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MODULATION OF DNA BINDING OF GLUCOCORTICOID RECEPTOR BY AURINTRICARBOXYLIC ACID*

VIRINDER K. MOUDGIL and VIRGINIA M. CARADONNA
Department of Biological Sciences, Oakland University, Rochester, MI 48063, U.S.A.

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Summary—Effects of aurintricarboxylic acid (ATA) were examined on the DNA binding properties of rat liver glucocorticoid–receptor complex. The DNA-cellulose binding capacity of the glucocorticoid–receptor complex was completely abolished by a pretreatment of receptor preparation with 0.1–0.5 mM ATA at 4°C. The half-maximal inhibition (i.d.50) in the DNA binding of [^{3}H]triamcinolone acetonide–receptor complex ([^{3}H]TARc) was observed at 130- and 40 μM ATA depending upon whether the inhibitor was added prior to or following the receptor activation. The entire DNA-cellulose bound [^{3}H]TARc could be extracted in a concentration-dependent manner by incubation with 2–100 μm ATA. The [^{3}H]TARc remained intact under the above conditions. the receptor in both control and ATA-treated preparations sedimented in the same region in salt-containing 5–20% sucrose gradients. The action of ATA appeared to be on the receptor and not on DNA-cellulose. The DNA-binding capacity of ATA-treated receptor preparations could be recovered upon exhaustive dialysis. The treatment with ATA did not appear to change the ionic behavior of heat activated GRc; the receptor in both control and the ATA-treated preparations showed similar elution profiles. Therefore, ATA appears to alter the binding to and dissociation of glucocorticoid–receptor complex from DNA. The use of ATA should offer a good chemical probe for analysis of the DNA binding domain(s) of the glucocorticoid receptor.

INTRODUCTION

Glucocorticoids are known to bring numerous physiological effects in a variety of target tissues. The molecular mechanism of glucocorticoid action has remained speculative and unclear. After their initial entry into target cells, glucocorticoids interact with intracellular soluble proteins termed "receptors". The complexes thus formed translocate into the nucleus onto certain sites on chromatin where they are thought to be involved in alteration of gene expression [1]. However, upon extraction from target cells at low temperature, receptors can be complexed with glucocorticoids to form non-activated complexes that have little affinity for isolated nuclei and DNA-cellulose. The DNA binding property can be acquired *in vitro* by various treatments of glucocorticoid–receptor complexes which cause alterations in the receptor collectively known as "activation". The activated glucocorticoid–receptor complexes acquire increased affinity for isolated nuclei, DNA and ATP-Sepharose, and altered mobility on ion-exchange resins [2–10].

One of the various approaches used toward understanding the mode of steroid hormone action is to study the interaction of receptors with various cellular constituents. Recently, a number of chemical agents have been identified which block the interaction of glucocorticoid receptors with DNA [11–15]. In this paper we report effects of another chemical agent, aurintricarboxylic acid, on the DNA binding properties of rat liver glucocorticoid–receptor complex.

Aurintricarboxylic acid (ATA) is a synthetic triphenylmethane dye which was initially employed for the quantitative determination of the aluminum ion [16] and was later introduced by Grollman and Steward[17] as an inhibitor of the protein synthesis in a cell-free system. In recent years ATA has been used quite extensively for blocking a wide variety of important processes that ultimately depend on the formation of a protein–nucleic acid complex [18–20].

It is now generally believed that steroid receptors interact with nuclear sites and perhaps with certain specific regions of DNA. Recently, purified glucocorticoid receptor has been shown to bind to specific mammary tumor virus (MTV) DNA sequences [21]. The choice of ATA as a putative modulator of DNA binding of glucocorticoid–receptor complex *in vitro* was, therefore, a reasonable one. Previous studies from this laboratory have shown that an incubation of receptor preparations with ATA blocks the acceptor binding of progesterone and estrogen receptors [22–24]. Recently, we have also reported results of our preliminary studies on the effects of ATA on the nuclear uptake of hepatic glucocorticoid receptor [25]. This is the first report in which we have undertaken a detailed characterization of DNA binding of rat liver GRc on the basis of its sensitivity to ATA.

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Note: The term activation refers to an alteration *in vitro* of cytosol receptor to a form which binds to isolated nuclei, ATP-Sepharose and DNA-cellulose.

