C₂₁ Steroid Side Chain Cleavage Enzyme from Porcine Adrenal Microsomes

PURIFICATION AND CHARACTERIZATION OF THE 17α-HYDROXYLASE/C₁₇,₂₀-LYASE CYTOCHROME P-450*

(Received for publication, July 19, 1983)

Shizuo Nakajin‡§, Masato Shinoda‡‡, Mitsuru Haniu¶‖, John E. Shively¶‖, and Peter F. Hall**‡‡

From the ‡Hoashi College of Pharmacy 2-4-41 Ebara, Shinagawa-ku, Tokyo 142, Japan, the *Division of Immunology, City of Hope Research Institute, Duarte, California 91010, and the **Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

The properties and the purity of a cytochrome P-450 (17α-hydroxylase) from porcine adrenal microsomes have been examined following a report that the corresponding enzyme from bovine adenocortical microsomes is inactive as a 17α-hydroxylase and fails to show a high spin spectrum on addition of substrate, once the enzyme has been purified (Bumpus, J. A., and Dus, K. M. (1982) J. Biol. Chem. 257, 12686-12704). The purity of the porcine enzyme was demonstrated by electrophoresis on polyacrylamide with sodium dodecyl sulfate, immunoelectrophoresis, and NH₂-terminal amino acid sequence (16 residues). The pure enzyme shows Mₐ = 54,000, heme content of >0.8 nmol/nmol of protein, and absorption spectra typical of cytochrome P-450. The enzyme is active with both Δ⁴ (progesterone) and Δ⁵ (pregnenolone) substrates as a 17α-hydroxylase and with the corresponding 17α-hydroxysteroids as a C₁₇,₂₀-lyase. All four substrates produce typical type I spectra with the enzyme (so-called high spin form). We conclude that: 1) porcine adrenal microsomes contain a 17α-hydroxylase/C₁₇,₂₀-lyase which is a single protein molecule readily purified to an enzymatically active form; 2) the C₁₇,₂₀-lyase activity is largely suppressed in the microsomes; and 3) the enzyme closely resembles that found in testicular microsomes. We propose that this enzyme be referred to as the adrenal C₂₁ steroid side chain cleavage enzyme.

The adrenal cortex of many mammalian species synthesizes cortisol which requires 17α-hydroxylation of progesterone. By contrast, the gonads synthesize C₁₉ steroids by 17α-hydroxylation followed by C₁₇,₂₀-lyase activity (Scheme 1).

Studies from this laboratory have shown that in pig, the hydroxylase and lyase activities of testicular microsomes result from the action of a single cytochrome P-450 (2-4). This observation has recently been confirmed in the guinea pig (5). In setting out to purify the adrenal 17α-hydroxylase, we expected to find an enzyme without C₁₇,₂₀-lyase activity. The limited production of C₁₉ androgens by the adrenal is usually attributed to the activity of a different zone of the adrenal cortex (6), so that we also anticipated the possibility that a second enzyme, resembling the testicular hydroxylase/lyase, might be found in small quantities, in whole adrenal extracts. We were surprised to find that pig adrenal contains a single 17α-hydroxylase which shows C₁₇,₂₀-lyase activity when purified and which cross-reacts with antibody to the testicular enzyme (7). At the same time, Bumpus and Dus (8) reported the isolation of a 17α-hydroxylase from bovine adenocortical microsomes that loses 17α-hydroxylase activity during purification (8). This enzyme also fails to show a type I spectral shift on addition of substrate; this spectral shift is usually regarded as the normal and inevitable consequence of substrate binding (4, 9). In addition, the purified bovine enzyme was reported to show side chain cleavage activity with cholesterol as substrate (8). In view of the anomalous behavior of the bovine enzyme, we decided to examine the properties of the purified porcine enzyme in greater detail and to compare the enzyme with the hydroxylase/lyase from porcine testicular microsomes on which NH₂-terminal amino acid sequence data are available (4). We report here that the adrenal enzyme can be readily purified in active form, that it shows a typical type I spectral shift with appropriate substrates, that it does not cleave the side chain of cholesterol, and that it closely resembles the testicular enzyme.

