HETEROGENEITY OF RAT LIVER SULFOTRANSFERASES

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Sulfotransferases (STs) active on androsterone (AD), cortisol (CS) and 4-nitrophenol (NP) were separated by diethylaminoethyl-cellulose chromatography from cytosolic fractions of female rat liver and were divided into five ST fractions (peaks I—V) with different activities toward three substrates. The precipitates obtained in the 68% of saturation of ammonium sulfate were passed through a Sephadex G-100 column and purified by agarose-hexane adenosine 3',5'-bisphosphate affinity chromatography. AD-ST isoenzyme (peak I) was purified 85-fold, had low CS-ST activity, was devoid of NP-ST activity and appeared to correspond to hydroxysteroid ST1. Peaks II and V appeared to consist mainly of hydroxysteroid ST and aryl ST, respectively.

**Keywords** — rat liver; sulfotransferase; isoenzyme; multiplicity; androsterone; cortisol; 4-nitrophenol

INTRODUCTION

Sulfation is generally an effective detoxification process by giving rise to the formation of ionized sulfates, which are more water soluble than the parent compounds and thus more easily excreted into urine or bile. However, some of the steroid sulfates have been recognized as important metabolic intermediates in steroid biosynthesis. Sulfation of an array of compounds, such as phenols, alcohols, amines and hydroxylamines, has provided the impetus to elucidate the multiple forms of sulfotransferase (ST). Recently, four aryl STs, three hydroxysteroid STs and a bile salt ST have been purified from rat livers. These enzymes have comparatively broad substrate specificity.

Previous studies from this laboratory have shown a wide variation in biliary metabolites of androsterone (AD) in Wistar rats. About half of the rats excreted large amounts of steroid glucuronides into the bile, whereas the other rats excreted mainly steroid sulfates into the bile. There was a discontinuous variation in hepatic uridine diphosphate-glucuronosyltransferase (UDP-GT) activity toward AD. On the other hand, a remarkable feature of ST activity toward AD was sex difference: ST activity toward AD was much higher in adult females than in adult males. Male low ST activity toward AD was increased by the administration of estrogen. These results prompted us to investigate which ST isoenzymes are responsible for sulfation of AD. The present paper describes the separation of ST isoenzymes active on AD, cortisol (CS) and 4-nitrophenol (NP) and the purification of AD-ST isoenzyme from female Wistar rats.

MATERIALS AND METHODS

**Chemicals** — [1,2-3H]AD (40.8 Ci/mmol) and [4-14C]CS (55 mCi/mmol) were purchased from New England Nuclear Corp., Boston, MA, U.S.A. AD and CS were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Diethylaminoethyl (DEAE)-cellulose (DE-52) and agarose-hexane adenosine 3',5'-bisphosphate (PAP-agarose) were provided by Whatman biochemicals, Clinton, New Jersey, U.S.A. and P-L Biochemicals, Milwaukee, WI, U.S.A., respectively. DEAE-Sephadex A-50 and Sephadex G-100 were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. NP was from Wako Pure
Chemical Ind. Ltd., Tokyo, Japan. 3'-Phosphoadenosine-5'-phosphosulfate (PAPS) was prepared by the method of Singer, and its purity was confirmed by high-performance liquid chromatography. All other reagents were of analytical grade.

Animals and Preparation of Cytosolic Fractions — Four female Wistar rats (210–350 g) were decapitated and livers were homogenized in three volumes of buffer A (0.25 M sucrose, 10 mM Tris–HCl, 3 mM 2-mercaptoethanol and 0.1 mM ethylenediaminetetraacetic acid (EDTA) adjusted to pH 7.4 at 25 °C) or buffer B (0.25 M sucrose, 50 mM Tris–HCl, 3 mM 2-mercaptoethanol and 0.1 mM EDTA adjusted to pH 7.4 at 25 °C) with a Teflon-glass homogenizer. All procedures for isolation and purification of ST were done at 0–4 °C. Cytosolic fractions were obtained by differential centrifugation (2 000 × g for 10 min, 16 000 × g for 45 min and 105 000 × g for 60 min).

