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CONVERSION FROM 4 S ANDROGEN RECEPTOR FROM RAT SUBMANDIBULAR GLAND TO HIGHER MOLECULAR FORM AND THE EFFECT OF SODIUM MOLYBDATE

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Summary—The androgen receptor from rat submandibular gland was transformed by exposure to ATP at 0°C. The transformed 4 S receptor converted to a higher molecular weight form in a low-salt glycerol gradient centrifugation when ATP was removed from the sample. The sedimentation coefficient of the converted receptor was similar in the absence or presence of 20 mM molybdate; 7.8 ± 0.5 S without molybdate and 7.6 ± 0.3 S with molybdate. However, the receptor converting in the presence of molybdate could markedly bind to DNA-cellulose, while an entity without molybdate could not. These results suggest that molybdate directly interacts with the DNA-binding domain on the 4 S androgen receptor and prevents this domain from being concealed by conversion in low-salt conditions.

INTRODUCTION

Androgen–receptor complexes, as well as other steroid–receptor complexes, are known to exist in at least two states, one of which can bind to the nucleus or DNA and the other can not. The conversion from DNA-unbound state to DNA-bound state is called transformation, occurring *in vitro* by brief heating [1, 2], or exposure to salt [3, 4] or ATP [5]. The transformed androgen receptor is distinguished from the nontransformed receptor by a smaller molecular weight and a weaker interaction with DEAE-cellulose than the nontransformed receptor. However, the molecular mechanism and the reversibility of transformation have not been well established. Colvard and Wilson[6] reported that the 8 S androgen receptor from rat prostate Dunning tumor could be reconstituted from the 4.5 S receptor and the 8 S promoting factor. Murayama *et al.* reported similar results on estrogen receptor of cow uterus [7] and progesterone receptor of hen oviduct [8]. On the other hand, there is evidence that the transformation process of steroid receptors is not reversible; pH-induced transformation of the rat hepatic glucocorticoid receptor was irreversible [9], and transformation of purified progesterone receptor from chick oviduct could not be reversed by addition of molybdate [10]. It has been recently reported that a 7–8 S form of glucocorticoid receptor from rat liver bound to DNA-cellulose or soluble DNA as well as the 4 S transformed receptor [11] and that 9.2 S glucocorticoid–receptor complexes from mouse brain also bound to DNA-cellulose after sucrose gradient centrifugation [12]. These reports suggest that a decrease of an apparent molecular weight is not necessary for glucocorticoid receptor to bind to DNA. In this report we describe the conversion of the transformed 4 S androgen receptor from rat submandibular gland and the significant effect of sodium molybdate on DNA-binding ability of converted receptors.

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EXPERIMENTAL

Materials

[17α-Methyl-^{3}H]trienolone (R1881, 87 Ci/mmol) was purchased from New England Nuclear. ATP and EDTA were obtained from Sigma. Dithiothreitol (DTT) was obtained from Boehringer. Leupeptin was a generous gift from Dr H. Umezawa. Calf thymus double-strand DNA-cellulose was from Wako Pure Chem. Co. (Japan). Sephadex G25, DEAE-Sephacel and Sephacryl S300 were obtained from Pharmacia. DEAE-cellulose (DE 52) was obtained from Whatman, and hydroxylapatite was from Seikagaku Kogyo (Japan). All other reagents were of analytical grade.

Buffers

The following buffers were used: buffer A, 50 mM Tris–HCl, 1 mM EDTA, 1 mM DTT, 0.5 mM leupeptin and 10% (v/v) glycerol, pH 7.5: buffer B, buffer A containing 20 mM sodium molybdate; buffer C, 10 mM Tris–HCl, 0.5 mM DTT, 10 mM sodium molybdate, pH 7.5; buffer D, 10 mM Tris–HCl, 1 mM EDTA, 1.5 mM 2-mercaptoethanol and 10% (v/v) glycerol, pH 7.5. The pH was adjusted at 20°C.