EXPERIMENTAL PROCEDURES

Methods for preparing the adrenal enzyme (7), for measuring enzymatic activities (2, 7), and for examining the properties of the cytochrome P-450 (3, 4) have been published elsewhere. The methods used to determine amino acid composition and sequence have also been published (3). NADPH-cytochrome P-450 reductase was purified from adrenal microsomes by the method of Yasukochi et al., devised for preparation of the liver enzyme (10). Antibodies were raised in rabbits as described previously (11). The purified enzyme remains stable for at least 6 months in 20% glycerol (v/v) and 0.2% Emulgen 913 (v/v) in potassium phosphate (50 mM; pH 7.4) at -70 °C. Measurement of side chain cleavage of cholesterol (12) and the preparation of adrenodoxin and adrenodoxin reductase from pig adrenal were performed as described elsewhere (13). The sources of

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‡ Recipient of the Oaki Research Grant, Japan, and Grant 57771585 from the Ministry of Education, Japan.
§ Recipient of Grant CA16434 from the National Cancer Institute.
¶‖ Recipient of Grant AM28113 from the National Institute of Arthritis and Metabolism.

Vol. 259, No. 6, Issue of March 25, pp. 3971-3976, 1984
Printed in U.S.A.
RESULTS

Purification of 17α-Hydroxylase—Preparation of the enzyme from 100 pig adrenal glands yield: 8–10 mg of homogeneous enzyme which represents between 2 and 4% of the total microsomal P-450 (Table I). The enzymatic activity of the 17α-hydroxylase during purification is also shown in Table I. The enzyme shows both 17α-hydroxylase and C17,20-lyase activities with a ratio of hydroxylase to lyase of 2.1 to 2.4 under conditions of V_{max}. When [3H]cholesterol was added to the enzyme together with NADPH, adrenodoxin, and adrenodoxin reductase, no evidence of side chain cleavage was observed and the substrate could be completely recovered after 30 min incubation within the limits of experimental error. At no stage during the purification procedure (microsomes to pure enzyme) was side chain cleavage of cholesterol observed.

Although the purification procedure used has been given elsewhere (7), the elution pattern for hydroxylapatite is shown in Fig. 1, because fractions from this column were examined in detail. It can be seen that considerable lyase activity is observed at this stage (Table I and Fig. 1). Enzyme activity was measured in the presence of Emulgen so that absolute values cannot be compared with those for the pure enzyme.

The purity of the enzyme is demonstrated by four methods. Firstly, electrophoresis on polyacrylamide with sodium dodecyl sulfate in the Laemmli system, with as much as 20 μg of protein/gel, showed a single band when stained with Coomasie blue (Fig. 2). Secondly, immunoelectrophoresis with antibody (IgG) raised against the analogous testicular enzyme shows a single band (Fig. 3). Antibody (IgG) raised against the adrenal enzyme shows a classical line of identity on double diffusion with the purified adrenal 17α-hydroxylase (Fig. 3). Thirdly, Table II shows the first 16 amino acids at the NH₂ terminus of the molecule. Each residue appeared as a single amino acid within the limits of the method (5%). Moreover, this NH₂-terminal sequence shows considerable homology (15 identical residues out of 16), with that of the analogous testicular enzyme which we have prepared in homogeneous form (5) (Fig. 4). It is interesting to notice that the adrenal enzyme shows an NH₂-terminal methionine like a number of other cytochromes P-450 including three from rat liver (P-450a, P-450b, and P-450c) (14). Fourth, antibody (IgG) to the testicular enzyme inhibits both enzymatic activities in parallel (Fig. 5). When the values for three separate studies like that shown in Fig. 5 were pooled and linearized by log-log transformation, the two lines (hydroxylase and lyase activities) showed no difference in slope (p > 0.7). The same result has already been reported for the testicular enzyme (3) and suggests that the two enzymatic activities are associated with a single protein. Similar inhibition was observed with antibody to the adrenal enzyme (not shown).

Properties of the Enzyme—The purified enzyme shows a molecular weight of 54,000 on sodium dodecyl sulfate gels (Fig. 2). A similar value was observed with column chromatography with buffer containing sodium dodecyl sulfate (not shown). The pure enzyme shows a heme content of 13–14 nmol/mg of protein. No significant difference in heme content was observed when measured by pyridine hemochromagen as opposed to CO difference spectra (not shown). The spectral properties of the enzyme are shown in Fig. 6. The enzyme is prepared in the low spin form with an absorbance maximum at 417 nm and no evidence of a high spin shoulder. When 0.5 mg of enzyme was extracted with methylene chloride, no steroids were found when the extract was examined by high pressure liquid chromatography. The oxidized enzyme also shows peaks at 565, 535, and 650 nm. On reduction, the principal change observed involves the formation of a single peak at 545 nm with a decrease and slight shift in the Soret peak (Fig. 6). The reduced CO spectrum shows a shoulder but no peak at 420 nm and a peak at 448 nm. The ratio of A448:A420 is 2.3. No evidence of P-420 has been seen in any of our preparations of this enzyme.

