NOTE

Partial Purification of Cytoplasmic Androgen Receptor in Rat Liver

MINORU OTA, NOBUKO SATO, SEIKO TAKAHASHI
AND SHIGERU ONO

*Department of Biochemistry, Iwate Medical University School of
Medicine, Morioka, Iwate 020*

Abstract

An attempt was made to purify androgen receptor of male rat liver cytosol which binds specifically with testosterone and androstenedione. A 30% ammonium sulfate precipitation afforded more than 5-fold purification of the androgen receptor and removed half of the amount of the enzymes involved in androgen metabolism.

There is evidence indicating the presence of protein in rat liver which has a binding affinity with 5 α -dihydrotestosterone (Milin and Roy, 1973) or androstenedione (Gustafsson *et al.*, 1975). Recent studies in our laboratory have demonstrated the presence of a macromolecular component which binds specifically to testosterone and androstenedione with a moderate affinity for the hepatic cytosol of male rats (Sato *et al.*, 1980a). This component was suggested as the androgen receptor, because it formed a complex with testosterone and then translocated to the nucleus (Ota *et al.*, 1981). This binding component in the cytosol was partially purified by gel filtration and was found to be a protein with a sedimentation coefficient of approximately 10 S (Ota *et al.*, 1980). However, this component is still in a crude form and purification is awaited.

We report further purification of this binding component obtained from rat livers by ammonium sulfate precipitation.

Materials and Methods

Preparation of original cytosol and partially purified cytosol

Male adult Wistar rats with body weights between 200 and 250 g were castrated by the abdominal route 15 h prior to the experiment. They were killed by decapitation and the livers were perfused with saline solution and quickly removed. The liver tissues were homogenized by Polytron PT-10 in 2 vol. of 0.01 M Tris-HCl buffer, pH 7.4, containing 0.01 M KCl, 0.001 M EDTA and 0.001 M dithiothreitol (TKED buffer) with five, 5-8 sec. bursts at power setting 6 at intervals of 15-20 sec. in an ice bath, and then centrifuged for 10 min at 25,000 $\times g$. Floating lipid was discarded and the supernatant was recentrifuged for 1 h at 105,000 $\times g$. The clear supernatant obtained was called "original cytosol fraction".

Saturated ammonium sulfate, neutralized with NH₄OH, was added dropwise to stirred "original cytosol" in ice until the final solution was 30% saturated in ammonium sulfate. To two aliquots of "original cytosol", saturated ammonium sulfate was added in the same manner as above to obtain a final concentration of 40% and 50% in ammonium sulfate saturation, respectively. Each solution was then stirred for 30 min and left to stand for over 45 min. The white precipitate was collected by centrifugation at 25,000 $\times g$ for 10 min. A TKED buffer was added to each precipitate obtained and the mixtures were gently homogenized using a Teflon homogenizer. The suspension obtained was about

one-tenth of the volume of the "original cytosol". The suspension of the precipitate obtained by 30% ammonium sulfate saturation was called "partially purified cytosol".

Measurement of binding activity

"Original cytosol", 30%, 40% or 50% saturated ammonium sulfate fraction prepared as described was incubated with 11.5 pmoles of [^3H]testosterone (specific radioactivity; 40 Ci/mmol, purchased from New England Nuclear) with or without a 1,000-fold excess of unlabeled testosterone at 0° C for 3 h. The final concentration of [^3H]testosterone was 9×10^{-9} M. Each sample was obtained from the same volume of "original cytosol". The protein content of "original cytosol", 30%, 40% or 50% ammonium sulfate saturation fraction was 18.00 mg, 1.42 mg, 2.80 mg and 3.88 mg in 1.25 ml, respectively. After the incubation, the bound radioactive steroids were measured by means of the Dextran-coated charcoal (DCC) method (Beato and Feigelson, 1972).

Metabolism of [^3H]testosterone by original or partially purified cytosol

Since "original cytosol" was previously observed to contain androgen-metabolizing enzymes which diminished bound-testosterone, the reduction of those enzymes by ammonium sulfate precipitation was checked. One ml of "original cytosol" or "partially purified cytosol" (each containing 8 mg protein in 1.0 ml) was incubated with 9 pmoles [^3H]testosterone for 3 h at 0° C. The incubation was terminated by adding methylene dichloride. The extraction of [^3H]steroids was carried out from the incubation medium and a suitable aliquot of the extract was chromatographed on a thin layer of silica gel according to the method of Ota *et al.* (1972). The radioactive metabolites were eluted with ethanol from silica gel and the radioactivity was measured as previously described (Sato *et al.*, 1980).

Other technique

The protein concentrations were determined by the method of Lowry *et al.* (1951) using crystalline egg albumin (Sigma) as a standard.

Results

Table 1 shows the purification of androgen receptor in rat liver cytosol by ammonium sulfate precipitation. The [^3H]testosterone binding ability per mg protein was increased by ammonium sulfate precipi-

itation 3 to 5 times that of the "original cytosol". The purification by ammonium sulfate precipitation reduced non-specific binding (data not shown). The highest purification was obtained by 30% ammonium sulfate saturation. At 50% saturation, the yield of receptor reached the highest (57% of receptors found in "original cytosol"), but the degree of purification was reduced in terms of disintegration per minute (dpm) per protein. Therefore, a 30% ammonium sulfate saturation fraction was used as "partially purified cytosol" in the present study.

