Requirement for Activation in the Binding of Progesterone Receptor to ATP-Sepharose†

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ABSTRACT: The present studies describe the effect of receptor activation on the binding of progesterone receptor to immobilized ATP. When fractionated by ammonium sulfate precipitation, the avian progesterone-receptor complex readily binds to ATP-Sepharose. In contrast to freshly prepared cytosol, the ammonium sulfate precipitated receptor is in the "activated" state in that it binds to isolated nuclei at 4 °C. By comparing "activated" with "unactivated" preparations of cytosol receptor, we have determined that activation is also required for receptor binding to ATP. Oviduct cytosol from estrogen-treated chicks was activated (1) by incubation at 23 °C or (2) by incubation in the presence of KCl. The proportion of receptor that bound ATP was tested by ATP-Sepharose chromatography at 4 °C. Less than 3% of the unactivated receptor bound ATP. However, ATP binding increased markedly with preincubation at 23 °C and under optimum conditions (60 min, 23 °C) about 60% of the total receptor bound ATP. This process was hormone dependent and paralleled the activation of receptor for nuclear binding. Salt treatment also increased ATP binding and was a more effective method than incubation at 23 °C. Following a 2-h incubation at 4 °C in 0.5 M KCl, and subsequent dilution to 0.25 M KCl, about 90% of the receptor bound ATP. Thus, ATP binding appears to be a property of the activated progesterone receptor. To determine if activated estrogen receptor would also bind ATP, intact rat uteri were incubated at 37 °C for 30 min with 10 nM [^{3}H]-estradiol. The nuclear and cytosol receptors were then obtained and tested for their ability to bind ATP. A majority of the activated or nuclear bound receptor was retained by ATP-Sepharose while only a small portion of the cytosol receptor was bound by the column. This demonstrates that ATP binding is also a property of the activated estrogen receptor and suggests that this might be a property of steroid receptors in general. Although the actual role of ATP in receptor function is unknown, these results suggest that ATP may be biologically important in a function of the activated receptor.

Although the avian progesterone receptor has been characterized by purification and physicochemical analysis, the actual biochemical function of this protein remains unknown. Some insight into the function of steroid receptors has come from the observations that progesterone receptor binds to DNA and chromatin (Schrader et al., 1972; Spelsberg, 1974). More recently, the interaction of progesterone receptor with a nucleotide has been demonstrated in our laboratory by the use of ATP-Sepharose chromatography (Moudgil and Toft, 1975, 1977). This interaction was shown to be reversible, ionic in nature, and to have a preference for ATP over other nucleoside triphosphates, AMP, and cAMP.[1] In these studies, the binding of progesterone receptor to ATP-Sepharose was demonstrated using receptor preparations that were fractionated by ammonium sulfate precipitation. In contrast, more recent studies show that freshly prepared cytosol has little or no affinity for ATP-Sepharose. Since ammonium sulfate fractionated receptor is in an "activated state" in that it can bind oviduct nuclei in a cell-free system at 4 °C (Buller et al., 1975a,b), we have tested the possibility that the progesterone receptor may first require an activation step before binding to ATP can occur. In this study, we will show that the activation of freshly prepared cytosol receptor is required for binding of receptor to ATP.

Experimental Procedures

Materials. [1,2-^{3}H]Progesterone (48 Ci/mmol) and [2,4,6,7-^{3}H]estradiol (91.6 Ci/mmol) were obtained from New England Nuclear; ATP was from Schwarz/Mann; Sepharose 4B and Dextran T-70 were from Pharmacia, Uppsala, Sweden; thioglycerol and activated charcoal were from Sigma; Basal Eagles medium was from Grand Island Biological Co. and Vi-Gain round pellets, containing 15 mg of diethylstilbestrol, were from Vineland Laboratories.

Preparation of ATP-Sepharose. ATP was covalently linked to Sepharose 4B as previously described (Moudgil and Toft, 1975). In this process, ATP was attached to Sepharose 4B through the hydroxyl groups of the ribose via a six-carbon bridge of adipic acid dihydrazide. These preparations contained 6–13 μmol of ATP per mL of packed Sepharose as determined by phosphate analysis (King, 1932).