Animals and preparation of cytosol

Intact female Wistar rats, 8–10-weeks-old were used in the experiments because the level of androgen receptor in the cytosol from submandibular gland is higher in females than in males and the characteristics of the receptor are similar in both sexes [13]. The rats were killed by decapitation and submandibular glands were excised, rinsed in ice-cold 0.9% NaCl. All procedures were performed at 0–4°C. Tissues were homogenized in 4 vol of buffer A or B, and cytosol was obtained as a clear supernatant following 150,000 *g* centrifugation for 30 min.

Isotope labeling and treatments with ATP

Cytosol obtained following centrifugation was immediately incubated with 5 nM [^{3}H]R1881 for 1.5 h in the presence or absence of a 100-fold excess of cold R1881. Labeled cytosol was divided into two portions and one portion was added to the ATP stock solution (0.173 M) to make a final concentration 10 or 20 mM, while another was kept without ATP. These cytosols were further incubated for 3 h, and then treated with a pellet of an equal volume of dextran-coated charcoal (DCC; 0.5% activated charcoal and 0.05% dextran T 70) suspension prepared in buffer A in order to avoid the dilution of cytosol. For the minicolumn binding assay, labeled cytosols were treated with a same volume of DCC suspension to diminish the concentration of ATP. Following 10 min incubation, the sample was centrifuged for 5 min at 3000 rpm, as described previously [13]. DCC-treated cytosol was subjected to the following experiments.

Sephadex G25 gel chromatography

In some experiments, DCC-treated cytosols containing ATP were passed through Sephadex G25 columns (1 × 11 cm) equilibrated with either buffer A or B to eliminate ATP.

Minicolumn binding assay

The minicolumn binding assay was carried out according to the method of Holbrook *et al.*[14] with a slight modification to determine the proportion of the transformed, nontransformed and mero receptors as described previously [5]. 0.25 ml each of DNA-cellulose (0.4 mg DNA), DEAE-cellulose and hydroxylapatite was packed separately in a 1 ml Pipetman tip. Three tips were directly connected (DNA-cellulose at the top, DEAE-cellulose in the middle, and hydroxylapatite on the bottom) and were equilibrated in buffer C. Aliquots (100 μl) of DCC-treated cytosols or (200 μl) of cytosols passed through Sephadex G25 were applied onto the top column and washed with 3 ml buffer C. Bound radioactivity in each column was extracted with 1 ml ethanol and counted with 32% efficiency as described previously [15]. The specifically bound was obtained from subtracting the nonspecifically bound (with cold R1881) from the total bound (without cold R1881) retained on the columns.

Glycerol gradient centrifugation

Linear glycerol gradient (5 ml) was prepared in buffer A either with or without 10 mM ATP, or in buffer B to make a final glycerol concentration 12.5–27.5% (v/v). Aliquots (200 μl) of the samples were layered on the gradients and centrifuged at 190,000 *g* in a Hitachi 55T2 rotor for 22 h. Eight-drop fractions (190 μl) were collected from the top with a Hitachi DGF-U fractionater. In the experiments in Fig. 3, 8-drop fractions were directly applied onto hydroxylapatite columns, corresponding to the total bound or DNA-cellulose columns containing 0.25 ml of each resin. Columns were separately prepared in 1 ml Pipetman tips, equilibrated and washed with buffer C. Bound radioactivity was extracted with 1 ml ethanol and counted. Sedimentation coefficients of the receptor complexes were determined by the following markers: horse γ-globulin (7.0 S), bovine serum albumin (4.6 S), and sperm whale myoglobin (2.0 S).

Sephacryl S300 gel chromatography

An aliquot (0.5 ml) of a DCC-treated cytosol was analyzed by gel chromatography with a column of Sephacryl S300 (1 × 55 cm) equilibrated in buffer D containing 10 mM sodium molybdate or 10 mM ATP. The following standards were used to calibrate the apparent molecular weight: blue dextran, horse γ-globulin (160 KDalton), bovine hemoglobin (64.5 KDalton), sperm whale myoglobin (17 KDalton), and vitamin B_{12} (1.4 KDalton).

DEAE-Sephacel chromatography

An aliquot (0.5 ml) of DCC-treated cytosol or an aliquot (1 ml) of the ATP-treated cytosol passed through Sephadex G25 was applied on columns (0.9 × 3 cm) of DEAE-Sephacel equilibrated in buffer C. The column was washed with 25 ml of buffer C and the adsorbed receptors were eluted with a 50 ml linear gradient from 0 to 0.4 M KCl in buffer C.

