNA-PM in the presence of THC: (+) mixture 1; (△) mixture 2; (×) mixture 3; (◇) mixture 4 in the smallest angles (a) and in the region of diffraction peak at $h = 2.66\,\mathrm{nm}^{-1}$ (b). Plots of the mixtures were taken at 37 °C. DNA, RNA-PM and apoA-I concentrations in mixtures 1–4 are shown in Table 4.

of the values of parameter n in each protein–DNA mixture, one can obtain the expression [7]:

$$n = \frac{M_s(T_{i0} - 1)}{M_p}, \quad (3)$$

where $T_{i0} = (I_i(0)/I_0(0))^{1/2}$; $I_i(0)$ and $I_0(0)$ are the intensities of scattering to the zero angle ($h = 0$) from the i-mixture and initial DNA preparation, respectively.

In ref. [7] at the same component concentrations we obtained the value n for apoA-I upon complexation with DNA in the presence of THC equal to 54 ± 1. Table 4 presents the calculation results for parameter n and the estimates of C_p/C_s balance for all RNA-PM mixtures with DNA used in the study in the presence of THC–apoA-I complexes, taking $I_0(0) = 1605$ imp/s. One can see that the n value in the mixtures does not exceed the values of the balance and in mixture 4 reaches its maximum at 6 ± 1. This corresponds to a 6:1 stoichiometry of the formed PnS complex.

In the above results, the sites of binding the THC–apoA-I complex to DNA remained unclear. Search for the supposed sequences over existing data bases revealed the presence of

Table 3
Average values of radius of gyration (R_g) and intensities of X-ray scattering to the zero angle ($I(0)$) obtained from the SAXS data, at molar concentrations of RNA-PM (C_p) and DNA (C_s) in the presence (+) and in the absence (−) of THC and apoA-I in the mixtures

THC	ApoA-I	C_p (mkM)	C_s (mkM)	R_g (nm)	$I(0)$ (imp/s)
−	−	−	0.043	39.1 ± 0.5	1450 ± 11
−	−	0.140	0.043	38.8 ± 0.7	1485 ± 15
−	3.55	0.140	0.043	39.3 ± 0.6	1490 ± 12
+	3.55	−	0.043	39.2 ± 0.4	1605 ± 10

Table 4
Average values of radius of gyration (R_g), intensities of X-ray scattering to the zero angle ($I(0)$), and the number of RNA-PM molecules (n) in PnS complexes obtained from the SAXS data, at molar concentrations of apoA-I, RNA-PM (C_p) and DNA (C_s) in the mixtures

Mixture	ApoA-I (mkM)	C_p (mkM)	C_s (mkM)	R_g (nm)	$I(0)$ (imp/s)	n	C_p/C_s
1	3.55	0.140	0.043	39.0 ± 0.6	1764 ± 5	2	3
2	3.55	0.238	0.043	38.9 ± 0.7	1881 ± 7	4	5
3	3.55	0.380	0.043	39.2 ± 0.5	1960 ± 8	5	9
4	3.55	0.568	0.043	39.7 ± 0.8	1988 ± 8	6	13

Measurements were made in the presence of THC in the mixtures. For the complex (DNA + apoA-I) at $C_s = 0.043$ mkM, $I(0) = 1605 \pm 9$ imp/s.

cis-element of $(GCC)_n$ type in the structure of human apoA-I gene (Fig. 1). It locates at the position of (+6)–(+16) nucleotide pairs from the translational stop-codon. Its position from the transcription starting point is (+896)–(+906) nucleotide pairs [7]. A similar sequence was revealed in the regulatory regions of many mammalian and human genes. It is quite conservative in terms of evolution and plays, probably, an important role in regulation of these genes expression.

Further studies were performed with three synthetic oligonucleotides, whose structure was presented above (see Section 2). Figs. 2 and 4 show SAXS plots obtained from solutions of apoA-I (Fig. 2c) and oligonucleotide 1 (Fig. 4). Values of some structural characteristics of these macromolecules were calculated from the analysis of experimental data. ApoA-I molecules were shown to possess a slightly oblong shape with the ellipsoid axes ratio $b/a = 3.6$, radius of gyration $R_g = 2.35$ nm and molecular mass $M = 27.4$ kDa, which is close to the values we obtained earlier [7]. It is known that macromolecules of short single-chain oligonucleotides in a solution can have a common shape of the particles like ellipsoid of revolution or cylinder [19]. Thus, the values of radius of gyration ($R_g = 1.2 \pm 0.06$ nm) and volume ($V = 6.308\,\text{nm}^3$) we obtained for oligonucleotide 1 correspond to the structure of cylinder with 0.727 nm radius and 3.8 nm height.