Affinity Chromatography. Columns containing 1 mL of packed Sepharose were thoroughly washed and equilibrated with buffer containing 40 mM Tris, 12 mM thioglycerol, pH 8.0 (TTG), and 0.01 M KCl. The samples were layered on the columns which were eluted first with TTG containing 0.01 M KCl and then with TTG containing 1 M KCl. For each elution, 15 or 20 fractions (30 drops; approximately 1.2 mL) were collected on a Gilson microfractionator with a flow rate of approximately 1 drop/s. Aliquots (0.5 mL) from each fraction were counted in 8 mL of scintillation cocktail containing 0.8 mL of water.

Preparation of Progesterone Receptor. Oviducts were removed from White Leghorn chicks in which two pellets of diethylstilbestrol (Des) had been implanted subcutaneously for 3–5 weeks. This treatment produced changes in oviduct wet weight and RNA, DNA, and protein contents which were comparable to those seen in oviducts from chicks injected with 5 mg of Des/day in oil (R. Webster unpublished observation). The tissue was rinsed and homogenized using a "Tissumizer" (Tekmar Model SDT) in 2 volumes of TTG buffer containing

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[1] Abbreviations used: cAMP, cyclic adenosine 3′,5′-monophosphate; TTG, buffer containing 40 mM Tris, 12 mM thioglycerol, pH 8.0; Des, diethylstilbestrol; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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10% glycerol. The homogenate was first centrifuged at 12 000*g* for 10 min and the supernatant was then centrifuged at 149 000*g* for 60 min to obtain the cytosol fractions.

Temperature Activation and Binding to ATP-Sepharose. In these experiments, elevated temperature (23 °C) or high salt conditions were employed as a means of receptor activation. For incubations at elevated temperatures, aliquots of chick oviduct cytosol were preincubated at 4 °C for 2 h with 20 nM [^{3}H]progesterone and then incubated at 23 °C for varying periods of time. The samples were then cooled on ice and tested for the binding of receptor to ATP-Sepharose. Some samples were first incubated without hormone at 23 °C for varying periods of time, cooled on ice, and then incubated at 4 °C for 2 h with 20 nM [^{3}H]progesterone before testing receptor binding to ATP-Sepharose or to nuclei.

Salt Activation and Binding to ATP-Sepharose. In these experiments, aliquots of cytosol containing 20 nM [^{3}H]progesterone were incubated at 4 °C with 0.01 to 0.5 M KCl. Following a 1-h incubation, each sample was diluted 1:1 with TTG containing 0.01 M KCl before determining the binding of receptor to ATP-Sepharose. Parallel samples were also run to measure nuclear binding.

Nuclear Binding Assay. Cytosol was incubated at 23 °C or with various concentrations of KCl as described above. The procedure for nuclear binding assays is described in Lohmar and Toft (1975b). Briefly, duplicate or triplicate aliquots (0.5 mL) from the preincubated solutions were cooled on ice and incubated for 1 additional h with 0.2 mL of TTG (pH 7.5) and 0.15 mL of purified oviduct nuclei containing 50–100 μg DNA. The nuclei were prepared and stored according to method II of Spelsberg et al. (1974) (omitting the use of Triton X-100) and were resuspended in buffer containing 10 mM Tris, 25% glycerol, and 2 mM $MgCl_2$ (pH 7.5). At the end of the nuclear incubation, 3.5 mL of buffer (0–4 °C) containing 10 mM Tris, 10% glycerol, 1 mM $MgCl_2$, and 0.1% Triton X-100 (pH 7.5) was added to each tube and the nuclei were sedimented at 800*g* for 10 min. The supernatants were aspirated off, the nuclei were resuspended in 3.5 mL of the same buffer and again recovered by centrifugation. These two washes were sufficient for removing unbound hormone while maintaining optimum nuclear binding. Each nuclear pellet was suspended in 0.5 mL of water and transferred to a scintillation vial. Each tube was rinsed with 0.5 mL of water which was also transferred to the scintillation vial. Five milliliters of scintillation cocktail was added and the radioactivity determined.

