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Effect of Tetrahydrocortisol–Apolipoprotein A-I Complex on the Secondary Structure of Eukaryotic DNA and Its Interaction with RNA-Polymerase

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Abstract—The *in vitro* effect of tetrahydrocortisol–apolipoprotein A-I complex on native adult rat liver DNA results in the formation of S1 nuclease sensitive fragments that are irregularly distributed throughout a genome. Low-angle X-ray scattering showed that after the interaction with the tetrahydrocortisol–apolipoprotein A-I complex, DNA can bind to RNA-polymerase with a high and dose-dependent cooperativity. This indicates that the effect of tetrahydrocortisol–apolipoprotein A-I complex on secondary eukaryotic DNA structure causes a local denaturation of the double helix, promoting high cooperativity of binding to RNA-polymerase. The reduced form of the hormone, tetrahydrocortisol, previously considered as an inactive metabolite, when complexed with apolipoprotein A-I, promotes a biological function similar to that of a transcription factor.

Key words: DNA secondary structure, apolipoprotein A-I, tetrahydrocortisol, S1-nuclease, RNA-polymerase, low-angle X-ray scattering

Earlier it was established that glycocorticoids and high-density lipoproteins cooperatively increase the rates of RNA and protein biosynthesis in white rat liver [1, 2]. The mechanism of this phenomenon differs from the known mechanism of glucocorticoid enzyme induction—gluconeogenesis [3]. Further research revealed that an active hormone form, functioning within the nucleus in cooperation with apolipoprotein A-I (Apo A-I), appears to be its reduced form, tetrahydrocortisol [4], which was previously believed to be an inactive metabolite [5]. Fluorescent probing of native rat liver DNA with acridine orange demonstrated that cortisol–Apo A-I complex exerted only a negligible effect on the secondary DNA structure, whereas tetrahydrocortisol–Apo A-I complex increased the number of single-stranded regions [6]. Low-angle X-ray scattering showed that the increment is 54 ± 1 per DNA molecule of 26,000 kD molecular weight [7]. Such a high number of interaction sites (one complex per 1000-2000 DNA base pairs (bp)) indicates the possibility of nonspecific interactions between tetrahydrocortisol–Apo A-I and DNA *in vitro*, depending on the concentration of the constituents, temperature, and pH of the solution.

This work presents enzymatic analysis of DNA treated with cortisol, tetrahydrocortisol, and their complexes with apolipoprotein A-I *in vitro*. S1 nuclease was used as an enzyme that cleaves DNA only within single-stranded regions [8, 9]. The ability of DNA with a specifically altered secondary structure to participate in the transcription process has been evaluated through its interaction with DNA-dependent T7 bacteriophage RNA-polymerase.

MATERIALS AND METHODS

Apolipoprotein A-I (Apo A-I) was extracted from the blood plasma of Wistar line rats and identified as described in [10]. DNA was isolated from adult rat liver according to [11] and analyzed by electrophoresis in 0.3% agarose gel. The DNA molecular weight was 26,000 kD (~42 kb). Phage λ DNA and its *Hind* III-restricted fragments was provided by Sibenzyme (Novosibirsk, Russia); S1 nuclease from *Aspergillus oryzae* strain with an activity of 7 units per μl (Sigma, USA). One activity unit (U) cor-

Abbreviations: THC) tetrahydrocortisol.

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responds to the enzyme amount catalyzing the formation of 1 μg of acid-soluble nucleotides in 1 min at 37°C. T7 bacteriophage RNA-polymerase with an activity of 200,000 units per optical unit (absorption wavelength 280 nm) and electrophoretic purity of 95% was provided by V. N. Ankilova (NIBC, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia). One activity unit of RNA-polymerase corresponds to the enzyme amount catalyzing the inclusion of 1 nmol of nucleoside triphosphate in acid-insoluble product in 1 h at 37°C. Cortisol was from Serva (USA), tetrahydrocortisol was kindly provided by Y. A. Pankov (Research Institute of Experimental Endocrinology, Russian Academy of Medical Sciences, Moscow).