To estimate by SAXS the effects of apoA-I interaction with oligonucleotides we used the equilibrium mixtures of oligonucleotides with apoA-I and its complexes with THC and cortisol. We prepared four main titration mixtures of apoA-I with oligonucleotide 1, two mixtures for estimating the effects of apoA-I interaction with oligonucleotides 2 and 3, and two mixtures for determining the THC and cortisol effect on apoA-I interaction with oligonucleotide 1. Average R_g values were calculated as well as differential intensities of scattering to the zero angle ($\Delta I(0)$) for all mixtures of apoA-I with oligonucleotides and steroids. Table 5 lists the values of calculated characteristics of the interaction, obtained from preparation apoA-I and its mixtures with oligonucleotides in the presence and in the absence of THC in the medium. One can see in Table 5 that substantial interaction of apoA-I was observed only with oligonucleotide 1. It should be noted that in the medium without THC or when it was substituted for cortisol, the effect of oligonucleotide 1 interaction with apoA-I was much lower as compared to the control mixture 2.

The changes of $\Delta I(0)$ value in mixtures 1–4 indicate that complexes of initial molecules form in the mixtures. The average size ($R_g = 2.38 \pm 0.07$ nm) of PmSn complexes (P is the aggregate of apoA-I with THC; S the oligonucleotide; m, n are the number of protein and oligonucleotide molecules

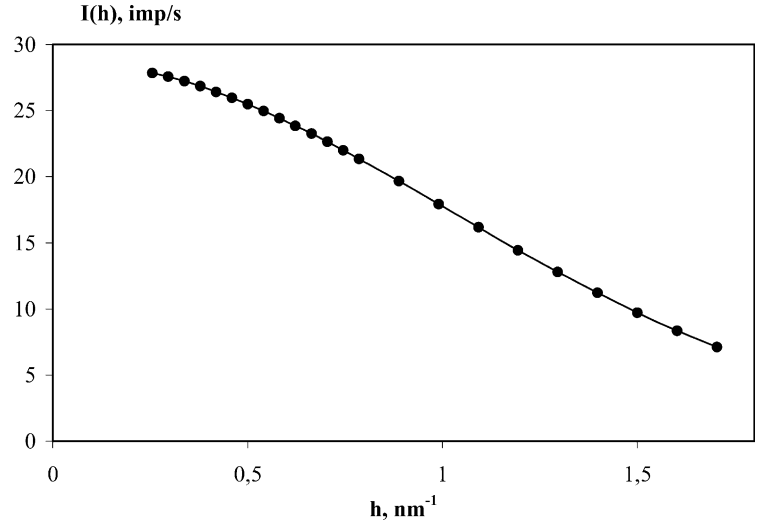

Fig. 4. SAXS plot of oligonucleotide 1 solution in coordinates $I(h)$, h. Oligonucleotide concentration 1.52 mg/ml with corrections for background scattering and collimation of X-ray beam.

Table 5
Average values of radius of gyration (R_g), experimental ($\Delta I(0)$) and simulated ($\Delta J(0)$) differential intensities of X-ray scattering to the zero angle obtained from the SAXS data, at molar concentrations of protein (C_p) and oligonucleotide (C_s) in the mixtures

Mixture	Oligonucleotide	C_p (mkM)	C_s (mkM)	R_g (nm)	$\Delta I(0)$ (imp/s)	$\Delta J(0)$ (imp/s)
1	Oligo-1	6.6	51.5	2.25 ± 0.04	3.2 ± 0.3	3.16
2	Oligo-1	9.9	25.7	2.32 ± 0.05	2.9 ± 0.4	2.93
3	Oligo-1	11.3	14.3	2.35 ± 0.05	2.1 ± 0.2	2.06
4	Oligo-1	12.1	8.6	2.36 ± 0.05	1.1 ± 0.3	1.40
5	Oligo-2	9.9	25.7	2.35 ± 0.05	0.4 ± 0.3	–
6	Oligo-3	9.9	25.7	2.37 ± 0.05	0.3 ± 0.2	–
7[a]	Oligo-1	6.6	51.5	2.33 ± 0.05	1.5 ± 0.3	–
8[b]	Oligo-1	6.6	51.5	2.28 ± 0.04	1.1 ± 0.2	–

In mixtures 1–6 THC is present with concentration 2×10^{-4} M. Primary structures of single-chain oligonucleotides: oligo-1 (oligonucleotide 1), oligo-2 (oligonucleotide 2), oligo-3 (oligonucleotide 3) are presented in Section 2.

[a] In mixture 7 hormones are absent.

[b] In mixture 8 cortisol is present with concentration 2×10^{-4} M.

which bound into P*m*S*n* complexes) is close to the size of apoA-I molecules in a free state, while the reliable changes were observed only in the values of $\Delta I(0)$ related to concentration and molecular mass of the complexes, similar to [7]; however, here $M = mM_p + nM_s$ (M is the molecular mass of P*m*S*n* complexes involved into scattering).