EXPERIMENTAL

All reagents were of analytical grade. [6,7(*N*)^{3}H]triamcinolone acetonide (30–35 Ci/mmol) and [6,7-^{3}H(N)]dexamethasone (38 Ci/mmol) were obtained from New England Nuclear (Boston, MA) and ammonium salt of aurintricarboxylic acid (ATA), Dextran T-70 and diethylaminoethyl (DEAE) Sephacel were purchased from Sigma Chemical Co. (St Louis, MO).

Buffers

The following buffers were prepared in deionized water at 23°C and were stored at 4°C. Buffer A: 50 mM Tris–HCl, 10 mM monothioglycerol, pH 8; Buffer B: 10 mM Tris–HCl, pH 8; Buffer C: 50 mM Tris–HCl, 12 mM monothioglycerol, 0.01 M KCl, 20% (v/v) glycerol, pH 8; Buffer D: 10 mM Tris–HCl, 12 mM monothioglycerol, 10 mM KCl, 20% (v/v) glycerol, pH 8; Buffer E: 50 mM Tris–HCl, 12 mM monothioglycerol, 1 mM EDTA, 10% (v/v) glycerol, pH 8; Buffer F: 10 mM Tris–HCl, 12 mM monothioglycerol, 40% (v/v) glycerol, pH 8; Buffer G: 10 mM Tris–HCl, 20% (v/v) glycerol, pH 8.

Preparation of glucocorticoid receptor

Male albino rats of Yale strain were obtained from King Animal Lab, Inc., Oregon, WI, U.S.A. The animals were bilaterally adrenalectomized, kept on 0.9% NaCl for 3 days, then sacrificed by cervical dislocation. The entire liver tissue was excised, rinsed with cold 0.9% NaCl, minced, and homogenized in 4 vol (v/v) of buffer E with a Tissumizer (Tekmar, model SDT). The homogenate was centrifuged at 12,000 *g* for 10 min and then at 150,000 *g* for 1 h. The resultant supernatant was incubated with [^{3}H]triamcinolone acetonide (1×10^{-8} M) at 4°C for 12 h to form a [^{3}H]TARc.

Activation of cytosol glucocorticoid–receptor complex

Freshly prepared [^{3}H]TARc were heat-activated by incubation at 23°C for 50 min and then chilled for 10 min at 4°C. The complexes were incubated for 5 min with pelleted Dextran-coated charcoal (0.5% Norit A, 0.05% Dextran T-80 made with buffer A) and were subsequently recovered by centrifugation at 1,000 *g* for 10 min. The glucocorticoid–receptor complexes were also activated by fractionation with 50% $(NH_4)_2SO_4$ saturation. The suspension was centrifuged for 20 min at 10,000 *g*. The resultant precipitate was redissolved in buffer G and dialyzed for 4 h at 4°C using buffer G. A clear supernatant obtained after centrifugation at 12,000 *g* for 20 min was used for DNA-cellulose binding assay.

Extraction of DNA-cellulose bound [^{3}H]triamcinolone acetonide-receptor complexes by aurintricarboxylic acid

Aliquots of the activated [^{3}H]TARc were first incubated with pellets of DNA-cellulose for 40 min at 4°C and centrifuged for 10 min at 1,000 *g*. The resulting pellets were rinsed 3 times with buffer D. The washed pellets were then incubated with different concentrations of ATA (3–400 μM) at 4°C for 30 min with gentle shaking. After centrifugation at 100 *g* for 10 min, the radioactivity in the supernatant was measured directly.

Preparation of DNA-cellulose

DNA-cellulose was prepared according to the methods of Alberts and Herrick [26]. Calf thymus DNA (type II) was linked to Cellex-410 and these preparations contained 1.5–2 mg DNA/g of packed DNA-cellulose as quantitated by the method of Burton [27].

DNA-cellulose binding assays

Aliquots of DNA-cellulose suspension (10 mg/ml) containing 15–300 μg DNA were incubated with activated glucocorticoid–receptor complexes for 40 min at 4°C. The DNA pellets were then washed 3 times using buffer D and the DNA–cellulose-bound complexes were recovered by incubation with 1 ml of buffer C for 30 min at 4°C. After centrifugation, the supernatant was drawn for measuring the radioactivity.

