A 17β-hydroxysteroid dehydrogenase of female rabbit liver cytosol

Purification and characterization of multiple forms of the enzyme

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(Received 11 July 1984/Accepted 10 September 1984)

Multiple forms of the soluble 17β-hydroxysteroid dehydrogenase of female rabbit liver were identified. NAD-dependent and NADP-dependent enzyme activities were separated by affinity chromatography on agarose-immobilized Procion Red HE3B, and three forms of the NADP-dependent enzyme activity were purified by chromatofocusing. These three enzyme forms are charge isomers and have no quaternary structure. The enzymes catalysed the C-17 oxidoreduction of oestrogens and androgens; with all enzyme forms the activity towards androgens was higher than that toward oestrogens. The enzymes also exhibited 3α-hydroxysteroid dehydrogenase activity towards androgens of the 5β-androstan series. Comparison of the relative activities of the enzymes towards a number of oestrogen and androgen substrates revealed differences among the enzyme forms for both the oxidative and the reductive reactions. In particular, one enzyme form had a significantly lower $K_m$ for the 3α-hydroxysteroid substrate and a higher 3α-/17β-hydroxysteroid dehydrogenase activity ratio than the other two enzyme forms.

17β-Hydroxysteroid dehydrogenase activity has been demonstrated in the liver of a variety of animals (Aoshima & Kochakian, 1963). Depending on the species, this enzyme activity may be soluble or membrane-bound and NAD- or NADP-dependent (Williamson, 1979). In general, the 17β-hydroxysteroid dehydrogenases of liver exhibit activity towards both oestrogens and androgens, although enzyme activity relatively specific for oestrogens (Renwick et al., 1981) and for androgens (Kobayashi & Kochakian, 1978) has been observed in some animals. Soluble 17β-hydroxysteroid dehydrogenases have been purified or partially purified from the liver of guinea pig (Kobayashi & Kochakian, 1978), chicken (Renwick et al., 1981) and rabbit (Thaler-Dao et al., 1972). These studies have revealed that multiple forms of this enzyme activity, differing in steroid and nicotinamide nucleotide specificity, are present in the liver.

We have been examining the role of the liver 17β-hydroxysteroid dehydrogenases in oestrogen metabolism in the female rabbit. Oestradiol-17β is metabolized to its 17α-epimer before excretion (Williams et al., 1968), and both 17α- and 17β-hydroxysteroid dehydrogenase activities are present in rabbit liver (Breuer & Pangels, 1960). We have isolated multiple forms of a soluble 17α-hydroxysteroid dehydrogenase from female rabbit liver with activity towards both oestrogens and androgens (Hasnain & Williamson, 1975, 1977). The purified enzymes also exhibit 3α-hydroxysteroid dehydrogenase activity towards androgens of the 5α-androstan series (Lau et al., 1982b). The present paper describes the purification and characterization of multiple forms of the soluble 17β-hydroxysteroid dehydrogenase of female rabbit liver. These studies were undertaken to compare the characteristics of the 17α- and 17β-hydroxysteroid dehydrogenases and to obtain information on the roles of the different enzyme forms in androgen and oestrogen metabolism in the rabbit.

