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ISOLATION OF AN ANDROGEN ACCEPTOR FROM SALT EXTRACT OF RAT PROSTATIC CHROMATIN

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SUMMARY

The soluble androgen acceptor has been isolated from 0.35 M NaCl extract of rat prostatic chromatin by affinity chromatography on DNA-cellulose. The acceptor activity was assayed by interaction with 5α-dihydrotestosterone-receptor. Native DNA enhances this interaction. Polyacrylamide gel electrophoresis of the acceptor under denaturing conditions reveals a single polypeptide of molecular weight of 14,000. Amino acid analysis shows that the acceptor protein contains a higher content of acidic amino acid residues than basic amino acid residues. In an in vitro RNA synthesizing system catalyzed by rat RNA polymerase II, addition of the acceptor stimulates RNA synthesis. Based on incorporation of [γ-^{32}P]ATP and [γ-^{32}P]GTP, the stimulation by the acceptor is mainly on the initiation of RNA chains.

INTRODUCTION

In rat ventral prostrate, administered testosterone or its metabolite, 5α-dihydrotestosterone, is retained as dihydrotestosterone-receptor complex in the cell nucleus (1-5). The translocated androgen-receptor is bound to chromatin binding sites (6-12), resulting in an altered chromatin structure and increased transcription (13,14). Interaction of androgen-receptor complex with specific chromatin binding sites or acceptor molecules thus represents one important stage in androgen action. Most of the nuclear retained androgen-receptor complex in rat prostate is recovered by extraction of the nuclei or chromatin with dilute salt solutions (2,4,5,15,16). The partial purification and some properties of an acceptor preparation from

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[2]Abbreviations used: [^{3}H]DHT, 5α-dihydro[1,2-^{3}H]testosterone; SDS, sodium lauryl sulfate; TEM, 10 mM Tris-HCl, pH 7.0, containing 1 mM EDTA and 1 mM 2-mercaptoethanol; PMSF, phenylmethanesulfonyl floride.

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salt extract of prostate nuclei have been reported by Tymoczko and Liao (15). In this communication, we describe the purification of an acceptor from 0.35 M NaCl extract of prostatic chromatin to electrophoretic homogeneity. An attempt has also been made to examine the possible role of the acceptor in transcription.

MATERIALS AND METHODS

Preparation of prostatic chromatin: Male Sprague-Dawley rats of about 300 g body wieght were bilaterally orchidectomized via the scrotal route and killed 48 h after the operation. The ventral prostates were dissected out and rinsed in ice-chilled 0.14 M NaCl. Nuclei were isolated from 200 rat prostates by the procedure of Blobel and Potter (17). The preparation of chromatin from nuclei was as described previously (11).

Isolation of androgen acceptor: All solutions used contained 1 mM PMSF. The prostatic chromatin was extracted with 100 vols of 0.35 M NaCl containing 20 mM Tris-HCl, pH 7.5, 1 mM EDTA and 1 mM 2-mercaptoethanol by stirring gently at 0-2°C for 15 min. The suspension was centrifuged for 10 min at 12,000 g and the supernatant was saved. The extraction of the pelleted chromatin with the buffered 0.35 M NaCl was repeated once more. The two supernatants were combined, dialyzed against 0.1 M NaCl containing TEM with two changes of 0.1 M NaCl-TEM, and centrifuged for 10 min at 12,000 g to remove insoluble material. The clear 0.35 M NaCl-soluble protein solution was loaded on a rat prostate DNA-cellulose column, prepared according to Alberts and Herrick (18), which had previously been equilibrated with 0.1 M NaCl-TEM. The column was washed with 0.1 M NaCl-TEM and the wash was discarded. The bound protein was eluted with 0.6 M NaCl-TEM. The eluate containing the DNA-binding protein was collected, dialyzed against 0.1 M NaCl-TEM, and mixed with BioRex-70 (Na^+ form, 10 mg protein/g resin) which had previously been washed with 0.1 M NaCl-TEM. The suspension was stirred at 0-2°C for 15 min and centrifuged for 15 min at 27,000 g. The supernatant, representing the acceptor preparation, was collected and stored frozen until use.