Steroid binding assays

Charcoal adsorption assays were performed to monitor the total and the non-specific steroid binding [8–10]. Duplicate aliquots of the receptor preparation were incubated at 4°C for 2 h with 10 nM [^{3}H]triamcinolone acetonide. The non-specific binding was determined by adding in duplicate samples, the unlabeled steroid (1 μM) before the addition of the radioactive steroid. In samples where complexes had already been made, the non-specific steroid binding was determined by heating the samples at 37°C for 60 min in the presence of excess unlabeled steroid (1 μM) to destroy the specific receptors.

Sucrose gradient analysis

Linear 5–20% sucrose gradients (4.4 ml) were prepared by Beckman gradient former. Aliquots (0.2 ml) containing the hormone receptor complexes with or without ATA treatment were layered onto the gradients and centrifuged at 150,000 *g* for 16 h at 4°C. The tubes were pierced at the bottom and 0.2 ml fractions were collected on an ISCO Golden Retriever fraction collector.

For measurement of radioactivity, the aqueous samples were combined with 5 ml of scintillation fluid consisting of toluene and Spectrafluor (Amersham, Arlington Heights, IL), 1000:42 (v/v), mixed thoroughly and cooled before counting.

Statistical evaluation of the results

The results were expressed (whenever appropriate) as the mean + SEM of duplicate or triplicate determinations in a single experiment. Each experiment was repeated 3–4 times with identical results.

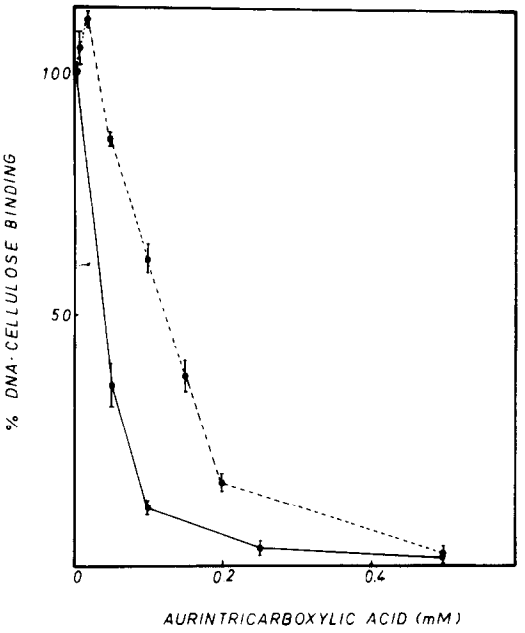

Fig 1. Effect of aurintricarboxylic acid on the activation and DNA-cellulose binding of [^{3}H]triamcinolone acetonide–receptor complex. Freshly prepared rat liver cytosol was incubated with 1×10^{-8} M [^{3}H]triamcinolone acetonide for 12 h at 4°C. Aliquots (0.5 ml) containing above complexes were incubated with buffer A (control) and different concentrations of ATA (10–500 μM) for 20 min at 4°C in a total volume of 1 ml (●– – –●). The mixtures were then brought to 23°C for 60 min to accomplish activation of complexes. The excess free or dissociated steroid was removed by treatment with Dextran-coated charcoal. The suspensions were centrifuged for 10 min at 1000 *g*. The resultant supernatant was then incubated with DNA-cellulose (1.75 mg DNA/g) and binding assays were performed as described in the Experimental section. For determining the effect of ATA on the DNA-cellulose binding of activated complexes (●– – –●), the cytosol was first heat-activated and then incubated with varying concentrations of ATA (10–500 μM). The values of the ordinates in this and subsequent figures represent specific DNA-cellulose or [^{3}H]triamcinolone acetonide binding. Non-specific steroid binding was determined by heating the samples at 37°C for 30 min in the presence of excess unlabeled steroid to destroy specific receptors. The values obtained from subtraction of non-specific binding from total binding represented the specific steroid binding. The non-specific DNA-cellulose binding was determined by measuring binding of complexes to cellulose, and averaged 5% of the total DNA-cellulose binding. The 100% binding corresponds to 4,430 cpm (●– – –●) and 3,400 cpm (●——●).