Other methods

The ATP stock solution was adjusted at pH 7.5 with Tris and the concentration was determined with ultraviolet absorption using $\varepsilon_{260} = 1.54 \times 10^4$. Protein concentration was measured by the method of Lowry *et al.*[16] using bovine serum albumin as the reference standard.

RESULTS

The cytosol prepared from female rat submandibular gland was labeled with [^{3}H]R1881 and analyzed by glycerol gradient centrifugation containing molybdate or ATP. As reported earlier on progesterone receptor [17], glucocorticoid receptor [18], and androgen receptor [5], [^{3}H]R1881–receptor com-

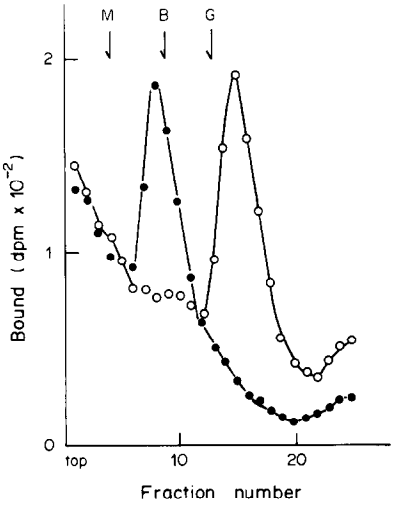

Fig. 1. Glycerol gradient centrifugation of androgen receptor in the absence or presence of ATP. Two hundred μl of labeled cytosols were analyzed on 12.5–27.5% (v/v) glycerol gradients in buffer A containing 10 mM ATP (●) or in buffer B (○). Centrifugation was performed at 190,000 *g* for 22 h at 2°C. Horse γ-globulin (G), bovine serum albumin (B), sperm whale myoglobin (M).

plexes sedimented at 8 S with 20 mM molybdate and at 4 S with 10 mM ATP (Fig. 1). The efficiency of transformation was measured by the minicolumn binding assay [14]. Table 1 represents a proportion of receptor complexes bound to DNA-cellulose, DEAE-cellulose or hydroxylapatite, corresponding to the transformed, nontransformed or mero receptor, respectively. Both 10 and 20 mM of ATP enhanced the DNA-cellulose binding from 6% to approx 40–50% within 3 h at 0°C. The similar degree of DNA-cellulose binding (ca. 50%) remained up to 27 h (data not shown). The complete transformation of the androgen receptor from submandibular gland was not obtained under these incubation conditions with either 10 or 20 mM ATP at 0°C, although most of [^{3}H]R1881–receptor complexes sedimented at 4 S even in 10 mM ATP as in Fig. 1.

When ATP was removed from the samples after 3 h incubation by Sephadex G25 gel chromatography equilibrated in the buffer without molybdate (buffer A), the DNA-cellulose binding markedly reduced to the similar extent of the nontreated cytosol (Table 1). On the contrary, the samples chromatographed with the buffer containing molybdate (buffer B) retained DNA-cellulose binding ability. In general, molybdate prevents the transformation of steroid receptors induced by gel chromatography as well as by exposure with high salt or high temperature [19, 20]. It is interesting to note that molybdate appears to affect in an opposite manner on the transformed 4 S androgen receptor passed through Sephadex G25; the transformed androgen receptor could bind to DNA-cellulose even after being chromatographed with the buffer containing molybdate, while the entity chromatographed without molybdate could not. These findings suggested that the once transformed 4 S androgen receptor converted to another form which was induced by the removal of ATP by Sephadex G25 gel chromatography. Then, ATP-treated cytosols were further examined with an ATP-lacking glycerol gradient following Sephadex G25 chromatography.

In contrast to the result in Fig. 1, androgen–receptor complexes sedimented at 7.6 S on the low-salt gradient in the absence of ATP (Fig. 2). The peak completely disappeared on a glycerol gradient after DNA-cellulose adsorption and a small peak was occasionally observed at 8.0 S, which might be the nontransformed receptor. [^{3}H]R1881–receptor complexes also sedimented at approx 7.6 S when ATP-treated cytosol was simply loaded on low-salt gradients without Sephadex G25 gel chromatography (Fig. 3). In these experiments ATP should be separated from androgen receptor during the centrifugation. Therefore, it was considered that a 4 S form of androgen receptor converted to a 7.6 S form in low-salt conditions, caused by the elimination of ATP.