Enzymatic activities as a function of substrate concentration with both Δ⁴ (progesterone) and Δ⁵ (pregnenolone) steroid substrates are shown in Fig. 7. Both hydroxylase and lyase activities are seen with Δ⁴ (progesterone) and Δ⁵ (pregnenolone) substrates. Binding of these substrates to the enzyme is shown in Figs. 8 and 9. It will be seen that all four substrates bind readily to the enzyme and that binding results in a typical type I difference spectrum. The findings from enzyme catalysis and substrate-induced difference spectra are summarized in Table III with corresponding values for the testicular enzyme (2, 4) for comparison. The two enzymes handle all four substrates in a remarkably similar manner. Table III also shows the greater affinity of both enzymes for the two Δ⁴ substrates when compared to the Δ⁵ substrates. Given the minor variations from preparation to preparation, the values for the two enzymes (adrenal and testicular) are very similar. With both enzymes, values for Kᵣ (concentration required to give half-maximal spectral shift) are lower for Δ⁴ than for Δ⁵ substrates.

The amino acid composition of adrenal 17α-hydroxylase is shown in Table IV together with values for the bovine adrenal enzyme and for the analogous testicular enzyme. It is clear that the amino acid compositions of the two porcine enzymes are extremely similar. The values shown are different from those reported by Bumpus and Dus for the enzyme from beef adrenal cortex (Table IV and Ref. 8) except for Pro, Phe, Lys, His, Arg, Trp, and Cys. The molecular weights of the bovine and porcine enzyme calculated from amino acid compositions

<table>
<thead>
<tr>
<th>Stage of Purification</th>
<th>Protein Total</th>
<th>Recovery</th>
<th>Total</th>
<th>Recovery</th>
<th>Specific content</th>
<th>Hydroxylase</th>
<th>Lyase</th>
<th>Ratio H/L</th>
</tr>
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<td>Microsomes</td>
<td>4000</td>
<td>100</td>
<td>4670</td>
<td>100</td>
<td>1.2</td>
<td>0.89</td>
<td>0.2</td>
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<td>4389</td>
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<tr>
<td>DEAE-cellulose</td>
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<td>2.4</td>
<td>13.1</td>
<td>6.9</td>
<td>3.1</td>
<td>2.2</td>
</tr>
</tbody>
</table>

# Table I

Purification of cytochrome P-450 (17α-hydroxylase) from porcine adrenal microsomes

- **Stage of Purification:**
  - Microsomes
  - Cholate extract
  - DEAE-cellulose
  - DEAE-Sepharose
  - CM-Sepharose
  - Hydroxylapatite
  - CM-Sepharose

- **Protein Total:**
  - Microsomes: 4000 mg
  - Cholate extract: 2867 mg
  - DEAE-cellulose: 671 mg
  - DEAE-Sepharose: 116 mg
  - CM-Sepharose: 39 mg
  - Hydroxylapatite: 25 mg
  - CM-Sepharose: 9 mg

- **Recovery %:**
  - Microsomes: 100%
  - Cholate extract: 72%
  - DEAE-cellulose: 17%
  - DEAE-Sepharose: 3%
  - CM-Sepharose: 1%
  - Hydroxylapatite: 0.6%
  - CM-Sepharose: 0.2%

- **Total Enzymatic Activity:**
  - Hydroxylase: nmol/mg protein
  - Lyase: nmol product/min/nmol

- **Ratio H/L:**
  - Microsomes: 4.5
  - Cholate extract: 0.2
  - DEAE-cellulose: 4.6
  - DEAE-Sepharose: 1.8
  - CM-Sepharose: 2.2

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additions) was started. Fractions (7 ml) were collected and examined. At
hydroxylapatite (2.1 mm; pH 7.4) containing 0.2% Emulgen 913 (v/v), 20% glycerol
(v/v), 0.1 mM EDTA, and 0.1 mM dithiothreitol (final concentrations). At a phosphate concentration gradient (with the same
additions) was started. Fractions (7 ml) were collected and exam-
ined as described in Ref. 7. The sample was applied to a column of
CM-Sepharose containing cytochrome P-450 were pooled and di-
verted to the left of the slide. A, double diffusion of adrenal P-450 (17α-
hydroxylase). Well A contained antibody (IgG) to adrenal 17α-
hydroxylase (300 µg of protein); wells B contained purified 17α-hydroxy-
lase (12.5 µg of protein); wells C contained cholate extract (110 µg
of protein).