Preparation of Estrogen Receptor. Holtzman female rats (22–26 days of age) were killed by cervical dislocation and the uteri stripped of fat and excised. The uteri were placed in Eagles medium (2 uteri/2 mL) containing 10 nM [^{3}H]estradiol and incubated in a shaking water bath under an atmosphere of 95% O_2 + 5% CO_2 at 37 °C for 30 min. The tissue was rinsed three times in ice-cold TTG, minced, and homogenized (0–4 °C) in TTG (5–10 uteri/mL) using a polytron homogenizer (Brinkman, Model PT 10-20-3550). The homogenate was first centrifuged at 800*g* for 60 min to obtain the cytosol fraction containing tritium-labeled receptor. The 800*g* × 10 min pellet was suspended in 1 mL of TTG containing 0.4 M KCl and incubated at 0 °C for 30 min to extract the nuclear bound receptor. After centrifugation at 12 000*g* for 10 min, the supernatant was diluted 1:1. Aliquots of nuclear and cytosol fractions were layered on ATP-Sepharose columns to test receptor binding to ATP.

Sucrose Gradient Analysis. Linear 5–20% sucrose gradients (4.5 mL) in TTG buffer containing 0.4 M KCl were prepared with a Beckman gradient former. Receptor preparations (0.2 mL) were layered on the gradients which were then centrifuged at 149 000*g* for 16 h. [^{14}C]Ovalbumin was layered on a separate gradient as a standard for determining sedimentation coefficients. The fractions (3 drops) were collected by piercing the bottom of each tube and the radioactivity determined.

Determination of Specific Receptor Binding. Duplicate aliquots were taken from steroid receptor preparations to estimate total and nonspecific hormone binding by charcoal assay. To determine nonspecific binding, approximately 1000-fold excess cold steroid was added to one aliquot which was then heated at 37 °C for 60 min to destroy specific receptor (Buller et al., 1975b). The other aliquot was left at 0 °C. An equal volume of Dextran-charcoal suspension (0.5% Norit A, 0.05% Dextran T-70 in TTG) was added to each tube for 5 min to remove free hormone. Specific binding was assumed to represent the radioactivity in the unheated minus heated samples.

Other Methods. Unless otherwise indicated, radioactivity was determined by combining aqueous samples with 5 mL of a scintillation cocktail consisting of toluene (Baker), Triton X-100 (RPI), and spectrofluor (Amersham/Searle), 1000: 521:42 (v:v:v). The counting efficiency for tritium was 33% in a Beckman LS250 liquid scintillation counter. Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. DNA was determined by the method of Burton (1968).

Results

Figure 1 shows the effect of temperature and salt activation on the binding of progesterone receptor to ATP. Cytosol which had been incubated with [^{3}H]progesterone at 0 °C and had not been incubated at room temperature showed little binding to ATP. However, subsequent incubation of receptor at 23 °C greatly enhanced receptor binding to ATP-Sepharose (Figure 1A). Using this method of receptor activation, the maximum extent of receptor binding to ATP-Sepharose was found to be between 40 and 60% of the total cytosol receptor.

Receptor binding to nuclei can also be enhanced by exposing receptor to high salt conditions (Higgins et al., 1973; Milgrom et al., 1973; Giannopoulos, 1975; Buller et al., 1975a,b). Figure 1B demonstrates that the binding of receptor to ATP-Sepharose was also increased by high salt treatment. The binding was very low using cytosol incubated in 0.01 M KCl but rose to a maximum in cytosol incubated with 0.5 M KCl. Cytosol incubated with 1 M KCl produced ATP binding which was comparable to cytosol incubated with 0.5 M KCl (data not shown). Under these conditions, 80–100% of the progesterone-receptor complex is able to bind to ATP-Sepharose. The increased ATP binding observed after temperature or salt activation could not be accounted for by a change in hormone binding activity as determined through charcoal adsorption assay. In addition, practically all of the column bound counts could be competed for by the addition of excess unlabeled steroid during the preincubation period indicating the absence of nonspecific binding.

Figure 2 compares the binding of progesterone receptor to ATP-Sepharose and to isolated nuclei. With cytosol incubated at 23 °C in the presence of progesterone (curves labeled "+P"), the binding of receptor to both ATP and to nuclei reached a maximum by 1 h of activation (Figures 2A and 2B) and then gradually decreased with time (data not shown). In addition, both binding activities are increased in the presence of progesterone. Parallel changes in receptor binding to ATP and to nuclei are also observed in cytosol incubated with varying concentrations of KCl (Figures 2C and 2D). This suggests that the same activation process may be required for both ATP binding and nuclear binding.