The sedimentation coefficient of the converted receptor was similar in the absence and presence of

Table 1. Minicolumn binding assay of ATP-treated androgen receptor from rat submandibular gland

Treatments	Experiment	Per cent of the total specifically bound		
		DNA	DEAE	HAP
None	1–3	6.3*	89.0*	4.7*
ATP (20 mM)	4	52.3	40.8	6.9
(20 mM)	5	40.7	57.0	2.3
(10 mM)	6	53.4	41.4	5.2
ATP (20 mM) + G25($-MoO_4^{2-}$)	4	3.4	87.5	9.1
ATP (20 mM) + G25($+MoO_4^{2-}$)	1–3	43.5*	51.5*	5.1*
	5	50.3	47.1	2.6

Labeled cytosol was incubated with 10 or 20 mM ATP or without ATP. After 3 h incubation, a part of the sample was treated with a same volume of DCC suspension and an aliquot (100 μl) was applied onto a minicolumn. A residual part (1 ml) of labeled cytosol incubated with 20 mM ATP was treated with a pellet of a same volume of DCC suspension, and an aliquot (0.6 ml) was passed through a Sephadex G25 column equilibrated in the absence (Experiment 4, using buffer A) or presence of molybdate (Experiments 1–3 and 5, using buffer B). An aliquot (200 μl) of the eluent in void volume was immediately applied onto a minicolumn. Results were expressed as per cent of the total specifically bound which was a summation of the specifically bound by DNA-cellulose, DEAE-cellulose and hydroxylapatite (HAP). Protein concentration of the cytosol and eluent from Sephadex G25 was 12–14 mg/ml and 3–4 mg/ml, respectively. *The results are the mean of three different experiments.

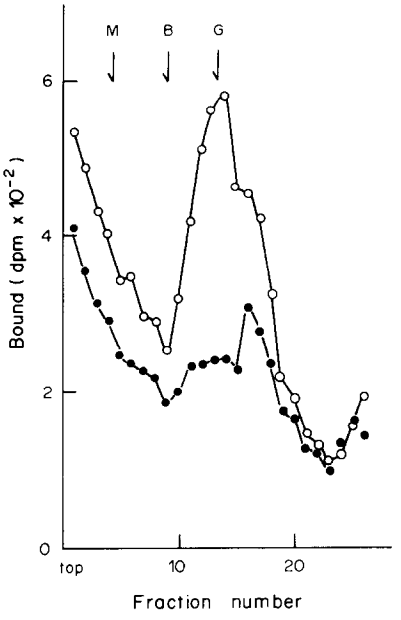

Fig. 2. Glycerol gradient centrifugation of the transformed androgen receptor after DNA-cellulose adsorption. Labelled cytosol in buffer A incubated with 10 mM ATP for 3 h was treated with a DCC pellet and passed through a Sephadex G25 column equilibrated in buffer B. A half of the eluent was kept alone (○) and another was incubated with DNA-cellulose resin (0.25 ml) for 20 min, then the supernatant was obtained by brief centrifugation (●). Aliquots (200 μl) of these samples were layered on the gradient in buffer B.

20 mM molybdate; 7.8 ± 0.5 S ($n = 10$) without molybdate, and 7.6 ± 0.3 S ($n = 6$) with molybdate. However, the dramatic difference in the ability of DNA-cellulose binding was observed on the converted receptors. The receptor converting in the absence of molybdate could not bind to DNA-cellulose (Fig. 3a), while an entity with molybdate could bind to DNA-cellulose (Fig. 3b), consistent with the results in Table 1. These findings seemed not to be specific for transformation induced with ATP, because [^{3}H]R1881–receptor complexes in the cytosol, briefly heated (25°C for 20 min) or treated with 0.4 M KCl, also sedimented around 7.6 S on the low-salt gradient prepared in buffer A or B at 2°C, although they sedimented at 4–4.3 S in the gradient containing 0.4 M KCl (data not shown).