Experimental

Materials

[1,2-3H]Androstenedione (sp. radioactivity 41 Ci/mmol), [1,2-3H]androsterone (sp. radioactivity 41 Ci/mmol), [1,2-3H]epitestosterone (sp.
radioactivity 50Ci/mmole), [1,2-3H]aeti chol- 
olone (sp. radioactivity 45Ci/mmole), [6,7-3H] 
oestradiol-17β (sp. radioactivity 40Ci/mmole), 
[6,7-3H]oestrone (sp. radioactivity 44Ci/mmole) 
and [7-3H]testosterone (sp. radioactivity 25Ci/ 
mmole) were purchased from New England 
Nuclear, Lachine, Que., Canada. The purity of all 
radioactive steroids was verified by t.l.c. on silica 
Gel N (Machery Nagel and Co., Düren, Germany) 
in the solvent system benzene/acetone (4:1, v/v). 
Non-radioactive steroids were purchased from 
Research Plus Steroid Laboratories, Bayonne, 
NJ, U.S.A., or Sigma Chemical Co., St. Louis, MO, 
U.S.A. LKB carrier amphotely, pH5–7 (40%, 
w/v), was obtained from Fisher Scientific Co., 
Ottawa, Ont., Canada. Polybuffer 74 and Poly- 
buffer Exchanger PBE 94 were purchased from 
Pharmacia, Dorval, Que., Canada. Agarose- 
imobilized Procion Red HED3B (Matrex Gel Red 
A) was obtained from Amicon, Oakville, Ont., 
Canada. All chemical reagents used were of 
analytical grade.

Enzyme assays

Hydroxysteroid dehydrogenase activity was measured as described by Lau et al. (1982a,b). The specific radioactivity of the steroid substrates employed in the assays was adjusted to 4.5μCi/ 
μmol by the addition of non-radioactive steroid 
substrate, and stock solutions (0.1mm) of the 
steroids were made up in methanol. The final 
concentration of the steroid substrates in the 
incubation medium was 1μM. Oxidation of 
hydroxysteroid substrates was assayed at 37°C in 
67mm-glycine/NaOH buffer, pH9.5, with 
NADP+ (167μM). Reduction of oxosteroid sub- 
strates was assayed at 37°C in 67mm-Tris/maleate 
buffer, pH7.6, with NADPH (167μM).

Protein was measured by the method of Bradford 
(1976), with bovine serum albumin as the 
standard. Enzyme activity is expressed as units/ml 
or units/mg of protein. One unit of activity is 
defined as the amount of enzyme catalysing the 
oxidation of 1μmol of substrate/min under the 
specified assay conditions.

Enzyme purification

All procedures were carried out at 0–4°C. Virgin 
female New Zealand White rabbits (2–3 months 
old) were killed by cervical dislocation. The livers 
were removed and rinsed in ice-cold 0.15M-KCl. 
The tissue (200g) was homogenized in a Sorvall 
Omni-Mixer with 4 vol. of 10mm-Tris/HCl buffer, 
pH8.0, containing 0.15M-KCl and 0.5mm-dithio- 
threitol. The homogenate was centrifuged at 
10000g for 30min, and the supernatant obtained 
was centrifuged at 105000g for 90min to yield the 
final supernatant fraction. This supernatant frac-
tion (10g of protein) was concentrated to approx. 
100ml by ultrafiltration on a Diaflo apparatus 
(Amicon) with a YM-10 membrane, and insoluble 
material was removed from the concentrate by 
centrifugation at 105000g for 90min.

The concentrated supernatant was divided into 
two equal portions, and each portion was applied to 
a column (5cm × 95cm) of Sephadex G-75 
(superfine grade) that had been equilibrated with 
10mm-Tris/HCl buffer, pH8.0, containing 0.5mm- 
dithiothreitol. Elution of the column was carried 
out with this same buffer at a flow rate of 20ml/h. 
Fractions (10ml) were collected and assayed for 
17β-hydroxysteroid dehydrogenase activity. Fractions 
from each column having enzyme activity 
were pooled and concentrated to 30ml by ultra-
filtration.