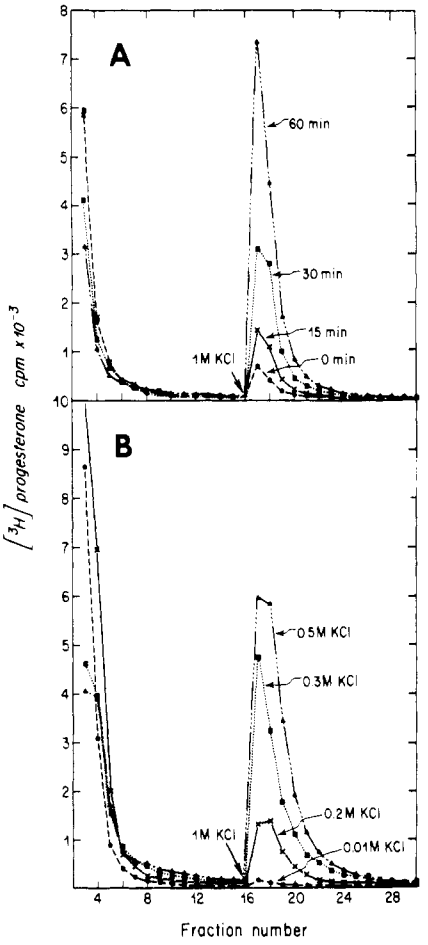

FIGURE 1: The effect of temperature and salt activation on the binding of progesterone receptor to ATP-Sepharose. (A) Chick oviduct cytosol was prepared in TTG buffer containing 10% glycerol. In the temperature activation experiments, aliquots (0.5 mL) of cytosol were preincubated at 4 °C for 2 h with 20 nM [^{3}H]progesterone and then incubated at 23 °C for the periods indicated. The amount of ATP binding by the 60-min sample represents 42% of the total progesterone receptor applied to the column. (B) For the salt activation experiments, aliquots (1.5 mL) of chick cytosol were incubated in buffer containing 20 nM [^{3}H]progesterone and the indicated concentrations of KCl. After a 1-h incubation, each sample was diluted 1:1 with buffer before determining binding to ATP-Sepharose. The amount of ATP binding by the 0.5 M KCl sample represents about 100% of the total progesterone receptor.

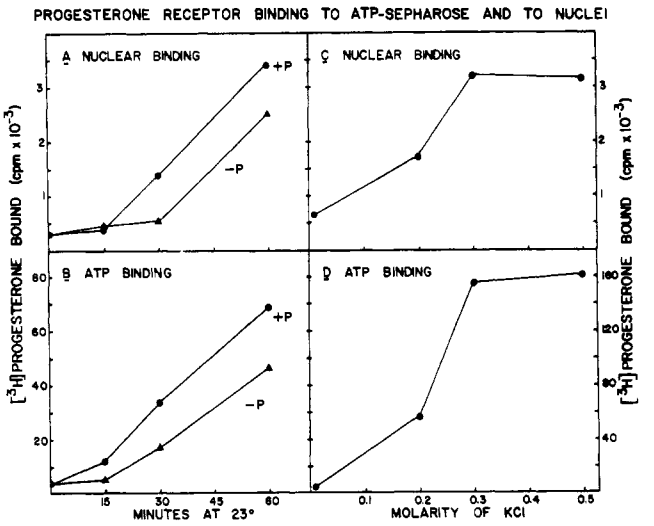

FIGURE 2: Progesterone receptor binding to ATP-Sepharose and to isolated nuclei. (A and B) As indicated in the curves labeled "+P", aliquots of chick cytosol were preincubated at 4 °C for 2 h with 20 nM [^{3}H]progesterone and then at room temperature for varying periods of time before testing the binding to ATP and to nuclei. Other aliquots of cytosol were first incubated at room temperature and then incubated with tritiated progesterone at 4 °C (labeled "−P"). (C and D) Aliquots of chick cytosol were incubated in buffer containing 20 nM [^{3}H]progesterone and the indicated concentrations of KCl. After a 1-h incubation at 4 °C, each sample was diluted 1:1 with buffer before determining binding to ATP-Sepharose and to isolated nuclei. The data for receptor binding to ATP-Sepharose are expressed as total cpm adsorbed to the column. The data for nuclear binding are expressed as the total cpm bound to the nuclei in each tube.