RESULTS

Incubation of the cytosol preparations with ATA reduced the ability of glucocorticoid–receptor complexes to bind to DNA-cellulose (Fig. 1). This inhibition in the binding of receptor to DNA depended on the concentration of ATA in the incubation mixture. The half-maximal inhibition (i.d.50) in the DNA binding of glucocorticoid–receptor complex differed depending upon whether ATA was added prior to or following the receptor activation. When the cytosol complexes were incubated at 23°C in the presence of ATA, inhibition in the DNA binding of glucocorticoid–receptor complex occurred with a half maximum at 130 μM ATA. However, heat-activated glucocorticoid receptor preparations appeared more sensitive to the presence of ATA and showed a half-maximal inhibition in the DNA binding at 40 μM. By raising the inhibitor concentration (0.2–0.4 mM ATA) the DNA binding of glucocorticoid–receptor could be completely abolished. On an average, one-fourth of the total glucocorticoid–receptor complexes were adsorbed on DNA-cellulose and represented a 100% DNA binding.

Since DNA binding is known to be a function of an activated receptor, the reduced DNA binding in the ATA-treated preparations could result due to an inhibition in the rate of receptor activation. To examine this possibility, cytosolic glucocorticoid–receptor complexes were heat-activated by exposure to 23°C for different time periods (0–120 min) in the presence and absence of ATA. The extent of receptor activation was then measured using DNA-cellulose. ATA (0.5 mM) presence completely inhibited the DNA-binding of the receptor throughout the incubation period suggesting that the action of ATA is not simply through effects on the rate of receptor activation.

The reduced DNA-cellulose binding of the glucocorticoid–receptor complex could result from a dissociation of the complex in the ATA-treated preparations. The steroid binding properties of the glucocorticoid receptor, however, remained intact under these assay conditions as judged by the charcoal adsorption assays and the sucrose gradient analysis (not shown).

The decreased DNA binding of glucocorticoid–receptor complex following an incubation with ATA (Fig. 1) could be due to either a complexing of the inhibitor with the receptor protein or with DNA-cellulose. Such possibilities were tested by first incubating a series of tubes containing DNA-cellulose with different concentrations of ATA, and then removing the free ATA by a thorough washing of the DNA-cellulose with low-salt buffer (buffer D). Following this, portions of cytosol containing activated glucocorticoid–receptor complexes were added to these tubes. The extent of the binding of the complexes to the DNA-cellulose was thus measured by performing batch assays as described in the Experimental section and Fig. 1. ATA-treated DNA-cellulose that was subsequently washed to remove the free inhibitor, retained its capacity to bind the [^{3}H]TA–receptor complexes at a level comparable to that seen with the untreated control DNA-cellulose (not shown).

Previous studies have shown that *in vitro* inactivation process (dissociation of steroid from the steroid–receptor complex or inability of receptor to bind steroid) of steroid receptor is related to receptor transformation; both of which are known to be