The concentrated enzyme fraction (5.4g of 
protein) was applied to a column (1.5cm × 40cm) 
of agarose-immobilized Procion Red HED3B that 
had been equilibrated with 10mm-Tris/HCl buffer, 
pH8.0, containing 0.5mm-dithiothreitol and 1mm- 
EDTA. The enzyme sample was applied at a flow 
rate of 6–8ml/h. The column was eluted first with 
150ml of 10mm-Tris/HCl buffer, pH8.0, containing 
0.5mm-dithiothreitol, 1mm-EDTA and 5mm-
MgCl2, followed by a 600ml linear gradient of 
NaCl from 0 to 1.0M in this same buffer and then 
by a 200ml linear gradient of NaCl from 1.0 to 
2.0M in 10mm-Tris/HCl buffer, pH8.0, containing 
0.5mm-dithiothreitol and 5mm-MgCl2. Finally the 
column was eluted with 400ml of 10mm-Tris/HCl 
buffer, pH8.0, containing 0.5mm-dithiothreitol, 
10mm-MgCl2 and 2.5mm-NaCl. The flow rate for 
elution of the column was 20ml/h, and 3.6ml 
fractions were collected. The major part of the 
17β-hydroxysteroid dehydrogenase activity was present 
in the protein eluted with the buffer containing 
2.5mm-NaCl. These fractions were pooled, glycerol 
was added to a final concentration of 20%(v/v) and 
the combined fractions were concentrated to 10ml 
by ultrafiltration. The concentrated fraction was 
dialysed against two changes of 4 litres of 10mm-
Tris/HCl buffer, pH8.0, containing 0.5mm-dithio-
threitol and 20% glycerol, and then centrifuged 
(10000g) to remove insoluble material.

Finally purification of the 17β-hydroxysteroid 
dehydrogenase was achieved by chromatofocusing. 
The concentrated and dialysed enzyme 
fraction (57mg of protein) obtained by affinity 
chromatography was applied to a column 
(1cm × 40cm) of Polybuffer Exchanger PBE 94 
that had been equilibrated with 10mm-imidazole/ 
HCl buffer, pH6.15, containing 0.5mm-dithio-
threitol. The eluting buffer was prepared by 
adjusting 50ml of a solution of Polybuffer 74 to 
pH5.15 with 1M-HCl and diluting the solution to 
400ml with deaerated distilled water. Dithio-
threitol was added to a final concentration of 0.5 mM. Elution of the column with this Polybuffer 74 solution was carried out at a flow rate of 15 ml/h, and 1.5 ml fractions were collected and assayed for 17β-hydroxysteroid dehydrogenase activity. The pH of appropriate fractions was measured at 4°C within 2 h of collection. Fractions comprising each peak of enzyme activity were pooled and concentrated by ultrafiltration. Each concentrated peak was subjected to chromatofocusing a second time under the conditions described above except that the column size was 1 cm x 10 cm and 100 ml of the diluted polybuffer solution was used for elution of the protein.

**Electrophoresis in polyacrylamide gels**

Polyacrylamide-gel electrophoresis was carried out as described by Davis (1964). The acrylamide monomer concentration was 10.5%. Gels were pre-run in the Tris/glycine buffer system (pH 8.3) before application of the protein sample (5–10 μg). Electrophoresis in polyacrylamide gels containing sodium dodecyl sulphate was performed as described by Weber et al. (1972).

Isoelectric focusing in polyacrylamide gels was carried out by a modification of the method of Hayes & Wellner (1969). Polyacrylamide gels (0.6 cm x 9 cm) were prepared from a solution containing the following components (final concentrations): acrylamide (8%, w/v), N,N'-methylenebisacrylamide (0.24%, w/v), carrier ampholytes, pH 5–7 (2%, w/v), ammonium persulphate (0.024%, w/v) and N,N'N'-tetramethylethlenediamine (0.24%, w/v). The cathode solution was 0.02 M-NaOH and the anode solution was 0.01 M-H₂PO₄. Before the application of the protein sample the gels were run at 4°C for 15 min at 200 V, then for 30 min at 300 V and finally for 30 min at 400 V. The NaOH solution was then removed from the cathode compartment, and the gel surfaces were rinsed with distilled water. Protein samples containing glycerol (20%, w/v) were applied to the tops of the gels and overlaid with the NaOH cathode solution. Isoelectric focusing was carried out at 4°C for 30 min at 400 V followed by 3–4 h at 500 V. Gels were stained for 17β-hydroxysteroid dehydrogenase activity with the substrate testosterone as described by Lau et al. (1982a).