As shown in Fig. 4, the apparent molecular weight of the nontransformed and transformed receptor was 220 KDalton and 80–85 KDalton, respectively. When ATP-treated cytosol was filtrated into a Sephacryl S300 column equilibrated in buffer B, the peak eluted between the nontransformed and transformed receptor with an apparent mol. wt of 140 KDalton. The converted receptor in the presence of molybdate appears to be slightly smaller than the nontransformed receptor as observed by both sedimentation analysis and gel chromatography.

The elution profiles on DEAE-Sephacel chromatography are illustrated in Fig. 5. ATP treated

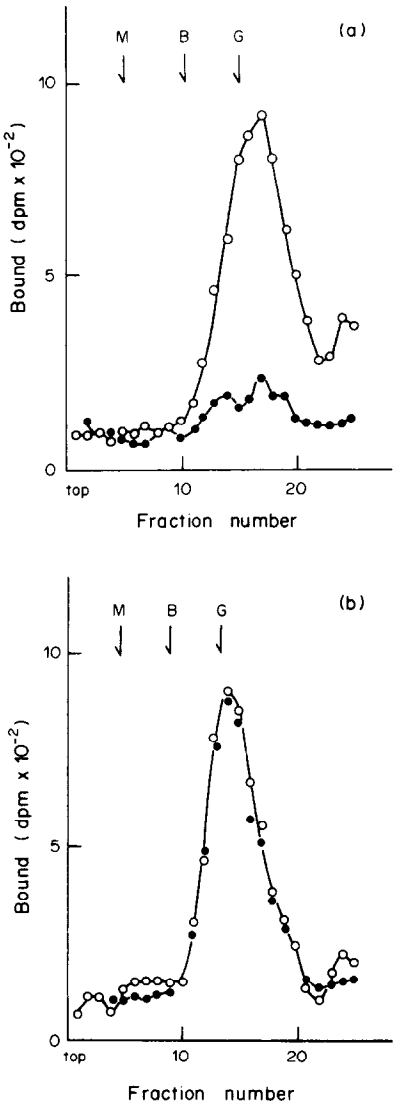

Fig. 3. DNA-cellulose binding ability of the androgen receptor converting on low-salt gradients in the absence or presence of molybdate. Labeled cytosol in buffer A was incubated with 20 mM ATP for 3 h and treated with a DCC pellet. Aliquots (200 μl) of ATP-treated cytosols, which had been omitted gel chromatography on Sephadex G25, were simply layered on glycerol gradients prepared in buffer A (a: lacking molybdate) or buffer B (b: containing molybdate). Eight-drop fractions were directly applied onto hydroxylapatite columns, corresponding to the total bound (○) or DNA-cellulose columns (●). The columns were washed with 3 ml buffer C and bound radioactivity was eluted with 1 ml ethanol and counted.

cytosol was directly applied to a DEAE-Sephacel column or applied to the column following Sephadex G25 column chromatography. The peaks of the specific binding eluted at 0.06–0.08 M KCl in both experiments. On the other hand, the nontransformed receptor eluted at 0.25–0.26 M KCl. Therefore, it was considered that the binding affinity between components in the converted recepter was relatively so weak that it caused dissociation from a 7.6 S form to