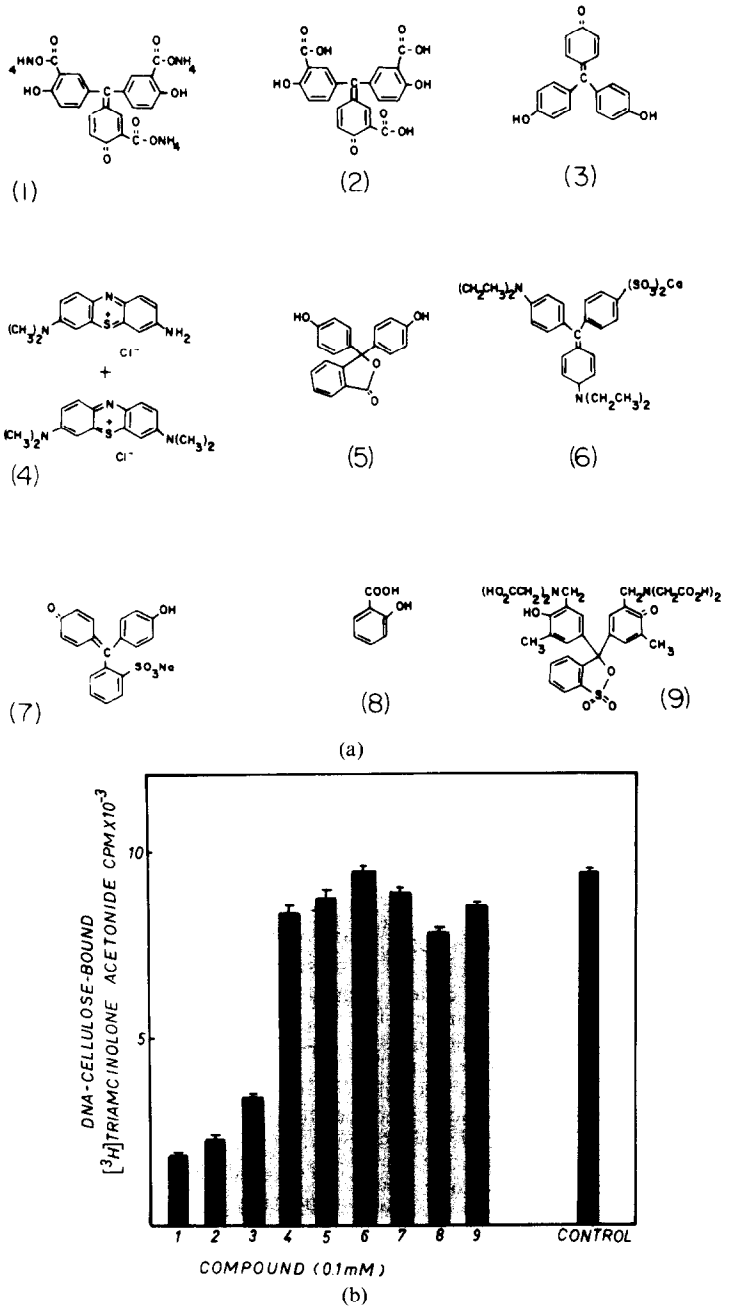

Fig. 2.A. Chemical structures of compounds tested to determine their effects on the DNA-cellulose binding of glucocorticoid–receptor complex: (1) Aluminon, ammonium salt of ATA; (2) aurintricarboxylic acid (ATA); (3) rosolic acid (aurin); (4) azure II, equal mixture of azure I and methylene blue; (5) phenolphthalein; (6) patent blue-violet; (7) phenol red; (8) salicyclic acid; (9) 3,3′*bis*-[*N*,*N*-di (carboxymethyl)aminomethyl-*o*-cresolsulfonephthlein] (xylenol orange). The compounds were dissolved in 5–10% dimethylsulfoxide; the latter did not appear to alter the steroid binding or DNA-cellulose binding properties of the glucocorticoid–receptor complex at that concentration. B. Relative effectiveness of the compounds [(1–9) listed in Fig. 2a] in blocking DNA-cellulose binding of activated glucocorticoid–receptor complex. The compounds were incubated with preformed [^{3}H]triamcinolone acetonide–receptor complexes for 40 min. Subsequently DNA-cellulose binding assays were performed as described in the Experimental section. The samples containing complexes incubated with buffer C only in the absence of the above compounds served as the controls.

blocked by sodium molybdate [28–30]. We examined the effect of ATA on the inactivation of glucocorticoid–receptor complex at elevated temperature (37°C). Warming the complexes at 37°C for 45 min destroyed the steroid binding completely, and the presence of ATA during this time had no protective function (not shown). In this regard, the mechanism via which ATA acts on the glucocorticoid