**Results**

**Purification of the 17β-hydroxysteroid dehydrogenase**

17-Hydroxysteroid dehydrogenase activities were measured in the 105000g supernatant of female rabbit liver. With NAD⁺ as cofactor 17α-dehydrogenase activity towards epistosterone (685 μunits/mg of protein) was higher than the 17β-dehydrogenase activity towards testosterone (560 μunits/mg of protein). 17β-Hydroxysteroid dehydrogenase activity was also present when NAD⁺ was used as the cofactor, although the activity towards testosterone with this nicotinamide nucleotide (230 μunits/mg of protein) was less than 50% of that observed with NAD⁺.

The procedure for the purification of the 17β-hydroxysteroid dehydrogenase is summarized in Table 1. The 17α- and 17β-hydroxysteroid dehydrogenase activities were co-eluted as a single peak on Sephadex G-75 gel filtration. This fraction was purified further by chromatography on agarose-immobilized Procion Red HE3B, and the enzyme elution profiles are shown in Fig. 1. Approx. 10% of the applied 17β-dehydrogenase activity was eluted in the wash buffer. This fraction also contained 14% of the total 17α-dehydrogenase activity. The remainder of the 17α-dehydrogenase activity was eluted by the 0–1 M-NaCl gradient, and partial resolution of the

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Protein (mg)</th>
<th>Total activity (munits)</th>
<th>Specific activity (munits/mg of protein)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 105000g supernatant</td>
<td>9980</td>
<td>5300</td>
<td>0.53</td>
<td>100</td>
</tr>
<tr>
<td>2. Sephadex G-75 gel filtration</td>
<td>5360</td>
<td>4300</td>
<td>0.80</td>
<td>81</td>
</tr>
<tr>
<td>3. Affinity chromatography</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash buffer</td>
<td>1720</td>
<td>410</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>0–1 M-NaCl</td>
<td>1160</td>
<td>220</td>
<td>0.19</td>
<td>71</td>
</tr>
<tr>
<td>1–2 M-NaCl</td>
<td>100</td>
<td>150</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>2.5 M-NaCl</td>
<td>57.0</td>
<td>3000</td>
<td>52.6</td>
<td></td>
</tr>
<tr>
<td>4. Chromatofocusing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak I</td>
<td>3.08</td>
<td>386</td>
<td>125</td>
<td>16</td>
</tr>
<tr>
<td>Peak II</td>
<td>1.31</td>
<td>121</td>
<td>92.4</td>
<td></td>
</tr>
<tr>
<td>Peak III</td>
<td>6.27</td>
<td>333</td>
<td>53.1</td>
<td></td>
</tr>
</tbody>
</table>
The 17β-hydroxysteroid dehydrogenase activity was recovered in the buffer containing 2.5M-NaCl and 10mM-MgCl₂ (Fig. 1 and Table 1). When this enzyme fraction was chromatofocused over the pH range 6.15–5.15 three peaks of enzyme activity were obtained (Fig. 2). Polycrylamide-gel electrophoresis of each enzyme peak, pooled as shown in Fig. 2, showed that there was cross-contamination among the enzyme forms. Therefore each peak was chromatofocused again under the conditions employed in Fig. 2 except that the column size was 1 cm × 10 cm and the volume of Polybuffer solution used for elution was 100 ml. This procedure yielded each of the enzyme forms in a homogeneous state, as established by polycrylamide-gel electrophoresis (Fig. 3). M₄ values of 37000, 40000 and 39000 were estimated for the enzyme forms I, II and III respectively by comparing the mobilities of the enzymes on sodium dodecyl sulphate/polycrylamide-gel electrophoresis with those of the reference proteins ribonuclease (M₄ 13000), chymotrypsinogen (M₄ 25700), fructose bisphosphate aldolase (M₄ 40000), ovalbumin (M₄ 45000) and bovine serum albumin (M₄ 68000).