For preparation of Apo A-I–hormone (cortisol, tetrahydrocortisol (THC)) complex, 57 ng of Apo A-I in 4 μl of 10 mM Tris-HCl buffer, pH 8, were supplemented with 1.38 ng of hormone (1 : 2, in other experiments the proportions were 3 : 1, 1 : 1, and 1 : 3), mixed with Vortex, and kept for 5 min at room temperature [12]. Then Apo A-I–hormone complex was added to an amount of 250 ng native DNA, dissolved in 4 μl of water, and the mixture was kept for 10 min at room temperature. Fivefold buffer for S1 nuclease [8] was added to the mixture up to final concentration of NaAc of 100 mM, Tris-HCl of 20 mM, and $ZnSO_4$ of 1 mM (pH 5). The protein/DNA ratios of five protein molecules (within Apo A-I–THC complex) per 1000 bp, one protein molecule per 5000 bp, one protein molecule per 1000 bp, and fifty protein molecules per 1000 bp were examined. The mixture was stirred and kept for 5 min at room temperature. S1 nuclease (0.2 U) was added to the solution obtained and incubated for 30 min at 37°C. The composition of the reaction mixture (15 μl volume) was: 250 ng of DNA, 2.27-570 ng of Apo A-I, 0.028-20.7 ng of hormone (THC or cortisol), S1-nuclease (0.2 U), 3 μl of 5-fold buffer for S1-nuclease, and water. Electrophoresis of the resulting DNA preparations was performed in 0.5 and 1% agarose gel [9]. For the estimation of DNA fragments size, the phage λ DNA and its *Hind* III-restricted fragments were used as markers. After electrophoresis the gel was treated with a 0.5 μg/ml solution of ethidium bromide and photographed in UV using Mikrat-izopan film (Tasma, Kazan, Russia).

A particular experiment was performed to reproduce the component ratio used in the previous work [6]: 166 ng/μl of DNA, 55.5 ng/μl of ApoA-I, and 0.22 ng/μl of hormone. The molar ratio in Apo A-I–hormone complex is 3 : 1, when the complex interacted with DNA the protein/DNA ratio was 7.5 Apo A-I molecules per 1000 bp.

The low-angle X-ray scattering (LAXS) technique, which was previously employed to study the interactions between DNA and Apo A-I–THC complex [7], was used in this work for the investigation of eukaryotic DNA and Apo A-I interactions with RNA-polymerase, both in the presence and the absence of hormones. LAXS patterns were recorded using a Siemens diffractometer (Germany). The X-ray wavelength was 0.154 nm (CuK_α). Homogeneous preparations of Apo A-I, RNA-polymerase, and DNA of initial concentrations of 0.40, 4.16, and 3.12 mg/ml, respectively, were used. For the investigation of native DNA and RNA-polymerase interactions in the presence of Apo A-I, the mixtures of DNA, Apo A-I and its complexes with THC or cortisol were prepared at the different concentrations, according to the above-mentioned molar ratios of the components. RNA-polymerase concentration in four investigated mixtures was 0.140, 0.238, 0.380, and 0.568 μM. The temperature of initial samples when recording a LAXS pattern was 20°C, whereas the temperature of DNA, Apo A-I, and RNA-polymerase mixtures was 37°C. LAXS patterns were obtained within the angle range of $0.0245 \le h \ge 3.423$ nm^{-1}, where $h = 4\pi\sin(\theta)/\lambda$, 2θ being the scattering angle. X-Ray patterns were corrected for background scattering, collimation, and smoothed. The results were evaluated using Student's *t*-criteria, with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

It is a well known that native rat DNA contains a number of single-stranded regions. Adult rats have twice the number of these regions compared to newborn rats [13]. In the present investigation DNA was extracted from adult rat liver under native conditions. Indeed, the treatment of the initial DNA preparation with S1-nuclease reveals some amount of single-stranded fragments (Fig. 1, lane *2*).