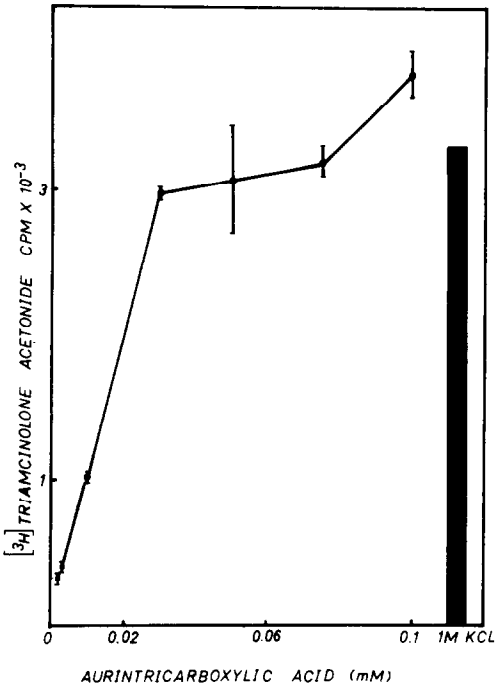

Fig. 3. Extraction of DNA-bound [^{3}H]triamcinolone acetonide–receptor complexes with aurintricarboxylic acid. Aliquots (0.5 ml) of heat-activated glucocorticoid–receptor complexes were incubated at 4°C for 40 min with 2 ml portions of DNA-cellulose (1.75 mg DNA/g). The DNA-cellulose suspensions were then washed three times with 3 ml of buffer D. The DNA–cellulose-bound complexes were then extracted by incubating the above washed pellets with 1 ml of buffer C, for 30 min at 4°C (control) and different concentrations of ATA (300–400 μM). The suspensions were then centrifuged at 1000 *g* for 10 min and the supernatant was used to measure the radioactivity. Duplicate aliquots of mixtures containing DNA–cellulose-bound complexes were also incubated with 1 ml of buffer C containing 1 M KCl.

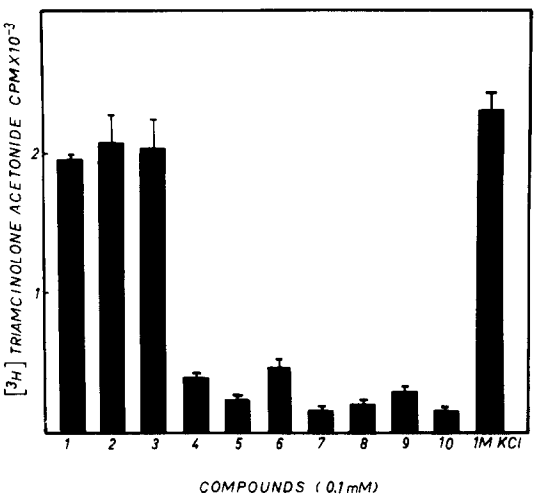

Fig. 4. Relative effectiveness of different compounds in the extraction of DNA-cellulose-bound glucocorticoid–receptor complexes. Series of tubes (in duplicate) containing equal amounts of DNA-cellulose-bound glucocorticoid–receptor complexes were incubated with compounds (1–9) listed in Fig. 2A. The extraction of receptor was achieved as described in Fig. 3. The background values for non-specific extraction of receptor were obtained by incubating the charged DNA-cellulose with buffer C (No. 10). The reference for the standard extraction of receptor was obtained by incubating the charged DNA-cellulose with high-salt buffer (buffer C plus 1 M KCl).

receptor appears to be different to that of sodium molybdate.

In order to get more insight into the probable domains of the receptor and functional group(s) of ATA that are involved in this interaction, several derivatives and analogs of ATA were tested for their ability to alter DNA-cellulose binding of receptor. The structures of these compounds are shown in Fig. 2a. These compounds were selected on the basis of their structural similarities with ATA and due to their previous use by other investigators [31, 32].

Figure 2b illustrates effects of the compounds listed in Fig. 2a on the DNA-cellulose binding capacity of heat-activated glucocorticoid–receptor complex. Each compound was preincubated at a fixed concentration (0.1 mM) with the activated [^{3}H]TA–receptor complex and its effect was determined by measuring the uptake of complexes by DNA-cellulose. Only compounds 1–3 (aluminon, ATA and aurin) were found most effective in inhibiting the DNA-cellulose binding of the receptor. Other compounds with close structural resemblance but without aurin structure with carboxylic groups were ineffective at concentrations tested. However, at higher concentrations their effects may become detectable, but such efforts were not made in the present studies.

If the incubation of receptor with ATA altered its DNA binding properties, then an incubation of the DNA-cellulose-bound [^{3}H]TA–receptor complexes with ATA may destabilize or disrupt this process. Results of Fig. 3 demonstrate that the [^{3}H]TA–receptor complexes can be extracted from DNA-cellulose by an incubation of the receptor-charged-resin with different concentrations of ATA. After the activated complexes were adsorbed on DNA-cellulose, and the resin was washed free of excess unbound material, a majority of the DNA-cellulose-bound radioactivity could be released by an incubation with 20 μM ATA (Fig. 3). Incubation of resin with higher concentrations of ATA resulted in increased extraction of radioactivity which was greater than that achieved by 1 M KCl. Upon gel-filtration and sucrose gradient analysis, the extracted radioactivity was found to be macromolecule-associated (not shown). The extraction of the [^{3}H]TA–receptor complex from DNA-cellulose was also noted to be specific for agents that possessed an aurin ring and carboxylic groups (Fig. 4). In all the cases, the recovery of receptor from DNA-cellulose by treatment with ATA or ATA-related compounds was quantitatively comparable to that achieved by 1 M KCl.