**FIG. 1.** Chromatography on hydroxylapatite. Fractions from
CM-Sepharose containing cytochrome P-450 were pooled and di-
alyzed as described in Ref. 7. The sample was applied to a column of
hydroxylapatite (2.1 x 10 cm) and eluted with potassium phosphate
(10 mM; pH 7.4) containing 0.2% Emulgen 913 (v/v), 20% glycerol
(v/v), 0.1 mM EDTA, and 0.1 mM dithiothreitol (final concentra-
tions). At a phosphate concentration gradient (with the same
additions) was started. Fractions (7 ml) were collected and exam-
ined for the various properties shown in the figure.

**FIG. 2.** Electrophoresis of purified adrenal P-450 (17α-hy-
droxy-
lase) with sodium dodecyl sulfate on Laemmli disc gels. Lanes A and B, standards and molecular weights as follows: phospho-
ylase (94,000); bovine serum albumin (67,000); ovalbumin (43,000);
carbonic anhydrase (30,000); and trypsin inhibitor (20,100). In addition lane B also shows adrenal cytochrome P-450 (17α-hydroxylase)
(2.5 µg). Lane C–E, P-450 5, 10, and 20 µg, respectively. Gels were
stained with Coomassie blue.

are 47,000 and 51,000, respectively, but differ from the mole-
cular weights estimated from sodium dodecyl sulfate gel electro-
phoresis (50,000 and 54,000, respectively).

**DISCUSSION**

The method described here for the purification of adrenal
17α-hydroxylase is based upon conventional chromatography.
As with the testicular hydroxylase/lyase (2), ion exchange
chromatography with different charges (DEAE and carboxy-
methyl) proved helpful. Preliminary studies revealed that w-
aminooctyl-Sepharose exerts some injurious influence on
17α-hydroxylase which makes it impossible to prepare the
enzyme in active form. The enzyme is also unstable in the
presence of cholate. The adrenal enzyme required five systems
of chromatography before the last contaminants could be
removed (see "Results"). The purified enzyme retains enzy-
matic activity, without detectable loss, for at least 6 months
if kept in 0.2% Emulgen 913 (v/v) and 20% glycerol (v/v) at
−70 °C. The enzyme shows both 17α-hydroxylase and C17,20-
lyase activities during and after purification, although the ratio
hydroxylase/lyase decreases as the enzyme is purified (Table I). In adrenal microsomes, lyase activity was not
detected with progesterone as substrate and very little lyase
activity was seen with 17α-hydroxyprogesterone (hydroxyl-
ase/lyase >10) (7). By contrast, testicular microsomes show
greater lyase than hydroxylase activity (hydroxylase/lyase 0.54), and this ratio reverses during purification to 2.5 (2); the
purified enzymes from both sources (testis and adrenal) show

**FIG. 3.** Immunochemical reactions of adrenal P-450 (17α-
hydroxylase). A, immunoelectrophoresis of purified adrenal 17α-hydroxy-
lase with antibody (IgG) to porcine testicular P-450 (hydroxylase/
lyase) (2). The enzyme (20 µg) was placed in the well and the antibody
(12.2 mg of protein/ml) in the trough. The support phase was agarose
(1.0 v/v). The method used is given in Ref. 3. The negative electrode
was to the left of the slide. B, double diffusion of adrenal P-450 (17α-
hydroxylase). Well A contained antibody (IgG) to adrenal 17α-hydroxy-
lase (300 µg of protein); wells B contained purified 17α-hydroxylase
(12.5 µg of protein); wells C contained cholate extract (110 µg
of protein).

**FIG. 4.** NH2-terminal sequences of porcine adrenal P-450
(17α-hydroxylase) and that of porcine testicular P-450 (hydroxylase/lyase) (3).