Enzyme Assay — ST activities toward AD, NP and CS were assayed by a modification of the method described previously. The incubation medium contained 0.1 M Tris–HCl buffer, pH 7.4, 10 mM MgCl₂, 0.1 mM PAPS, 0.1 mM EDTA and 0.086 mM [³¹P]AD (0.012 μCi), 0.063 mM NP or 0.040 mM [¹⁴C]CS (0.025 μCi) in a total volume of 1.0 ml. The incubation was performed at 37 °C for 20–30 min. The blank values were obtained from control incubations in which PAPS was omitted.

Protein concentrations were determined by the method of Lowry et al. and the method of Bradford, with bovine serum albumin as the standard.

DEAE-Sephadex A-50 Chromatography — ST activities were separated by a modification of a method described by Singer. The cytosolic fractions prepared in buffer B were applied to a DEAE-Sephadex A-50 column (5 cm × 40 cm) equilibrated with buffer B. The column was washed with 1 500 ml of buffer B and eluted with a linear KCl-gradient (1 000 ml of 0.3 M KCl in buffer B in the reservoir and 1 000 ml of buffer B in the mixing chamber).

DEAE-Cellulose Chromatography — ST activities were separated by a modification of a method of Lyon et al. The cytosolic fractions prepared in buffer A were fractionated on DEAE-cellulose column (2.5 cm × 70 cm) equilibrated with buffer A. The column was washed with 1 000 ml of buffer A and eluted with a linear NaCl-gradient (1 500 ml of 0.2 M NaCl in buffer A in the reservoir and 1 500 ml of buffer A in the mixing chamber).

Ammonium Sulfate Precipitation and Sephadex G-100 Chromatography — Finely ground solid ammonium sulfate was slowly added to ST fraction separated by DEAE-cellulose chromatography to 68% saturation. The mixture was centrifuged at 15 000 × g for 10 min. The precipitate was redissolved in 10 ml of buffer A.

The ammonium sulfate fraction was applied to a Sephadex G-100 column (2 cm × 70 cm) equilibrated with buffer A and eluted with buffer A.

PAP-Agarose Chromatography — ST fraction from Sephadex G-100 chromatography was loaded on PAP-agarose column (2 ml) equilibrated with buffer A. The sequential elution was performed by the addition of 50 ml of buffer B, followed by 30 ml of buffer B containing 0.5 M NaCl and finally by 30 ml of buffer B containing 0.5 M NaCl and 4 mM adenosine diphosphate (ADP). Most of the ST activity was eluted in buffer B containing 0.5 M NaCl. Purified ST fraction was dialyzed against 50 volumes of buffer B overnight.

Gel Electrophoresis — Polyacrylamide gel electrophoresis was performed on 15% polyacrylamide slab gel in the presence of 0.1% sodium dodecyl sulfate (SDS). The standard proteins used in gel electrophoresis were obtained from Sigma Chemical Co., Rockford, St. Louis, MO, U.S.A.

RESULTS AND DISCUSSION
Separation of Rat Liver STs by DEAE-Sephadex A-50 and DEAE-Cellulose Chromatography

Cytosolic fractions of female rat livers were separated by DEAE-Sephadex A-50 chroma-
tography with a linear KCl-gradient in buffer B. Fig. 1 shows the elution profile of ST activities toward AD, CS and NP. There were three partly resolved peaks of ST activities toward CS in accord with the results described by Singer et al.\textsuperscript{5}) ST activities toward AD overlapped with CS-ST activities, though the highest central peak of AD-ST was separated from that of CS-ST. A small peak of NP-ST coincided with the highest peak of CS-ST and the highest peak of NP-ST was eluted in a later fraction.