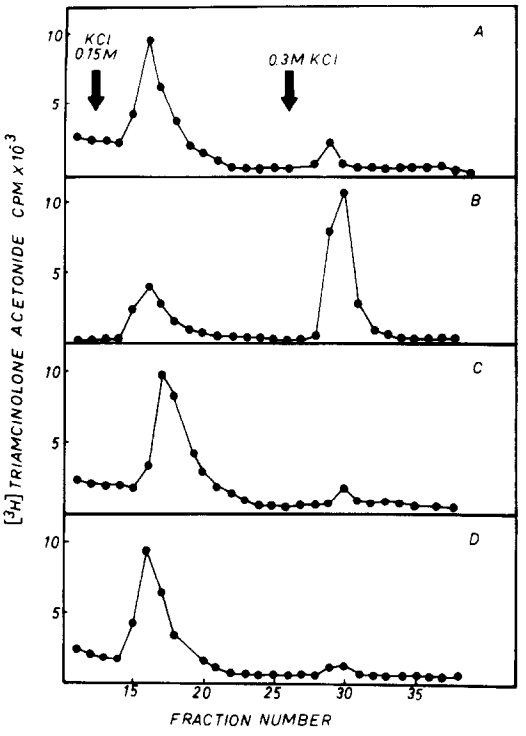

Fig. 5. Effect of aurintricarboxylic acid on the resolution of non-activated and activated glucocorticoid–receptor complexes. The cytosol containing the complexes was divided into 4 groups (A–D). Each group was separately treated with buffer A (A,C), 5 mM Na_2WO_4 (B), 0.2 mM ATA (D) for 30 min at 4°C. Subsequently samples were added with equal volume of saturated $(NH_4)SO_4$. After 30 min at 4°C, suspensions were centrifuged at 10,000 *g* for 10 min. The pellets were redissolved in buffer A using 1:10 volume of cytosol. The sample C aliquot was incubated with 0.2 mM ATA. All 4 samples (0.5 ml) were then chromatographed over separate but identical columns (0.9 × 7 cm) of DEAE-Sephacel equilibrated with buffer B. Fifteen fractions (1 ml) were collected each with buffer B, buffer B plus 0.15 M KCl and buffer B plus 0.5 M KCl. The entire fractions were used for measurement of radioactivity. A, cytosol + $(NH_4)_2SO_4$; B, cytosol + 5 mM Na_2WO_4, $(NH_4)_2SO_4$; C, cytosol + $(NH_4)_2SO_4$, 0.2 mM ATA; D, cytosol + ATA, $(NH_4)_2SO_4$.

The effect of ATA on the DNA binding of the glucocorticoid receptor appeared reversible. When the cytosolic [^{3}H]TA–receptor complexes were fractionated with 50% saturation of $(NH_4)SO_4$, incubated with different concentrations of ATA (0–200 μM) and subsequently dialyzed for 4 h at 4°C, the inhibitory action of ATA was abolished (not shown). The extent of DNA-cellulose binding of the dialyzed (ATA-treated) cytosol [^{3}H]TA–receptor complex was comparable to that seen with receptor from control (no ATA treatment) samples. The reversibility of ATA action has a potential for its use in the purification and characterization of glucocorticoid receptors.

In order to test whether the action of ATA involved an ionic interaction with the glucocorticoid receptor, the receptor preparations were incubated with ATA (50 μM) in the presence of increasing KCl concentrations (0–0.45 M). The samples were subsequently diluted to lower the salt concentration for performing DNA-cellulose binding assays. The effects of ATA were not reversed due to a prior incubation of receptor preparation with KCl (not shown), suggesting that ionic linkages may not contribute to the observed inhibitory effects of ATA on the DNA binding.

One of the problems with the use of an inhibitor to study the process of receptor activation or binding of activated receptor to acceptors is the interpretation of results. For example, receptor activation can be blocked in the presence of an inhibitor, but measuring this quantitatively involves incubation of complexes with DNA-cellulose or other acceptors. However, this problem can be addressed to some extent by employing ion-exchange chromatography [6, 7].