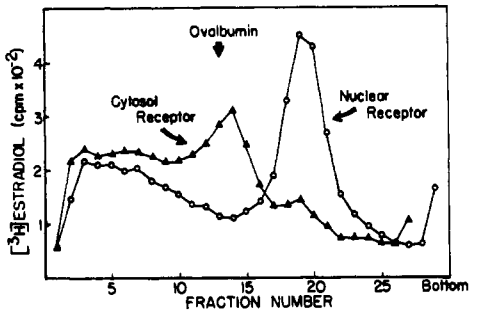

FIGURE 3: Sedimentation patterns of nuclear and cytosol estrogen receptor on sucrose gradients. Intact uteri from 22–26-day-old rats were incubated in 10 nM [^{3}H]estradiol at 37 °C for 30 min. Cytosol and nuclear fractions containing tritium-labeled receptor were prepared as indicated in methods and layered on 5–20% sucrose gradients containing 0.4 M KCl. The gradients were centrifuged for 16 h at 149 000g. The arrow represents the sedimentation of ovalbumin (3.7 S).

These experiments demonstrate that ATP binding appears to be a property of the activated progesterone–receptor complex and suggests that ATP binding is another measure of receptor activation. To test this hypothesis further, nuclear and cytosol receptor was obtained from intact tissue and tested for ATP binding. This was accomplished using estrogen receptor from rat uterus since, as seen in Figure 3, the cytosol and nuclear forms of the receptor can be readily identified by their sedimentation properties (Jensen and DeSombre, 1972). Sucrose gradient analysis (Figure 3) showed that the nuclear bound receptor had a sedimentation coefficient of approximately 5 S and the cytosol receptor had a sedimentation coefficient of approximately 4 S with a small fraction also in the 5S form. Figure 4 shows that these receptor preparations had very different binding patterns on ATP-Sepharose. When total hormone binding was estimated by sucrose gradient centrifugation, approximately 55% of the activated or nuclear bound receptor and 15% of the cytosol receptor were bound to the column. In additional experiments, a more quantitative charcoal assay was used to determine specific hormone binding. Under these conditions, as much as 95% of the nuclear receptor and 25% of the cytosol receptor were retained by ATP-Sepharose (data not shown).

Discussion

Receptor activation is thought to involve structural changes in the receptor molecule which enable it to occupy acceptor sites on nuclear chromatin. However, the actual molecular events in receptor activation remain uncertain and present methods for monitoring this process are usually cumbersome. The mammalian estrogen receptor appears to dimerize with activation and this can be detected by sedimentation analysis (Notides and Nielsen, 1975). However, with the avian progesterone receptor, any structural changes are apparently quite subtle and do not alter the size or sedimentation properties of the receptor (Buller et al., 1975b). Thus, activation is generally

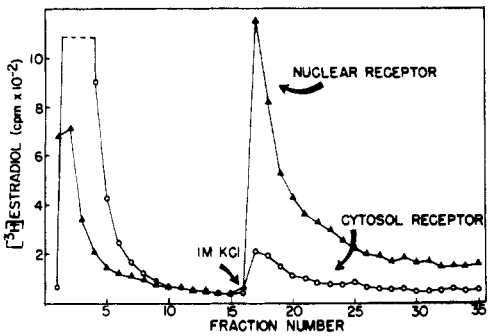

FIGURE 4: Estrogen receptor binding to ATP Sepharose. Tritium-labeled estrogen receptor was prepared from cytosol and nuclear fractions as described in Experimental Procedures and tested for binding to ATP. When total hormone binding was estimated by sucrose gradient centrifugation, approximately 55% of the activated or nuclear receptor and 15% of the cytosol receptor were retained by ATP-Sepharose.