To estimate the maximum stoichiometry of the complex formed as a result of interaction between oligonucleotide 1 (further oligonucleotide) and apoA-I we used the SAXS experimental data from the first four mixtures, the highly cooperative equilibrium scheme, and the methodology we developed earlier [18,19]:

$$m \times \mathrm{P} + n \times \mathrm{S} \Leftrightarrow \mathrm{P}m\mathrm{S}n,$$
$$K_{mn} = \frac{[\mathrm{P}]^m \times [\mathrm{S}]^n}{[\mathrm{P}m\mathrm{S}n]}, \quad (4)$$
$$R(m, n, K_{mn}) = \frac{2}{p} \frac{\sum |\Delta I_i(0) - \Delta J_i(0)|}{\Delta I_i(0) + \Delta J_i(0)},$$

where K_{mn} is the dissociation constant for P*m*S*n* complex; [P], [S] and [P*m*S*n*] are the equilibrium concentrations of protein, oligonucleotide and complex, respectively; R the optimization criterion; $\Delta I_i(0)$ and $\Delta J_i(0)$ are experimental and simulation values of differential intensities of scattering to the zero angle ($h = 0$).

It follows from the data of Table 6 that the minimum value of R criterion corresponds to 1:1 stoichiometry of the formed complex ($m = 1$, $n = 1$) at the association constant (K_{ass}) $= 1.66 \times 10^6\ \mathrm{M}^{-1}$. Using the $\Delta I_i(0)$ values obtained for mixtures 7–8 (see Table 5), we estimated the K_{ass} values for these mixtures, which appeared equal to $5.37 \times 10^5\ \mathrm{M}^{-1}$ without THC in the mixture and $3.65 \times 10^5\ \mathrm{M}^{-1}$ when THC was substituted for cortisol.

Table 6
Values of optimization criterion R (%) for the $m \times n$ matrix calculated from the SAXS experimental data

m	n		
	1	2	3
1	2.8 ($K_{ass} = 1.66 \times 10^6\ \mathrm{M}^{-1}$)	27.3	36.9
2	27.4	5.9	8.9
3	40.5	26.5	15.5

4. Discussion

An action of HDL on chromosomal DNA from the different tissue and cells attracts considerable attention of researches. At present, the mitogen-stimulating function of HDL is widely discussed in the literature. The stimulating effect of HDL was demonstrated for the proliferation of lymphocytes [25], epithelial [26], endothelial [27], smooth muscle [28], and tumor [29] cells. It is known that in the G_1-period of mitotic cycle the mRNA and protein synthesis increases, in the S-period the mRNA, rRNA and protein synthesis increases, in the G_2-period the mRNA, rRNA and protein synthesis proceeds and proteins-initiators are formed, in the M-period proteins-initiators are activated and DNA is reduplicated. In the absence of protein synthesis the cells cannot pass the mitosis stage and enter the next mitotic cycle. The causes of these cell changes in premitotic period are still not clear.

Structural analysis of the genes presented in International banks (GenBank, EMBL DNA library) revealed the presence of recurrent $(GCC)_n$. These recurrences are found in both prokaryotic and eukaryotic genes, in human genes as well. The number of such recurrences varies and may be very great. Fig. 1 shows the structure of human apolipoprotein A-I gene, where three such recurrences are present. Such recurrences (“packets”) stabilize the DNA structure, at the same time they hamper its reduplication. They imply a special mechanism favoring the rupture of hydrogen bonds in such “packets”. Our studies allow to conclude that the formation of THC–apoA-I complex may serve as such mechanism.

Formation of this complex is related to the resident macrophages, which control the intracellular regeneration

and proliferation of cells. They possess a remarkable ability to restore double bonds in A ring of steroid hormones yielding tetrahydrocompounds. This process is catalyzed by α- and β-reductases [30]. Our studies showed that tetrahydrocortisol and other steroid hormones form the biologically active complex with apoA-I enhancing the synthesis of DNA, RNA, and protein [7,10]. In this case, apolipoprotein A-I acts as a targeted carrier to the cells nuclei. This complex is formed also in macrophages. The source of apoA-I are HDL, which are cooperatively captured by macrophages together with the products of cells degradation [9].

Thus, for the first time in the present work a specific interaction of THC–apoA-I complex with eukaryotic DNA and the kinetics of this interaction were described, structural characteristics of apoA-I, oligonucleotide $CC(GCC)_5$, and their complexes in the presence of cortisol and THC were determined. It was assumed that THC–apoA-I complexes interact with DNA regions containing $(GCC)_n$ elements, which results in the rupture of hydrogen bonds between the pairs of DNA nitrous bases. Partially untressed sections of DNA double chain are the targets for RNA-PM, whose binding and functioning are greatly affected by local denaturation of DNA and formation of single-stranded regions [6], though it is known that RNA-PM can bind also to the native DNA. It was shown for the first time that the reduced forms of glucocorticoids, tetrahydrocompounds, are the biologically active form of hormone involved in the enhancement of gene expression, entering the composition of THC–apoA-I complex. In this case, apoA-I acts as a targeted carrier, and reduced form of hormone favors the rupture of hydrogen bonds between the pairs of DNA nitrous bases, causing its local denaturation and creating prerequisites for interaction of DNA-dependent RNA-polymerase with single-stranded DNA regions. The described mechanism of gene expression enhancement can be the cause of protein biosynthesis activation in hepatocytes under the action of THC–apoA-I complex [7].

Acknowledgements

The work was supported by the Russian Foundation for Basic Research, grant no. 00-04-49261.

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