**TABLE II**

*Microsequence analysis of porcine adrenal cytochrome P-450 17α-
hydroxylase*

The sample (3 nmol) was subjected to microsequence analysis
without prior treatment with 4-sulfophenylisothiocyanate. The
absolute yield of valine at cycle 3 was 34%.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Amino acid</th>
<th>Yield (pmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Met</td>
<td>923</td>
</tr>
<tr>
<td>2</td>
<td>Trp</td>
<td>1059</td>
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<tr>
<td>3</td>
<td>Val</td>
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</tr>
<tr>
<td>4</td>
<td>Leu</td>
<td>862</td>
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<td>Leu</td>
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<td>7</td>
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<td>Phe</td>
<td>779</td>
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<td>9</td>
<td>Leu</td>
<td>224</td>
</tr>
<tr>
<td>10</td>
<td>Leu</td>
<td>122</td>
</tr>
<tr>
<td>11</td>
<td>Thr</td>
<td>102</td>
</tr>
<tr>
<td>12</td>
<td>Leu</td>
<td>88</td>
</tr>
<tr>
<td>13</td>
<td>Thr</td>
<td>72</td>
</tr>
<tr>
<td>14</td>
<td>Tyr</td>
<td>56</td>
</tr>
<tr>
<td>15</td>
<td>Leu</td>
<td>107</td>
</tr>
<tr>
<td>16</td>
<td>Phe</td>
<td>53</td>
</tr>
</tbody>
</table>
The enzymatic activities with Δ⁴ and Δ⁵ substrates are similar to those observed with the testicular enzyme (Table III and Ref. 2), revealing greater affinity for Δ⁵ substrates than for the corresponding Δ⁴ steroids. The values for \( K_a \) are consistent with values for \( K_r \) derived from substrate-induced difference spectra (Table III). Moreover, all four substrates induce typical type 1 spectra (Figs. 8 and 9) so that the cytochrome shows the expected transition to the high spin form in the presence of substrate (4, 9).

The properties of the purified enzyme that are attributable to the heme moiety and its interaction with the protein are typical of other cytochromes P-450. The heme content of 13-14 nmol/mg of protein corresponds to a specific heme content of >0.8 nmol of heme/nmol of protein which is higher than other steroidogenic cytochromes P-450 which lose heme during purification (2, 16, 17). The spectral properties of the adrenal 17α-hydroxylase are unremarkable.

The properties of the pure enzyme are of interest in relation to its role in steroid synthesis. In the first place, the preference for the Δ⁴ substrates is consistent with the use of the so-called Δ⁵ pathway by pig adrenal in vivo (18); that is, cleavage of the C₁₇,₂₀ bond precedes, to a large extent, conversion of the 3β-hydroxysteroid to the Δ⁴ 3-ketone form (1). The findings with the pure enzyme suggest that the preference for the Δ⁴ pathway by the pig may be, at least partly, attributable to the higher affinity of the enzyme for pregnenolone as opposed to progesterone (Table III). The same preference was also seen with the testicular enzyme (4).

In the second place, the low level of lyase activity in the adrenal microsome raises the question of where and how androgens are synthesized in the adrenal. Circumstantial evidence suggests that androgen synthesis may be confined to the zona reticularis (6). According to this view the zona fasciculata would produce 17α-hydroxy-C₁₇α-steroids by pos- sissing a 17α-hydroxylase without lyase activity. By contrast the cells of the reticularis would resemble the steroidogenic (Leydig) cells of the testis in having both hydroxylase and lyase activities; this would lead to the production of C₁₇α androgens. The properties of the pure enzyme suggest that the adrenal 17α-hydroxylase is also associated with lyase activity so that the fasciculata has the potential for making androgens. The site and extent of androgen synthesis may therefore be determined by local factors in the endoplasmic reticulum capable of regulating the expression of lyase activity. The important point is that in the present studies we have exhaustively examined discarded fractions of adrenal extracts (i.e. fractions not on the purification pathway), without finding any additional 17α-hydroxylase activity unaccompanied by lyase activity. We are forced to conclude that the bulk of adrenal 17α-hydroxylase activity is attributable to an enzyme that also possesses lyase activity. If traces of a pure 17α-hydroxylase do exist, it is doubtful whether such traces could account for the high production of 17α-hydroxy-C₁₇α- steroids by porcine adrenal.
Adrenal 17α-Hydroxylase

### FIG. 8. Substrate-induced difference spectra of adrenal microsomal P-450 (17α-hydroxylase) with \( \Delta^5 \) substrates.

Each cuvette contained 1.3 nmol of P-450 (0.1 mg of protein) in potassium phosphate (100 mM; pH 7.4) containing 20% glycerol (v/v), EDTA (0.1 mM), and dithiothreitol (0.1 mM). The sample cuvette contained the concentrations of steroid shown in C. The reference cuvette contained ethanol without steroid. C shows double reciprocal plots of mean values for two experiments including those shown in A and B.