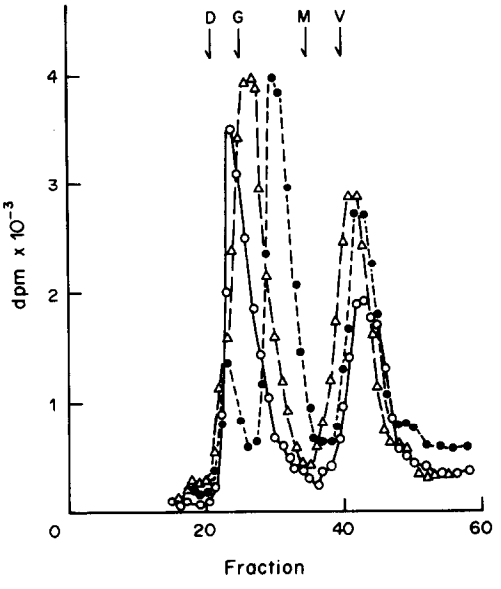

Fig. 4. Sephacryl S300 gel chromatography of the androgen receptor. Following DCC treatment, labeled cytosol prepared in buffer B (0.5 ml) was chromatographed on Sephacryl S300 equilibrated in buffer D containing 10 mM molybdate (○). Labeled cytosol prepared in buffer A was incubated with 10 mM ATP for 3 h and treated with a DCC pellet. Then aliquots (0.5 ml) were loaded on a column in buffer D containing 10 mM molybdate (△) or buffer D containing 10 mM ATP (●). Twenty-drop fractions (1.1 ml) were collected and the radioactivity was counted. Blue dextran (D), horse γ-globulin (G), sperm whale myoglobin (M), vitamin B_{12} (V).

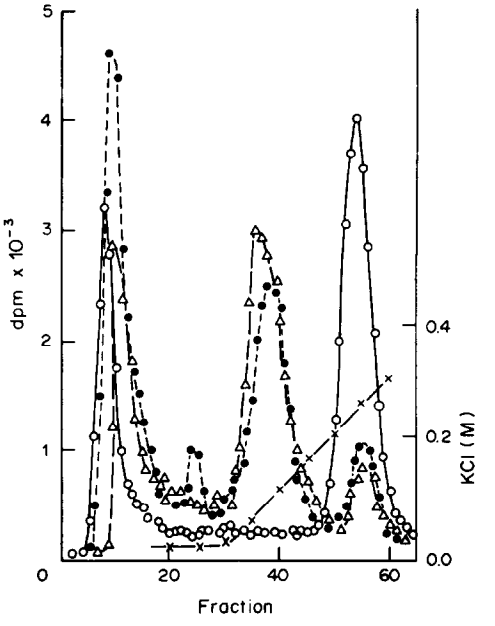

Fig. 5. DEAE-Sephacel chromatography of the androgen receptor. Following DCC treatment, an aliquot (0.5 ml) of labeled cytosol prepared in buffer B (○) was applied on a DEAE-Sephacel column equilibrated in buffer C. Labeled cytosol in buffer A incubated with 10 mM ATP for 3 h was treated with a DCC pellet. An aliquot (0.5 ml) of ATP-treated samples was directly applied (●) or an aliquot (1 ml) of the eluent from a Sephadex G25 column equilibrated in buffer B was applied (△) on a DEAE-Sephacel column equilibrated in buffer C. Twenty-drop fractions (1.1 ml) were collected and 0.5 ml of each fraction was counted.

a 4 S form during DEAE-Sephacel chromatography even in the presence of molybdate. It is less likely that the converted receptor has similar electrostatic properties with the transformed receptor.

DISCUSSION

Exposure of the cytosol from rat submandibular gland to ATP induced transformation of androgen receptor at 0°C. When the efficiency of transformation was measured by the minicolumn binding assay, the DNA-cellulose binding was approx 50% of the total androgen receptor within 3 h and further increase in the DNA-binding did not occur even in the prolonged incubation up to 27 h. On the other hand, most of androgen receptor appeared to be transformed on the glycerol gradient containing 10 mM ATP. We do not know the reason for these differences. However, the separation of the 4 S receptor from some associating factors might occur during glycerol gradient centrifugation, and then, most of the androgen receptors in the cytosol might become transformed under centrifugation conditions.

The conversion from the transformed 4 S androgen receptor from rat submandibular gland to a 7.6–7.8 S form was observed in low-salt conditions caused by elimination of ATP. The molecular size of the converted receptor was slightly smaller than the nontransformed receptor, and the elution profile on the DEAE-Sephacel chromatography was clearly distinguished from the nontransformed receptor. The failure of complete reassociation from a 4 S form to the nontransformed state may be due to the loss of some factors associating with the nontransformed receptor or the irregular reassociation of the components. Colvard and Wilson [6] reported that rat submandibular glands as well as other target tissues contained the 8 S promoting factor, which reconstituted the 8 S androgen receptor from the 4 S form. The conversion of androgen receptor from rat submandibular gland after removal of ATP may be caused by the binding of 8 S promoting factor to the 4 S receptor. Recently, Rowley *et al.*[21] reported that a 4.4 S androgen receptor from Dunning prostatic tumor reaggregated to a 7–8 S form which bound to DNA-cellulose when KCl was removed. Our findings also indicated that the conversion from a 4 S form to a 7.6–7.8 S form required the withdrawal of transformation inducing materials (KCl or ATP) or conditions (high temperature).