A significant increase in the number of S1 nuclease-sensitive regions in the native rat liver DNA was observed upon the influence of Apo A-I–tetrahydrocortisol complex (Fig. 1, lanes *3-6*). Apo A-I complex with cortisol did not display such an effect, i.e., the number of S1 nuclease sensitive regions remained comparable to the initial DNA sample (Fig. 1, compare lanes *6* and *7*). DNA treatment with Apo A-I alone neither led to any remarkable change in a number of single-stranded fragments. The interaction of DNA with Apo A-I–hormone complex was previously investigated in Tris-HCl buffer with pH 7.5 [6, 7]. pH 5 is an optimal value for S1 nuclease [8]. In this connection, both buffers were used in the present work (Fig. 2). As was shown previously, Apo A-I–hormone complex is preserved within the pH range 5-8 [12]. Figure 2 illustrates that the S1 nuclease cleavage pattern after DNA treatment with cholesterol, cortisol, THC, or their complexes with Apo A-I is not affected when a pH 7.5 buffer is replaced with a pH 5 buffer (Fig. 2, compare lanes *3-6* and *7-10*). In our opinion, this is an indication of a minor contribution of electrostatic interactions but a major contribution of hydrophobic interactions for steroids and steroid–Apo A-I complexes bind-

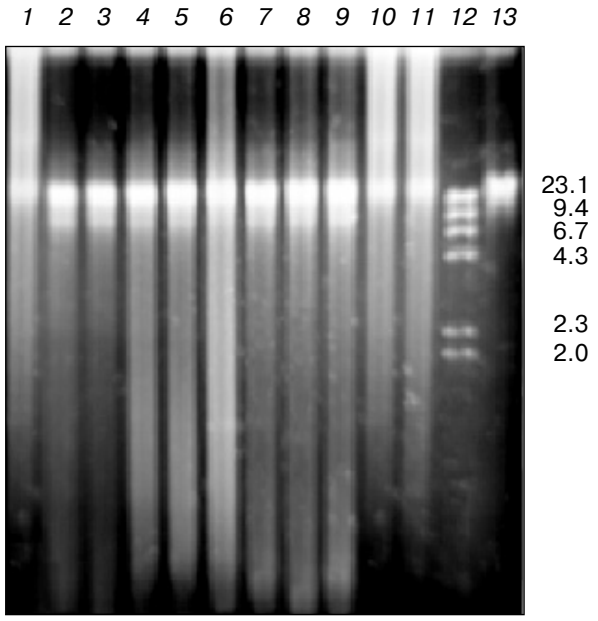

Fig. 1. Electrophoretic DNA separation in 1% agarose gel: *1*) control DNA; *2-9*) S1-nuclease treated DNA (8 U/ml) (DNA + Apo A-I–THC (*3-6*), one protein molecule per 5000 bp, one protein molecule per 1000 bp, five protein molecules per 1000 bp, and 10 protein molecules per 1000 bp, respectively; DNA + Apo A-I–cortisol (*7*), 10 protein molecules per 1000 bp; DNA + Apo A-I (*8*, *9*), one protein molecule per 5000 bp, five protein molecules per 1000 bp, respectively); *10*, *11*) DNA + Apo A-I (without S1-nuclease) (one protein molecule per 5000 bp, five protein molecules per 1000 bp, respectively); *12*) DNA λ *Hind* III; *13*) DNA λ. The gel was treated with ethidium bromide. To the right, DNA fragments size expressed as thousands of base pairs.

ing to DNA. It should be noted that the interaction of cholesterol with DNA was not detected under any conditions, which correlates with the literature data [14], where the formation of *in vitro* adducts was shown for DNA and cortisol, tetrahydrocortisol, as well as a number of other steroid hormones, but not their precursor, cholesterol.