DEAE-cellulose chromatography was carried out by washing and eluting with buffer A and a NaCl-gradient in buffer A, respectively. When buffer A was substituted for buffer B as in the case of DEAE-Sephadex A-50, all the ST activities were eluted by washing with buffer B due to the higher concentration of Tris-HCl buffer. DEAE-cellulose chromatography of cytosolic fractions provided an improved separation of these ST activities (Fig. 2). Five peaks I—V were named in order of their elution from the column. Peak I (fractions 54—64) contained the highest ST activity toward AD and low ST activities toward CS and NP. Peak II (fractions 65—80) showed the highest ST activities toward CS and comparatively high ST activities toward AD and NP. Peak III (fractions 81—90) contained relatively high ST activities toward AD and CS and low ST activity toward NP. Peak IV (fractions 91—104) had a relatively high ST activity toward NP and low ST activities toward AD and CS. Peak V (fractions 105—120) showed the highest activity toward NP and low ST activities toward AD and CS. Each peak was subjected to further purification.

Purification of DEAE-Cellulose Pools

Each peak (I—V) obtained by DEAE-cellulose chromatography was precipitated with 68% of saturation of ammonium sulfate and passed through a Sephadex G-100 column. ST activity was eluted as a sharp peak and was clearly separated from salts.

ST fraction from Sephadex G-100 chroma-

![Graph](image)

FIG. 1. DEAE-Sephadex A-50 Column Chromatography of Rat Liver ST Activities toward AD, CS and NP

The column procedure is described in the Materials and Methods section. Cytosolic fractions prepared in buffer B were applied to a DEAE-Sephadex A-50 column. Buffer B (1 500 ml) was passed through the column before application of a linear 0—0.3 M KCl gradient (—). Fractions (20 ml) were collected and assayed for ST activities toward AD (○), CS (△) and NP (●). ST activity is expressed as nmol/min per ml fraction. Protein (■) was determined at 280 nm.
Rat Liver Sulfotransferases

Histochemistry was purified by affinity chromatography on PAP-agarose. The column was washed with buffer B and eluted with buffer B containing 0.5 M NaCl. When buffer B was substituted for buffer A, ST activity was not eluted completely from the column with buffer A containing 0.5 M NaCl or 0.5 M NaCl and 4 mM ADP.

SDS-Polyacrylamide Gel Electrophoresis of Purified STs

Peaks I—V obtained by DEAE-cellulose chromatography were further purified and their subunit molecular weights were estimated by SDS-polyacrylamide gel electrophoresis (Fig. 3). Peaks I, III and IV showed a prominent polypeptide band with a subunit molecular weight of 29 000. Peak II had subunit molecular weights of 28 000 and 36 000, whereas peak V had subunit molecular weights of 29 000 and 36 000.

Several authors separated and purified ST isoenzymes and these STs were named in order of their elution from DEAE-cellulose or DEAE-Sephadex column. Lyon et al. purified hydroxysteroid ST 1, 2 and 3 by DEAE-cellulose, hydroxyapatite or isoelectric focusing procedures and reported that these isoenzymes have subunit molecular weights of 28 000, 32 000 and 60 000, respectively. Singer and his coworkers reported the purification of ST I, II and III active on glucocorticoids (CS as substrate) by DEAE-Sephadex A-50 and PAP-agarose chromatography. Singer described that ST I and III have subunit molecular weights of about 31 000 and 30 000, respectively and that ST III is more abundant than ST I in female rats. He suggested that ST II should be 3β-hydroxysteroid ST, which probably corresponds to hydroxysteroid ST 1. However, direct comparison has not yet been made between hydroxysteroid STs 1—3 and STs I—III. Aryl ST I, II, III and IV were purified by Sekura et al. Aryl ST I and II have subunit molecular weight of 35 000, while aryl ST III and IV have subunit molecular weight of 33 500. Small differences in subunit molecular weight estimation among the investigators may reflect different molecular species and/or the limited accuracy of SDS-gel technique.