In order to determine whether forms of the 17β-hydroxysteroid dehydrogenase were lost or generated during purification, enzyme activity throughout this procedure was monitored by isoelectric focusing in polycrylamide gels. Densitometer scans of gels focused over the pH range 5–7 and stained for 17β-dehydrogenase activity are shown in Fig. 4. Four major bands of 17β-dehydrogenase activity are present in the 105000g supernatant of female rabbit liver (Fig. 4b), three forms having similar isoelectric points and the fourth form being more acidic. This same enzyme-activity profile was obtained with gels of the Sephadex G-75 enzyme fraction (Fig. 4c). Affinity chromatography separated the acidic enzyme activity, which was eluted from the column by the wash buffer (Fig. 4d), from the three more basic enzyme forms, which were eluted from the column by buffer containing 2.5M-NaCl (Fig. 4e). These latter enzyme forms were subsequently separated and purified by chromatofocusing (Fig. 2). The data in Fig. 4 show that enzyme forms were neither lost nor generated during the purification procedure.

The acidic 17β-dehydrogenase activity eluted in the wash buffer during affinity chromatography differs in nicotinamide nucleotide specificity from the more basic enzymes eluted from this column in buffer containing 2.5M-NaCl (Figs. 1 and 4). The specific activity of the acidic enzyme fraction is 14-fold higher with NAD⁺ than with NADP⁺,
whereas the more-basic enzymes are specific for NADP+ (results not shown). The elution sequence of the NAD+- and NADP+-dependent 17β-dehydrogenase on affinity chromatography is consistent with the properties of agarose-immobilized Procion Red HE3B, which has been shown to have a higher affinity for NADP-dependent dehydrogenases than for NAD-dependent dehydrogenases (Watson et al., 1978). The data in Fig. 4 suggest that more than one form of the 17β-dehydrogenase is present in the acidic fraction. However, this could not be clearly established, owing to the presence of an achromatic band in the region of the acidic 17β-dehydrogenase activity produced under the substrate-staining conditions employed. This band appeared as a trough in the densitometer scans (Figs. 4c and 4d) and was caused by a cyanide-insensitive superoxide dismutase (Weisiger & Fridovich, 1973). This enzyme can inhibit the reduction of Nitro Blue Tetrazolium and thus prevent the formation of the coloured bands normally produced with this substrate-staining technique (Beauchamp & Fridovich, 1971). Further purification of the acidic 17β-dehydrogenase form(s), which accounted for 10% of the NADP-dependent 17β-hydroxysteroid dehydrogenase activity of rabbit liver cytosol, was not undertaken.

**Substrate specificities of the purified enzymes**

The activities of the 17β-hydroxysteroid dehydrogenases towards a number of androgen and oestrogen substrates are shown in Table 2. The enzymes catalyse the reversible oxidation of 17β-hydroxy C19 and C19 steroids and exhibit a pH optimum of 9.5 for the oxidative reaction and 7.6 for the reductive reaction. We have observed a variation in the stability of the enzyme forms during their purification and subsequent storage at 4°C. Enzyme III, which has the lowest isoelectric point, is the least stable enzyme form. Because of this variation in enzyme stability it is not possible to compare the specific activities of the enzymes directly. However, the data in Table 2 do show the relative activity of each enzyme towards the different steroid substrates, and these profiles for the three enzymes can be compared. Enzyme III is the most distinct in its 17β-dehydrogenase activity profile. In the oxidative reaction the specific activity of this enzyme towards testosterone is 47-fold higher than towards oestradiol-17β. This difference is 26-fold for enzyme I and 11-fold for enzyme II. In the reductive reaction the specific activity of enzyme III towards androstenedione is only 2-fold higher than towards oestrone, whereas for enzymes I and II this difference is approximately 6-fold. All forms of the 17β-hydroxysteroid dehydrogenase exhibit 3α-hydroxysteroid dehydrogenase activity. This activity is relatively specific for androgen substrates of the 5β-androstane series, the activity towards the 5β-substrate aetiocholanolone being at least 6-fold higher than towards the 5α-substrate androsterone (Table 2). With all enzyme forms the 3α-hydroxysteroid
dehydrogenase activity is lower than the 17β-hydroxysteroid dehydrogenase activity under the experimental conditions of the enzyme assays.