The DNA treatment with any of the hormones used in this study (THC, cortisol) followed by the treatment with S1 nuclease resulted in a nonspecific cleavage (Fig. 2, lanes *4*, *5*, *8*, *9*). It should be mentioned that the emergence of a certain number of single-stranded fragments under hormone influence on DNA was also detected in our previous work, where fluorescent probes were used [6]. This work revealed no difference between the effects produced by cortisol and tetrahydrocortisol on native rat liver DNA in the absence of Apo A-I. Neither LAXS technique [7] allows the detection of such a small increment in a number of single-stranded fragments within a large DNA molecule influenced only by hormones. At the same time, hormone behavior within the complex with Apo A-I was dramatically different—only the reduced hormone form (THC) within the complex with Apo A-I caused the formation of an appreciable number of single-stranded DNA regions. These were also registered earlier by two independent methods, fluorescent probes [6] and the LAXS [7]. Furthermore, S1 nuclease cleavage of single-stranded fragments formed upon the DNA interaction with Apo A-I–tetrahydrocortisol revealed highly specific DNA binding sites with this complex. As seen in Fig. 3 (lanes *3-7*), the fragments of ~6,000 and 5,500 bp are accumulated with the increase of complex concentration. A significant number of these fragments is detected only when protein to hormone molar ratios of 1 : 2 are present in the complex. It seems likely that the Apo A-I carrying two hormone molecules has a conformation allowing preferable detection of highly specific DNA sites. The accumulation of 6,000 and 5,500 bp fragments could be detected under photographing of 0.5% agarose gel only with long exposures. Electrophoregrams in 1% agarose gel were scanned and the DNA amount hydrolyzed by S1

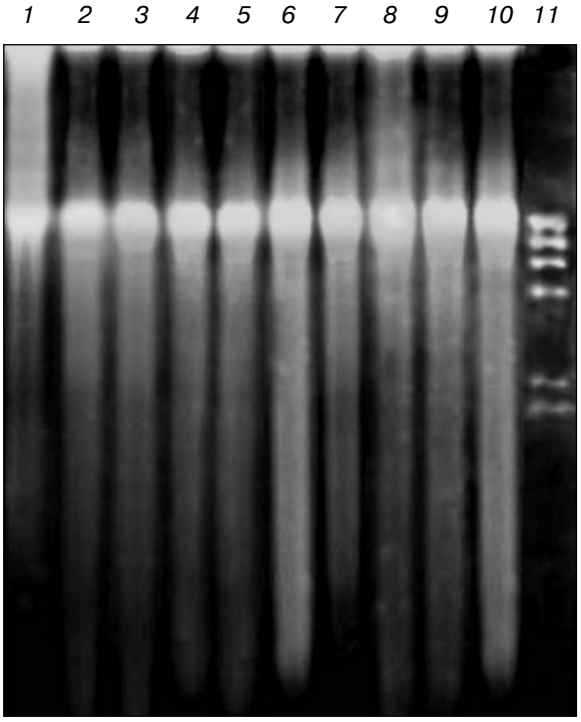

Fig. 2. Electrophoregram of DNA treated with steroids (one steroid molecule per 1000 bp) or by Apo A-I–THC (1 : 1) complex at pH 5.0 (*3-6*) and 7.5 (*7-10*) (1% agarose gel treated with ethidium bromide): *1*) control DNA; *2-10*) S1-nuclease treated DNA (0.35 U/ml) (DNA + cholesterol (*3*, *7*), DNA + cortisol (*4*, *8*), DNA + THC (*5*, *9*), DNA + Apo A-I–THC (*6*, *10*)); *11*) DNA λ *Hind* III.

nuclease after the treatment with THC–Apo A-I (2 : 1) complex was estimated. The obtained value was 3-5% of the initial DNA amount, which corresponds to the average value of 1 to 2 S1 nuclease sensitive regions per 100,000 bp. As seen in Fig. 1, the length of unrestricted by S1 nuclease fragments ranges from 500 to 15,000 bp, i.e., THC–Apo A-I interaction sites with DNA are irregularly distributed throughout the genome, with exception for those which formed 6,000 and 5,500 bp fragments after the cleavage.