We also examined the reversibility of the transformation in low-salt conditions with androgen receptors from rat prostate and thymus and glucocorticoid receptor from rat liver and thymus. The

conversion from a 4 S form to a 6 S form was observed with androgen receptors from both tissues, although the sedimentation coefficient of the converted form was smaller than that of androgen receptor from submandibular gland (data not shown). However, we have not succeeded in clearly demonstrating the conversion of the glucocorticoid receptor. The sedimentation coefficient of glucocorticoid receptor from rat liver and rat thymus was slightly changed from 4.5 S in the presence of ATP to 5.1 S in the absence of ATP (data not shown). Recently, Reker *et al.*[22] reported the existence of 5.2 S, oligomeric glucocorticoid receptor as the second transformed species from mouse AtT-20 cells.

The transition metals which include Mo, V and W are known to form weak covalent coordination complexes with a radical such as SH, NH_2 or OH [23], and they stabilize the 8 S receptors and inhibit transformation caused by high salt, temperature or dilution [19, 20]. Moreover, Weisz *et al.*[24] reported that molybdate directly interacted with the glucocorticoid receptor from rat liver and induced an apparent dimerization. In this study, we examined the effect of molybdate on the conversion of the 4 S androgen receptor. When ATP was eliminated from cytosol samples in the absence of molybdate, the converted receptor did not bind to DNA-cellulose. Contrarily, the converted receptor could bind to DNA-cellulose following withdrawal of ATP in the presence of molybdate, despite the sedimentation coefficients of the converted receptors were similar in both conditions (Table 1 and Fig. 3). These findings suggest that molybdate may interact with the 4 S androgen receptor, probably with the DNA-binding domain, then prevent this domain from being concealed during the conversion in low-salt conditions. There is evidence that lysine [25], arginine [26] and sulfhydryl groups [27] associate with DNA-binding domain. These reactive groups might play an important role in the interaction with molybdate.