Figure 5 illustrates the results of the analysis of glucocorticoid–receptor complex over columns of DEAE-Sephacel. The cytosol [^{3}H]TARc was activated by fractionation with $(NH_4)_2SO_4$ and chromatographed over the ion-exchange resin. The activated receptor with presumably more positive charges on its surfaces, eluted with a lower salt concentration and was represented by the first peak of radioactivity (Fig. 5A). The cytosol preparations were also pretreated with Na_2WO_4 to block receptor activation [15]. This non-activated, more acidic receptor form appeared to bind more strongly to the resin and eluted at a higher salt concentration (Fig. 5B). When cytosol containing [^{3}H]TARc was first fractionated with $(NH_4)SO_4$ and then treated with 0.2 mM ATA, the resulting [^{3}H]TARc still appeared in the low salt fractions on the ion exchange resin corresponding to the activated receptor form (Fig. 5C). Alternatively, the reverse protocol was also applied. The aliquots of cytosol were first treated with ATA and subsequently fractionated with $(NH_4)_2SO_4$; the [^{3}H]TARc still appeared in the first peak following a profile of an activated receptor form (Fig. 5D). The results of these studies suggest that ATA-treated receptor still remains in an activated form. The effects of ATA, therefore, may result due to its binding either to or adjacent to a DNA binding site, or to another site whose location and conformation plays an important role in the DNA binding process. ATA, however, does not appear to interfere with the process of receptor activation.

DISCUSSION

We have identified aurintricarboxylic acid as an inhibitor of the DNA binding of rat liver glucocorticoid–receptor complex. The DNA–bound receptor complexes are equally sensitive to ATA presence and can be extracted upon incubation with low concentration of the inhibitor. Treatment with ATA does not appear to alter the steroid binding properties of receptor or accelerate dissociation of the

complex. We have presented evidence that ATA does not inhibit the process of receptor activation and that it may bring its effects via an interaction with the DNA binding site(s) of the glucocorticoid receptor. This interaction may also result in an alteration in the conformation of the activated receptor to a form unfavourable for optimal DNA binding. The activated receptor remains in an activated state after ATA treatment suggesting that the inhibitor may be complexing its DNA binding site.

The site of ATA action appears to be located on the receptor, and both the unique aurintriphenyl methane ring system and the carboxylic acid groups appear necessary for inhibition of DNA binding. Tsutsui *et al.* [19] have proposed that ATA may bring its effects via interactions with histones. Such binding may alter the nuclear structure and result in the loosening of the chromatin and the subsequent release of certain proteins. Our results are in agreement with those of Kreamer *et al.* [32] who observed inhibition of cell-free protein synthesis by ATA and several of its related synthetic analogs. These investigators ascribed the effects of ATA to the aurintriphenyl methane ring and carboxylic acid groups. Igarashi and Zmean [31] tested various triphenylmethane compounds and observed that the charge properties of the side chains of the dye are not critical since both negatively and positively charged compounds exhibited inhibitory action on the aminoacyl-*t*RNA synthetase at approximately the same concentrations. It appears that the skeletal structure of triphenylmethane, rather than the side chains, is the site of inhibitory action of ATA [32].

ATA has been used as an inhibitor of RNA synthesis and used as an anti-viral agent [17]. Relationships between steroid receptors and enzymes of nucleic acid metabolism can be suggested based on the sensitivity of the two systems to ATA. Furthermore, steroid receptors have been proposed to be metalloproteins [33–37]. Recently, specific and direct correlations between enzyme activities and steroid receptors have been reported by Schmidt *et al.* [36] who have proposed antigenic similarities between DNA polymerase-α and the glucocorticoid receptor. Although the mode of action of steroid hormones is still obscure, our results further point out similarities between steroid receptors and enzymes of nucleotide metabolism.

Previous studies have shown that ATA combines with the template binding site of nucleic acid binding proteins [18]. Since steroid receptors are known to interact with nuclear components for their biological function, the use of ATA has a potential as a chemical probe for the characterization of acceptor site(s) of steroid receptors. The evidence presented above only indirectly argues in favor of an interaction between ATA and glucocorticoid receptor. Studies are now in progress to demonstrate by direct means a binding site for ATA by the use of affinity chromatography (Samokyszyn and Moudgil, unpublished). However, an indirect action of ATA cannot be ruled out, but appears unlikely.

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