FIG. 2. DEAE-Cellulose Column Chromatography of Rat Liver ST Activities toward AD, CS and NP

The column procedure is described in the Materials and Methods section. Cytosolic fractions prepared in buffer A were applied to a DEAE-cellulose column. Buffer A (1 000 ml) was passed through the column before application of a linear 0—0.2 M NaCl gradient (—). Fractions (18 ml) were collected and assayed for ST activities toward AD (○), CS (△) and NP (■). ST activity is expressed as nmol/min per ml fraction. Protein (■) was determined at 280 nm.
Profiles of Purified STs

The highest central peak of AD-ST activity obtained by DEAE-Sephadex A-50 chromatography appears to be similar to ST II,\(^{19}\) which has been assigned to hydroxysteroid ST I,\(^{6}\) separated by DEAE-cellulose chromatography. In fact, separation of AD-ST by DEAE-cellulose column provided the highest AD-ST activity in peak I, which should correspond to hydroxysteroid ST I, on the basis of the chromatographic elution profiles. As described in the previous section, the subunit molecular weight of peak I was in good agreement with that of hydroxysteroid ST I. Though characterization of this enzyme is still incomplete, the close correspondence of chromatographic profiles and subunit molecular weights suggests that AD-ST isoenzyme (peak I) should be hydroxysteroid ST I. The results of a typical purification experiment of peak I are shown in Table I. Application of peak I to an affinity column resulted in a 85-fold purification of AD-ST activity from cytosolic fractions. Peak I isoenzyme had comparatively low CS-ST activity and was devoid of NP-ST activity. Lyon et al.\(^{8}\) purified hydroxysteroid ST I (dehydroepiandrosterone as substrate) by 317-fold, employing DEAE-cellulose, ammonium sulfate precipitation and hydroxypatite steps.

Brief mention of the other ST peaks should be made here. Peak II appears to be a mixture of ST III and aryl ST I, whereas peak V should mainly consist of aryl ST II. Peak III and IV could not be specified at present.

In this study, we demonstrated the existence of four partially resolved ST peak I, II, III and IV active on AD. A previous study\(^{12}\) revealed a biphasic development of female ST activity toward AD. The enzyme activity increased after birth in parallel in both sexes, attained the highest activity at about 20 d of age, and began to decline thereafter. In contrast to males, female ST activity toward AD increased again to high levels after 40 d of age. ST activity toward AD appears to be regulated by gonadal and progestational hormones.\(^{13}\) These studies indicate that further studies are required to clarify which isoenzymes are responsible for developmental alteration, sex difference, induction or suppression.

![FIG. 3. SDS-Polyacrylamide Gel Electrophoresis of Rat Liver STs after Purification by DEAE-Cellulose (Peaks I—V) and PAP-Agarose Chromatography](image-link)

A, bovine albumin (66 000), egg albumin (45 000) and carbonic anhydrase (29 000); B, trypsinogen (24 000); C, peak I; D, peak II; E, peak III; F, peak IV; G, peak V. Migration is from top to bottom.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Protein (mg)</th>
<th>AD SA</th>
<th>AD TA</th>
<th>AD RP</th>
<th>CS SA</th>
<th>CS TA</th>
<th>CS RP</th>
</tr>
</thead>
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<tr>
<td>Cytosolic fractions</td>
<td>1820</td>
<td>0.21</td>
<td>382</td>
<td>1</td>
<td>0.032</td>
<td>58.2</td>
<td>1</td>
</tr>
<tr>
<td>DEAE-cellulose (peak I)</td>
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<td>1.30</td>
<td>131</td>
<td>6</td>
<td>0.058</td>
<td>5.9</td>
<td>2</td>
</tr>
<tr>
<td>Sephadex G-100</td>
<td>27</td>
<td>2.88</td>
<td>77</td>
<td>14</td>
<td>0.137</td>
<td>3.7</td>
<td>4</td>
</tr>
<tr>
<td>PAP-agarose</td>
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<td>43</td>
<td>85</td>
<td>1.140</td>
<td>2.7</td>
<td>36</td>
</tr>
</tbody>
</table>

*SA is the specific activity in nmol/min per mg protein. TA is the total activity in nmol/min. RP is the relative purification. ST activities toward AD and CS were assayed as described in the Materials and Methods section.*
of AD-ST activity.

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**REFERENCES**