Preparation of [^{3}H]DHT-receptor complex: To prepare the cytosol fraction, rat prostates were homogenized in 3 vols of 0.25 M sucrose containing 0.05 M Tris-HCl, pH 7.5, 25 mM KCl and 5 mM $MgCl_2$. The homogenate was centrifuged at 3,000 g for 10 min from which the supernatant was further centrifuged at 133,000 g for 45 min. The supernatant from this high speed centrifugation was collected as the cytosol. To the cytosol, saturated ammonium sulfate solution was added slowly to 40% saturation. After standing at 0-2°C for 10 min, the mixture was centrifuged for 10 min at 12,000 g. The precipitate was collected, dissolved in and dialyzed against 0.1 M NaCl-TEM. [^{3}H]DHT-receptor complex was prepared by mixing 2.5 nmoles of [^{3}H]DHT (100 µCi) with 100 mg of the ammonium sulfate-precipitated cytosolic protein in 6 ml of 0.1 M NaCl-TEM, allowed to stand at 0-4°C for 2 h and frozen at -20°C overnight. After thawing, the mixture was passed through a Sephadex G-50 column to remove the free steroid.

Interaction of acceptor with [^{3}H]DHT-receptor complex: This was done according to Tymoczko and Liao (15). The acceptor sample, in varying amounts, was incubated at 0-4°C with 0.7 mg protein of the [^{3}H]DHT-receptor complex, either in the presence or absence of native prostatic DNA, in a final volume of 1.5 ml of 0.1 M NaCl-TEM. The complex formed was collected on a

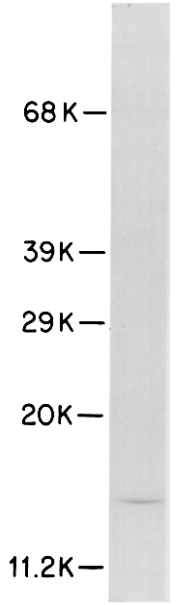


Fig. 1. Electrophoresis of the soluble androgen acceptor in 10% SDS-acrylamide gel performed according to Laemmli (19).

Millipore RA filter (1.2 μm) and washed with 15 ml of 0.2 M NaCl-TEM. Controls without the acceptor preparation or DNA were run in parallel to serve as background. The complex retained on the filter was counted in toluene scintillation cocktail containing NCS(Amersham).

RNA synthesis in vitro: Preparation of RNA polymerase II from rat liver nuclei and in vitro incorporation of labeled nucleotides into RNA were carried out as described elsewhere (14).

RESULTS AND DISCUSSION

The acceptor isolated from 0.35 M NaCl extract of prostatic chromatin, purified as described under Materials and Methods, appeared as a single polypeptide when analyzed by SDS-acrylamide gel electrophoresis (Fig. 1). The molecular weight of the acceptor was estimated to be 14,000. Amino acid composition analysis of the acceptor, shown in Table 1, revealed a higher proportion of acidic to basic amino acid residues. The amide content of the acidic residues was not determined. However, since the acceptor preparation was not bound by BioRex-70 at pH 7.0, the acceptor is considered to be an acidic protein.

TABLE I

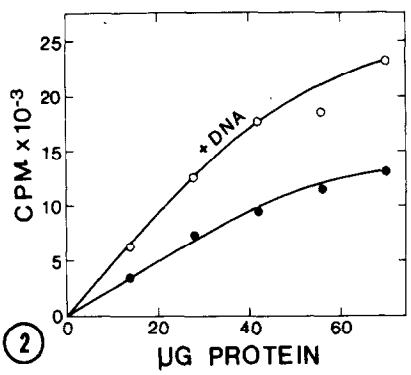
Amino Acid Composition of the Soluble Acceptor

Amino acid	Molar % of recovered residues
Aspartic acid	9.3
Threonine	4.5
Serine	8.9
Glutamic acid	14.0
Proline	5.6
Glycine	15.0
Alanine	7.4
Valine	5.9
Methionine	1.6
Isoleucine	3.0
Leucine	4.8
Tyrosine	0.1
Phenylalanine	3.9
Lysine	8.5
Histidine	2.5
Arginine	5.0