measured by the ability of receptor to bind isolated nuclei (Buller et al., 1975a,b; Lohmar and Toft, 1975b). More recently, activated receptors have been shown to acquire an ability to bind to DNA-cellulose (Kalimi et al., 1975; Toft, 1973; Yamamoto and Alberts, 1972; Milgrom et al., 1976), phosphocellulose (Atger and Milgrom, 1976; Schrader et al., 1975), and to polyanionic resins in general (Milgrom et al., 1973). These are all ionic interactions that can be easily disrupted by increasing the ionic conditions to that of 0.2 to 0.3 M KCl. On the other hand, the binding of progesterone receptor to ATP is stable under these salt conditions and is not disrupted until a concentration of about 0.5 M KCl is reached (Moudgil and Toft, 1977). This offers significant advantages over other methods for monitoring receptor activation in that the binding can be conducted under salt conditions that minimize protein aggregation and nonspecific binding. In addition, this method is more easily applied in studying the activation of receptor by high salt treatment.

The studies reported here demonstrate that ATP-Sepharose binding is a characteristic of the activated form of the progesterone receptor. Freshly prepared cytosol receptor showed little binding to ATP-Sepharose. However, when cytosol was activated either by exposure to 23 °C or high salt (Figure 1), the binding of receptor to ATP-Sepharose was greatly enhanced. Comparison of ATP binding with nuclear binding experiments shows that activation for ATP binding has a time course which is similar to the time course of activation for nuclear binding (Figure 2). In addition, the binding of temperature activated receptor to both ATP and nuclei is increased in the presence of progesterone. This suggests that the same activation process may be required for both ATP binding and nuclear binding.

Activation by high salt treatment proved to be the most effective since this procedure avoids exposing the labile receptor to elevated temperature and can be used to bind 80–100% of the receptor to ATP-Sepharose (Figure 1B). The ability of ATP-Sepharose to bind activated receptor provides a tool to more clearly characterize the process of salt activation. In this way, we have found that salt activation is a rather slow process requiring approximately 1 h in 0.5 M KCl (data not shown). When cytosol is incubated with lower concentrations of salt, activation occurs but at a much slower rate, i.e., requiring approximately 120 min to complete activation with 0.3 M KCl. Maximal receptor activation is usually not achieved at salt concentrations below 0.5 M KCl. Unlike the process of temperature activation, maximal activation by 0.5 M KCl can occur in the absence of added hormone (data not shown). The reason for this is unknown but may suggest that the hormone is not needed to stabilize the progesterone-receptor during salt activation at 4 °C but is able to protect the progesterone receptor, at least to some extent, during exposure to 23 °C.

The studies using estrogen receptor from intact uteri labeled in vitro confirmed the results found in the cell-free system. When rat uterine estrogen receptor was tested for ATP binding, it was found that the activated or nuclear bound receptor also showed enhanced ATP binding compared to nonactivated or cytosol receptor (Figure 4). These results from two hormone systems suggest that ATP binding might be a property of activated steroid receptors in general.

Since ATP is more readily bound by activated receptor, this interaction may be involved either in the activation process itself or in a function of the receptor at sites on nuclear chromatin. Recent studies in our laboratory have shown that nucleotides can either inhibit or accelerate the rate of progesterone receptor activation depending upon the concentration used (Toft et al., 1977a,b). This process is inhibited by low concentrations of nucleotides but is stimulated when the nucleotide concentration is above 1 mM. Since appropriate concentrations of ATP stimulate the rate of receptor activation and since activated receptor has a much greater affinity for ATP, it is possible that the binding of ATP stabilizes the receptor during the activation process so as to enhance receptor activation. This could explain the stimulatory effect of ATP; however, at the present time, there is no evidence which indicates that ATP is a necessary factor in the activation process.

Additional studies in our laboratory show that the metal chelator, *o*-phenanthroline, and the antibiotic, rifamycin AF/013, inhibit binding of the progesterone-receptor complex to nuclei (Lohmar and Toft, 1975b) and to ATP-Sepharose (Toft et al., 1977a,b). These results suggest that nucleotide binding sites on the receptor may be closely associated with the region that participates in the nuclear binding process. However, these two binding processes are not identical and it is possible that two separate interactions occur both of which are altered by the inhibitors. Receptor binding to nuclei and to ATP differ in affinity since nuclear binding can be disrupted by 0.3 M KCl whereas the salt concentration must be above 0.5 M KCl to disrupt ATP binding (Moudgil and Toft, 1975). While the binding of receptor complex to nuclear acceptor sites may involve the ATP binding site, this is a more complex interaction which appears to involve nuclear acidic proteins on the chromatin and DNA (Spelsberg, 1974).