Table 2 shows the relative radioactivity in the percentage of remaining substrate, androstenedione and other metabolites. More residual testosterone was recovered from the media incubated with "partially purified cytosol". This observation suggests that the partial purification by 30% ammonium sulfate precipitation reduced the amounts of enzymes involved in androgen metabolism.

Discussion

The androgen receptor in hepatic cytosol is a labile protein, and is present in minute amounts unlike those present in other target organs. Moreover, physiological androgens are easily metabolized by the liver cytosol preparation which contains a large amount of androgen-metabolizing enzymes. In the present study, purification of the original cytosol receptor preparation was attempted by ammonium sulfate preparation, because it is known that androgen receptor proteins are precipitated at low concentrations of ammonium sulfate (Fang and Liao, 1971; Mainwaring and Peterken 1971; Verhoeven *et al.*, 1975). The amount of bound [^3H] testosterone metabolized was 24% in the "original cytosol", but was 14% in "partially purified cytosol". Thus, the enzymes related to androgen metabolism were remarkably removed from "original

Table. 1. Purification of cytoplasmic androgen receptor by ammonium sulfate precipitation

	Total volume (ml)	Total protein (mg)	Total bound to receptor ^b (dpm)	Specific activity (dpm/mg protein)	Fold purification ^c
Original cytosol	30	540	185×10^3	343	1
30% Ammonium sulfate precipitation ^a	3	42.6	78×10^3	1826	5.3
40% Ammonium sulfate precipitation ^a	3	84.0	96×10^3	1143	3.3
50% Ammonium sulfate precipitation ^a	3	116.4	106×10^3	908	2.6

^aEach ammonium sulfate saturation solution was prepared from 30 ml of "original cytosol"

^bSpecific activity based on tritiated testosterone

^cBased on specific activity recovered from "original cytosol"

Table. 2. Metabolism of [³H]testosterone by cytosol or partially purified cytosol

	Original cytosol (percentage radioactivity)	Partially purified cytosol (percentage radioactivity)
Testosterone	76.4%	86.2%
Androstenedione	1.7%	5.6%
Others	21.9%	8.2%

The conditions of incubation and extraction of metabolites are described in the text. Each value is expressed as the relative radioactivity in percentage of tritiated metabolites and the unmetabolized substrate per total recovered radioactivity.

cytosol" by 30% ammonium sulfate precipitation, resulting in a 5-fold purification of the binding sites. This purification is important in the assessment of androgen binding sites.

Recent studies in our laboratory have shown that "original cytosol" bound with [³H] testosterone can be separated into two radioactive peaks by Sephadex G-100 column chromatography; one appeared in and near the void volume and the other was eluted after the bovine serum albumin which was

used as a marker (Sato *et al.*, 1980a). This later eluted peak had no specific binding ability for testosterone and androstenedione, because the binding site was not saturable (Ota *et al.*, 1980). This peak containing low molecular weight substances disappeared following 30% ammonium sulfate precipitation (the data will be reported elsewhere).

In conclusion, the ammonium sulfate precipitation procedure was useful in isolating testosterone-binding protein (receptor) from nonspecific binding proteins and also in removing half of the amount of metabolizing enzymes present in the rat liver cytosol preparation.

Acknowledgments

We are grateful to Ms. Virginia L. Braymer and Miss Mitsuki Takahashi for their help in the preparation of the manuscript.

References

- Beato, M. and Feigelson, P. (1972). *J. Biol. Chem.*, **247**, 7890-7896.
- Fang, S. and Liao, S. (1971). *J. Biol. Chem.*, **246**, 16-24.
- Gustafsson, J. -A., Pousette, A., Stenberg, A. and Wrangé, O. (1975). *Biochemistry*, **14**, 3942-3948.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). *J. Biol. Chem.*, **193**, 265-275.
- Mainwaring, W. I. P. and Peterken, B. M. (1971). *Biochem. J.*, **125**, 285-295.
- Milín, B. and Roy, A. K. (1973). *Nature, New Biology*, **242**, 248-250.
- Ota, M., Sato, N. and Obara, K. (1972). *J. Biochem.*, **72**, 11-20.
- Ota, M., Sato, N., Takahashi, S. and Ono, S. (1980). *Endocrinol. Japon.*, **27**, 321-328.
- Ota, M., Sato, N., Takahashi, S. and Ono, S. (1981). *Experientia*, **37**, 318-319.
- Sato, N., Ota, M. and Obara, K. (1980a). *Endocrinol. Japon.*, **27**, 315-319.
- Sato, N., Takahashi, S. and Ota, M. (1980b). *Endocr. Res. Comm.* **7**, 241-252.
- Verhoeven, G., Heyns, W. and De Moor, P. (1975). *Steroids*, **26**, 149-167.