The purified acceptor interacted with [^{3}H]DHT-receptor, approaching saturation level with increasing concentrations of the acceptor (Fig. 2). Addition of native (Fig. 2) but not heat-denatured (data not shown) DNA stimulated the interaction between [^{3}H]DHT-receptor and the acceptor by about 80%. Since the values due to stimulation by DNA were obtained after subtracting the controls containing only DNA and [^{3}H]DHT-receptor, the values represent the participation of the acceptor in the complex formation. These results support the original observations of Tymoczko and Liao (15).

The purified soluble androgen acceptor stimulated transcription of DNA *in vitro*. As shown in Fig. 3, incubation of DNA-templated RNA polymerase II reaction mixture in the presence of the acceptor resulted in 65-70% increase in incorporation of [^{3}H]UMP into acid-insoluble material. Preheating the acceptor for 5 min at 60°C abolished its stimulatory effect on RNA synthesis as well as its ability to interact with [^{3}H]DHT-receptor complex.

Using [γ-^{32}P]ATP and [γ-^{32}P]GTP as the labeled substrates, the acceptor stimulated incorporation of the radioactive labels into RNA, comparable to the level of the acceptor-stimulated [^{3}H]UMP incorporation (Table II). There



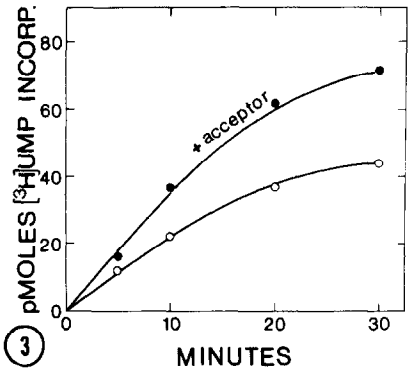


Fig. 2. Interaction of acceptor with [^{3}H]DHT-cytosolic receptor complex. The [^{3}H]DHT-receptor preparation (0.7 mg protein) was incubated with varying amounts of the purified acceptor (0-70 μg) and assayed in the absence (●) and presence (○) of 100 μg of native prostate DNA as described under Materials and Methods. The Millipore filter-retained complex was counted in NCS (Amersham)-toluene cocktail.

Fig. 3. Effect of soluble acceptor on RNA synthesis in vitro. Transcription of native prostate DNA in vitro by rat RNA polymerase II was carried out as described under Materials and Methods in the absence (○) and presence (●) of the soluble acceptor (7 μg per assay).

was little difference in the calculated average chain lengths of RNA synthesized in vitro in the presence and absence of the acceptor. The effect of acceptor on transcription is mainly stimulation of RNA initiation.

These results suggest a possible role for DNA in androgen action. Since both androgen-receptor and the soluble acceptor are DNA-binding, DNA

TABLE II

Effect of Soluble Acceptor on the Initiation and Synthesis of RNA in vitro

Addition	Time of synthesis (min)	pmoles of labeled nucleotides incorp. [^{3}H]UMP	[γ-^{32}P]ATP	[γ-^{32}P]GTP
None	5	48	0.35	0.62
	20	118	0.58	1.07
Acceptor (20 μg)	5	66	0.54	0.92
	20	186	0.90	1.56

The assays were carried out as described under Materials and Methods

could facilitate the receptor-acceptor interaction via an initial binding of androgen-receptor to DNA; the androgen-receptor then locates the acceptor on DNA in chromatin. The effect of the acceptor on transcription may also be considered in relation to this receptor-acceptor interaction. Chromosomal proteins soluble in dilute salt solutions have been shown to exert a positive effect on transcription (20,21) and suggested to play a regulatory role in gene control. The acceptor, being a nonhistone chromosomal protein of prostatic chromatin, may function as a possible regulatory molecule. Interaction of androgen-receptor with the acceptor presumably effects a structural change of the prostatic chromatin (14), allowing initiation of RNA chains on new DNA regions made available, perhaps, through this structural alteration of chromatin.

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