A more defined function of ATP is indicated by other recent studies from this laboratory (Moudgil and Toft, 1976). We have found that receptor preparations that have been extensively purified are able to catalyze an ATP-pyrophosphate exchange reaction. These results suggest that the receptor has an enzymatic function with involves ATP. However, this possibility has not been demonstrated using totally pure receptor and a complete enzymatic process has not yet been described.

The fact that receptor activation is required for the binding of progesterone receptor to ATP offers additional support for the biological significance of this interaction. We have discussed some possible functions of ATP binding and these are presently under investigation.

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Kinetic and Equilibrium Studies on Steroid Interaction with Human Corticosteroid-Binding Globulin†

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ABSTRACT: Kinetic and equilibrium studies on the interaction of steroids with human corticosteroid-binding globulin (CBG, transcortin) were performed with pH, temperature, and steroid structure as variables. Dissociation rate constants were determined fluorometrically; the values for cortisol, corticosterone, deoxycorticosterone, and progesterone are 0.031, 0.047, 0.10, and 0.16 s^{-1}, respectively, at 20 °C, pH 7.4. The pH dependence of the dissociation rate constant for the corticosterone complex below pH 10.5 at 20 °C is given by $k_{off} = 0.043\ (1 + [H^+]/10^{-6.50})\ s^{-1}$; above pH 11, $k_{off} = 0.030\ (1 + 10^{-12.15}/[H^+])\ s^{-1}$. A temperature-dependence study of k_{off} for the cortisol and progesterone complexes gave values of 0.0028 s^{-1} and 0.012 s^{-1} at 4 °C, respectively, and 0.88 s^{-1} and 4.5 s^{-1} at 37 °C, with progesterone dissociating about four to five times faster over the entire temperature range. The affinity constants, determined by equilibrium dialysis, for the binding of cortisol, corticosterone, and progesterone at 4 °C were 7.9, 7.2, and 7.0 × 10^8 M^{-1}; values of 0.40 and 0.26 × 10^8 M^{-1} were determined at 37 °C for cortisol and progesterone. The close similarity of the affinity constants of the three steroids combined with differing dissociation rates implies that the association rate changes with steroid structure, in contrast to our earlier findings with progesterone-binding globulin.

Human corticosteroid-binding globulin (CBG)[1] was the first high-affinity steroid binding protein to be reported (Daughaday, 1956; Bush, 1957; Sandberg and Slaunwhite, 1958). It has been purified in several laboratories and some of its physicochemical properties have been described in detail (Westphal, 1971; Le Gaillard et al., 1975). Advances in affinity chromatography have simplified the purification of CBG (Rosner and Bradlow, 1971; Le Gaillard et al., 1974). However, only a few studies have been published on the mechanism of steroid interaction with the high-affinity binding protein. In the present report, we attempt, by means of kinetic and equiilibrium studies, to establish an understanding of the binding of steroids to CBG.

The kinetic studies on CBG–steroid interaction utilized the method of fluorescence quenching (Stroupe et al., 1975). The intrinsic fluorescence of the binding protein is quenched upon

† From the Department of Biochemistry, University of Louisville School of Medicine, Health Sciences Center, Louisville, Kentucky 40232. *Received July 22, 1977.* This is report No. 37 in the series Steroid-Protein Interactions; for the preceding paper, see Stroupe et al. (1977). Part of this work was presented previously (Stroupe, S. D., Forsthoefel, M. W., and Harding, G. B. (1977), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 36,* 781). Our studies were supported by a grant from the National Institute of Arthritis, Metabolism, and Digestive Diseases (AM-06369) and a research career award (U.W.) from the Division of General Medical Sciences (GM-K6-14,138) of the United States Public Health Service.

[1] Abbreviations used: CBG, corticosteroid-binding globulin or transcortin; E_a, energy of activation; PBG, progesterone-binding globulin; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

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