### TABLE III

**Enzymatic activities of porcine adrenal P-450 (17α-hydroxylase)**

The values for kinetic constants represent the means and ranges of three determinations made as described in the legend to Fig. 7. Values for \( K_s \) represent means of two determinations of substrate-induced difference spectra performed as described in the legend to Fig. 8. \( k_p \) is the concentration of substrate required to give half-maximal spectral shift. Values in parentheses are for the testicular hydroxylase/lyase (2-4).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( V_{max} ) nmol product/min/nmol P-450</th>
<th>( K_s ) ( \mu M )</th>
<th>( K_c ) ( \mu M )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>6.9 ± 0.74 (4.6)</td>
<td>1.8 ± 0.74 (1.5)</td>
<td>1.0 ± 0.74 (0.9)</td>
</tr>
<tr>
<td>17α-OH-progesterone</td>
<td>3.1 ± 0.3 (2.6)</td>
<td>2.5 ± 0.3 (2.4)</td>
<td>5.0 ± 0.3 (5.0)</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td>3.7 ± 0.10 (3.3)</td>
<td>0.8 ± 0.10 (0.3)</td>
<td>0.8 ± 0.10 (0.7)</td>
</tr>
<tr>
<td>17α-OH-pregnenolone</td>
<td>2.0 ± 0.10 (2.1)</td>
<td>0.9 ± 0.10 (0.3)</td>
<td>0.8 ± 0.10 (0.7)</td>
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</table>

The results reported by Bumpus and Dus (8) for the bovine enzyme are interesting but difficult to interpret. The enzyme shows no 17α-hydroxylase activity and no high spin transition with progesterone. It is therefore either denatured or it represents another enzyme. No evidence has so far been found for any P-450 that is denatured without being converted to P-420. Since the enzyme reported by these authors shows no evidence of P-420, but does show side chain cleavage of cholesterol (8), it may be that the enzyme represents a mitochondrial contaminant, efforts to exclude this possibility not-

### TABLE IV

**Amino acid compositions of porcine and bovine adrenal cytochrome P-450 (P-450\( _{17α} \)) and porcine testicular cytochrome P-450 (P-450\( _{17α} \))**

Data for porcine adrenal enzyme from this paper, for bovine adrenal from Bumpus and Dus (8), and for porcine testes from Nakajin et al. (3). The data of Bumpus and Dus refer to their preparation of 17α-hydroxylase.

<table>
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<tr>
<th>Amino acid</th>
<th>Porcine adrenal P-450( _{17α} ) (M, total)</th>
<th>Bovine adrenal P-450( _{17α} ) (M, total)</th>
<th>Porcine testicular P-450( _{17α} ) (M, total)</th>
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<tr>
<td>Asx</td>
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<tr>
<td>Cys(^a)</td>
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<td>5</td>
<td>6</td>
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*Values for cysteic acid after performic acid oxidation.

\(^a\) Value obtained from sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis.
Adrenal 17α-Hydroxylase

withstanding (8). We have made two observations concerning the preparation of porcine adrenal cytochrome P-450 that may prove relevant. Firstly, as stated above, ω-amino-octyl-Sepharose renders the 17α-hydroxylase inactive and the enzyme is very unstable in the presence of cholate. Secondly, the use of a blender to prepare adrenal hemogenate is associated with considerable release of mitochondrial P-450 and adrenodoxin. The form of the C27 side chain cleavage enzyme released during homogenization may differ from that of the same enzyme remaining in the mitochondrial membrane so that, for example, binding of the two forms of the enzyme to adrenodoxin may be different (8). Finally, when our procedure is applied to bovine adrenocortical microsomes, a 17α-hydroxylase is isolated which is enzymatically active and which shows spectral properties (including substrate-induced difference spectra) like those reported here (data not shown).

The adrenal enzyme appears closely to resemble the testicular enzyme. The amino acid compositions are very similar and NH2-terminal amino acid sequence show one difference in the first 16 residues. Molecular weights, enzymatic activities, and kinetic and binding constants are all similar (this paper and Refs. 2–4). However, the two proteins are not identical as revealed by significant differences in amino acid composition (Table IV). Determination of complete amino acid sequence will demonstrate the extent and nature of the differences between the two enzymes.

Throughout this manuscript, we have referred to this enzyme as adrenal 17α-hydroxylase. In view of the findings presented here and in a preliminary paper (7), we now propose that the enzyme be referred to as adrenal C21-steroid side chain cleavage enzyme (17α-hydroxylase/C21SCC), as in the case of the testicular enzyme. This name can be conveniently abbreviated to C21SCC.

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S Nakajin, M Shinoda, M Haniu, J E Shively and P F Hall


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