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REFERENCES

1. Fang S. and Liao S.: Androgen receptors. *J. biol. Chem.* **246** (1971) 16–24.
2. Mainwaring W. I. P. and Irving R.: The use of deoxyribonucleic acid-cellulose chromatography and isoelectric focusing for the characterization and partial purification of steroid–receptor complexes. *Biochem. J.* **134** (1973) 113–127.
3. Wilson E. M. and French F. S.: Effects of proteases and protease inhibitors on the 4.5 S and 8 S androgen receptor. *J. biol. Chem.* **254** (1979) 6310–6319.
4. Kovacs W. J., Griffin J. E. and Wilson J. D.: Transformation of human androgen receptors to the deoxyribonucleic acid-binding state. *Endocrinology* **113** (1983) 1574–1581.
5. Nemoto T., Ohara-Nemoto Y., Sato N., Kyakumoto S. and Ota M.: Characterization of nontransformed and transformed androgen receptor from rat submandibular gland. *Biochim. biophys. Acta* **839** (1985) 249–257.
6. Colvard D. S. and Wilson E. M.: Identification of an 8 S androgen receptor-promoting factor that converts the 4.5 S form of the androgen receptor to 8 S. *Endocrinology* **109** (1981) 496–504.
7. Murayama A., Fukai F. and Hazato T.: Estrogen receptor of cow uterus. *J. Biochem.* **88** (1980) 963–968.
8. Murayama A., Fukai F. and Yamamoto T.: Progesterone receptor system of hen oviduct. *J. Biochem.* **88** (1980) 1305–1315.
9. Bodine P. V., Schmidt T. J. and Litwack G.: Evidence that pH induced activation of the rat hepatic glucocorticoid–receptor complex is irreversible. *J. steroid Biochem.* **20** (1984) 683–689.
10. Puri R. K. and Toft D. O.: Transformation of highly purified avian progesterone receptor. *Endocrinology* **115** (1984) 2453–2463.
11. Tymoczko J. L., Phillips M. M. and Vernon S. M.: Binding of the rat liver 7–8S dexamethasone receptor to deoxyribonucleic acid. *Arch. Biochem. Biophys.* **230** (1984) 345–354.
12. Luttge W. G., Densmore C. L. and Emadian S. M.: Increased binding to DNA-cellulose of the unactivated and activated glucocorticoid–receptor complex from mouse brain following sucrose density gradient ultracentrifugation. *Brain Res.* **295** (1984) 385–388.
13. Ohara-Nemoto Y., Nemoto T., Sato N., Kyakumoto S. and Ota M.: Comparison of androgen receptor in male and female rat submandibular glands. *Jpn. J. Oral Biol.* **27** (1985) 679–684.
14. Holbrook N. J., Bodwell J. E., Jeffries M. and Munck A.: Characterization of nonactivated and activated glucocorticoid–receptor complexes from intact rat thymus cells. *J. biol. Chem.* **258** (1983) 6477–6485.
15. Kyakumoto S., Sato N., Nemoto T., Ohara-Nemoto Y. and Ota M.: Binding of [^{3}H]methyltrienolone to androgen receptor in rat liver. *Biochim. biophys. Acta* **800** (1984) 214–219.
16. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
17. Moudgil V., Kruczak V., Eessalu T., Paulose C. S., Taylor M. and Hansen J.: Activation of progesterone receptor by ATP. *Eur. J. Biochem.* **118** (1981) 547–555.
18. Holbrook N. J., Bodwell J. E. and Munck A.: Effects of ATP and pyrophosphate on properties of glucocorticoid–receptor complexes from rat thymus cells. *J. biol. Chem.* **258** (1983) 14885–14894.
19. Noma K., Nakano K., Sato B., Nishizawa Y., Matsumoto K. and Yamamura Y.: Effect of molybdate on activation and stabilization of steroid receptors. *Endocrinology* **107** (1980) 1205–1211.
20. Baulieu E. E., Binart N., Buchou T., Catelli M. G., Garcia T., Gasc J. M., Groyer A., Joab I., Montcharmont B., Radanyi C., Renoir M., Tuohimaa P. and Mester J.: Biochemical and immunological studies of the chick oviduct cytosol progesterone receptor. In *Nobel Symposium on Steroid Hormone Receptors: Structure and Function* (Edited by J. A. Gustafsson and H. Eriksson). Elsevier, Amsterdam (1983) pp. 45–70.
21. Rowley D. R., Premont R. T., Chang C. H. and Tindall D. J.: Transformation properties of androgen receptor from Dunning prostatic tumor. In *Abstracts 7th International Congress of Endocrinology*. No. 2154. Excerpta Medica, Amsterdam (1984).
22. Reker C. E., Kovačič-Milivojević B., Eastman-Reks S. B. and Vedeckis W. V.: Transformed mouse glucocorticoid receptor: Generation and interconversion of the 3.8S, monomeric and 5.2S, oligomeric species. *Biochemistry* **24** (1985) 196–204.
23. Stiefel E. I.: In *Progress in Inorganic Chemistry* (Edited by S. J. Lippard). John Wiley, New York, Vol. 22 (1977) pp. 3–203.

24. Weisz A., Baxter J. D. and Lan N. C.: Interaction of sodium molybdate with highly purified glucocorticoid receptor. *J. steroid Biochem.* **20** (1984) 289–293.
25. DiSorbo D. M. and Litwack G.: The use of pyridoxal 5-phosphate as a tool in the study of steroid receptors. In *Biochemical Actions of Hormones* (Edited by G. Litwack). Academic Press, New York, Vol. IX (1982) pp. 205–219.
26. Müller R. E., Mrabet N. T., Traish A. M. and Wotiz H. H.: The role of arginyl residues in estrogen receptor activation and transformation. *J. biol. Chem.* **258** (1983) 11582–11589.
27. Bodwell J. E., Holbrook N. J. and Munck A.: Evidence for distinct sulfhydryl groups associated with steroid- and DNA-binding domains of rat thymus glucocorticoid receptors. *Biochemistry* **23** (1984) 4237–4242.