Earlier the LAXS technique was successfully employed to investigate the interactions between individual tRNA and synthetases [15], and also for oligonucleotides and methyl-transferases [16-18]. It was shown that this method provides valuable information about the molecular interaction mechanisms, specifically, to determine the stoichiometry and equilibrium constants for macromolecular complexes and structural and weight characteristics of the macromolecules and their aggregates [15-18].

It is well known that the homogeneity of a secondary DNA structure can be estimated by the presence of the diffraction maximum in the angular range $h \approx 2.66$ nm^{-1}, which corresponds to the DNA double helix diameter, ~2.2 nm [19]. The analysis of LAXS data has shown that RNA-polymerase, Apo A-I, and native DNA preparations are essentially monodisperse when their structural parameters and weight characteristics were determined by us earlier [1, 7, 20]. Figure 4 presents LAXS patterns for native DNA, Apo A-I, and RNA-polymerase mixtures in the absence (curve *1*) and in the presence of THC (curve *2*), when the peak height is reliably lower. Appreciable RNA-polymerase binding was observed only after DNA interaction with Apo A-I–THC complex, but not with Apo A-I–cortisol, as reported in [20]. The maximum rate of RNA-polymerase binding to DNA corresponds in this case to the stoichiometry of 6 : 1. Publications [1, 6, 7] provide evidence of DNA secondary structure disruption that is caused by DNA interaction with THC–Apo A-I complex accompanied by the formation of single-stranded regions. The comparison of LAXS data presented in Fig. 4 with those obtained in [20] indicates that at least some of the newly formed single-stranded sites become a target for DNA-dependent RNA-polymerase. It is well known that T7 phage RNA-polymerase conducts synthesis only from a double-stranded phage promotor with a specific nucleotide sequence [21], and this enzyme is widely used in structure–functional research due to being well explored, and also because the availability of the source and simpler structure in comparison to eukaryotic polymerases. The results of LAXS depend on dispersion properties, i.e., molecular weight and shape of interacting molecules and their aggregates. That is why the mono-subunit structure of T7 RNA-polymerase became the main factor governing the choice of a model system.

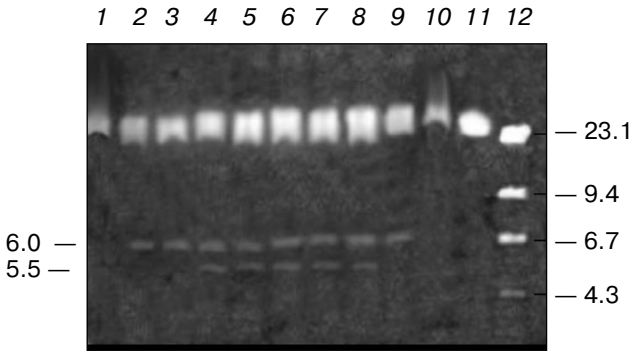

Fig. 3. Electrophoregram of DNA treated with Apo A-I–THC complex (protein/steroid ratio is 1 : 2) and S1-nuclease (8 U/ml) (0.5% agarose gel treated with ethidium bromide): *1*) control DNA; *2-9*) DNA treated with S1 nuclease (DNA + Apo A-I–THC (*3-7*), one protein molecule per 5000 bp, one protein molecule per 1000 bp, five protein molecules per 1000 bp, 10 protein molecules per 1000 bp, and 50 protein molecules per 1000 bp, accordingly; DNA + Apo A-I–cortisol (*8*), five protein molecules per 1000 bp); DNA + Apo A-I (*9*), five protein molecules per 1000 bp); *10*) DNA + Apo A-I (without S1 nuclease), one protein molecules per 1000 bp; *11*) DNA λ; *12*) DNA λ *Hind* III. To the left, size of rat DNA fragments; to the right, size of DNA λ fragments in thousands of nucleotide pairs.