Enzyme III exhibits the highest 3α-dehydrogenase activity relative to its 17β-dehydrogenase activity.

A comparison of the kinetic constants for the 17β- and 3α-dehydrogenase activities of each enzyme, determined with testosterone and aetiocholanolone respectively, is shown in Table 3. Preliminary studies showed that under the experimental conditions employed and at steroid concentrations ranging from 0.3 to 3 times \( K_m \) the reaction rates were linear for 30 min. With testosterone as substrate enzymes I and II obeyed Michaelis–Menten kinetics, whereas enzyme III exhibited substrate inhibition at testosterone concentrations above 4 \( \mu M \). All enzymes showed substrate inhibition with aetiocholanolone. This occurred at steroid concentrations above 4 \( \mu M \) with enzymes I and II and above 0.5 \( \mu M \) with enzyme III. The major difference in the kinetic constants among the enzymes was observed for enzyme III. The \( K_m \) of this enzyme with aetiocholanolone is 17 times lower than the \( K_m \) with testosterone and at least 10 times lower than the \( K_m \) with aetiocholanolone for either enzyme I or II.

### Discussion

Earlier studies by Thaler-Dao et al. (1972) had shown that female rabbit liver cytosol has 17β-hydroxysteroid dehydrogenase activities that differ in steroid and nicotinamide nucleotide specificity. The heterogeneity of this enzyme activity has been confirmed in the present studies. We have purified, for the first time, three forms of the NADP-dependent 17β-hydroxysteroid dehydrogenase by the use of methods based on the

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**Table 2. Substrate specificities of the multiple forms of the soluble 17β-hydroxysteroid dehydrogenase of female rabbit liver**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Testosterone</th>
<th>Oestradiol-17β</th>
<th>Androstenedione</th>
<th>Oestrone</th>
<th>Aetiocholanolone</th>
<th>Androsterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>125</td>
<td>4.9</td>
<td>96.1</td>
<td>16.2</td>
<td>32.8</td>
<td>3.9</td>
</tr>
<tr>
<td>II</td>
<td>92.4</td>
<td>8.4</td>
<td>76.3</td>
<td>14.0</td>
<td>36.6</td>
<td>1.5</td>
</tr>
<tr>
<td>III</td>
<td>53.1</td>
<td>1.1</td>
<td>12.1</td>
<td>5.6</td>
<td>35.1</td>
<td>5.3</td>
</tr>
</tbody>
</table>