The direct comparison of the phage and eukaryotic RNA-polymerase effect on the same template was performed in [22] using phage SP6 RNA-polymerase and yeast RNA-polymerase III. It was demonstrated that

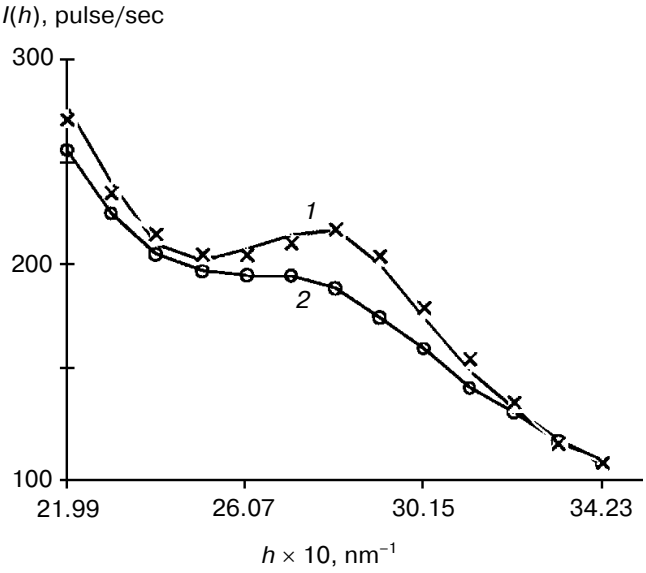

Fig. 4. LAXS patterns for DNA mixtures with Apo A-I and RNA-polymerase without THC (*1*) and in the presence of THC (*2*). Concentration of DNA, 0.043 μM; Apo A-I, 1.22 μM; THC, 2.55 μM; RNA-polymerase, 0.568 μM.

both polymerases conduct the transcription in a similar way, e.g., the histone octamer was transferred through the same distance, which might reflect a similarity between these transcription initiation mechanisms. This is an indication of some fundamental polymerase features, although in reality the eukaryotic transcription is accomplished without phage polymerases. Investigation of transcription mechanisms involving RNA-polymerase II has been hampered by the absence of an adequate experimental model [23].

Thus, only one out of 54 interaction regions of THC–Apo A-I complex with DNA previously detected by LAXS [7] results in the formation of a single-stranded DNA capable of S1 nuclease cleavage. Probably a partial binding of RNA-polymerase is also caused by nonspecific interactions. Apparently, a fraction of RNA-polymerase has been bound to DNA independently from newly formed single-stranded (S1 nuclease-sensitive) fragments. That is not surprising since RNA-polymerase can also bind to native DNA [21]. It is important to mention that partially unwound regions of the double-stranded DNA are in this case the targets for RNA-polymerase, i.e., the detected specific alteration of a secondary DNA structure, affected by THC–Apo A-I complex, initiates the cooperative and dose-dependent RNA-polymerase binding. The authors of [24] have shown that the formation of a specific transcription elongation complex with RNA-polymerase II *in vitro* can be completed without a promotor-dependent initiation if protruding 3′-ends are available, and from those the initiation is run. The model proposed by us is characterized by the fact that transcription initiation *in vivo* probably occurs in newly formed inner single-stranded DNA regions under regeneration conditions in a number of adaptive processes.

As shown previously [4, 20], the reduced form of the hormone (THC) is a biologically active form that participates in amplification of gene expression within THC–Apo A-I complex. In this case Apo A-I plays a role of a target hormone transporter, and the reduced form of the hormone promotes disruptions of the hydrogen bonds between heterocyclic base pairs, thus resulting in local DNA denaturation and favoring the interactions between RNA-polymerase and single-stranded DNA regions. Generally, the role of THC–Apo A-I complex in this case is similar to that of the transcription factor, which alters the DNA structure [25] in the vicinity of binding sites, initiating the interaction with RNA-polymerase. The proposed mechanism of gene expression amplification can be the reason for the activation of protein biosynthesis in hepatocytes under the influence of THC–Apo A-I complex as described in the literature [1, 2, 4, 20].

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