Enzyme activity was measured at pH 9.5 in the oxidative direction and at pH 7.6 in the reduction direction. The experimental details are given in the text. The steroid concentration was 1 \( \mu M \) and the NADP concentration was 167 \( \mu M \). For the assays with testosterone the enzyme concentration was 0.9–1.8 \( \text{nm} \). With other substrates the enzyme concentration was adjusted so that the extent of oxidation did not exceed 30%.
differences in nicotinamide nucleotide specificity and isoelectric points among the different 17β-dehydrogenase activities of rabbit liver cytosol. It is unlikely that the multiple forms of the 17β-hydroxysteroid dehydrogenase are artifacts produced during purification. The enzyme forms could be identified in the initial liver cytosol and enzyme forms were neither lost nor generated throughout the purification procedure (Fig. 4). Furthermore, the same relative amounts of the enzyme forms were obtained from different rabbit liver preparations, and storage of the liver cytosol at 4°C for several days did not alter the enzyme pattern. Heterogeneity appears to be a feature common to several mammalian 17β-hydroxysteroid dehydrogenases, including those of human placenta (Engel & Groman, 1974), guinea-pig liver (Kobayashi & Kochakian, 1978), guinea-pig kidney (Shen & Kochakian, 1978) and pig testis (Inano et al., 1980). More recently, two forms of the enzyme have been identified in human skin (Hodgins et al., 1982) and human testis (Leinonen, 1982). The characteristics of the multiple forms of the soluble 17β-hydroxysteroid dehydrogenase of female rabbit liver purified in the present study indicate that the enzymes are charge isomers. Their Mr values, determined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, are all within 3% of the average value of 38700. This Mr value is consistent with the elution of the enzyme forms on Sephadex G-75 and reveals that the enzymes have no quaternary structure. These enzymes are similar in size to the multiple forms of the 17α-hydroxysteroid dehydrogenase present in this tissue (Hasnain & Williamson, 1977) and in rabbit kidney (Lau et al., 1982a) and to the 17β-hydroxysteroid dehydrogenases of guinea-pig liver and kidney (Kobayashi & Kochakian, 1978; Shen & Kochakian, 1978) and pig testis (Inano et al., 1977). However, they differ from the 17β-hydroxysteroid dehydrogenase in human placenta, which is a dimer having a subunit Mr of 34000 (Burns et al., 1972).

All forms of the soluble 17β-hydroxysteroid dehydrogenase of female rabbit liver exhibit higher activities towards androgens than towards oestrogens (Table 2). Similar specificities have been reported for the soluble 17β-hydroxysteroid dehydrogenases of guinea-pig liver (Kobayashi & Kochakian, 1978) and kidney (Liu & Kochakian, 1972). In contrast, oestrogens are the preferred substrates for the 17β-hydroxysteroid dehydrogenases of chicken liver (Renwick et al., 1981), human placenta (Langer et al., 1959), sheep ovary (Kautsky & Hagerman, 1970) and human ovary (Pittaway et al., 1977).

Bifunctional enzyme activity has been reported for a number of hydroxysteroid dehydrogenases (Battais et al., 1977; Shen & Kochakian, 1978; Edwards & Orr, 1978; Strickler et al., 1981; Sharaf & Sweet, 1982; Lau et al., 1982a,b). The present studies have demonstrated that the multiple forms of the soluble 17β-hydroxysteroid dehydrogenase of female rabbit liver exhibit bifunctional enzyme activity. Enzymes that are homogeneous by the criterion of polyacrylamide-gel electrophoresis catalyse the oxidation of both 17β- and 3α-hydroxy-steroids (Table 2). The 3α-dehydrogenase activity is relatively specific for steroids of the 5α-androstan series, the activity towards aetiocholanolone being at least 6-fold higher than towards androsterone. The multiple forms of the 17α-hydroxysteroid dehydrogenases of female rabbit liver and kidney also have 3α-dehydrogenase activity. However, androgens of the 5α-androstan series are the preferred substrates for these enzymes (Lau et al., 1982a,b). Furthermore, in contrast with the 17β-hydroxysteroid dehydrogenases, the 3α-dehydrogenase activity of the 17α-hydroxysteroid dehydrogenases of rabbit liver exceeds their 17α-dehydrogenase activity.

Although the general substrate specificities of the multiple forms of the female rabbit liver 17β-hydroxysteroid dehydrogenases are similar, there are differences among the enzyme forms. Enzyme III is the most distinct in its substrate specificity. This enzyme has the highest relative 3α-dehydrogenase activity and a distinctly low Km with aetiocholanolone (Table 3). The molecular basis for the differences in substrate specificity of the three
forms of the 17β-hydroxysteroid dehydrogenase, as well as the physiological significance of these differences, must await a detailed comparison of the structural and kinetic properties of these enzymes.

This work was supported by a grant from the Medical Research Council of Canada.

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