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SOLUBILIZATION AND PROPERTIES OF BOVINE ADRENAL CORTICAL CYTOCHROME P-450 WHICH CLEAVES THE CHOLESTEROL SIDE CHAIN

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SUMMARY

A cytochrome P-450 with cholesterol side-chain cleaving activity was solubilized from beef adrenal mitochondria with sodium deoxycholate and glycerol and purified by chromatography on DEAE-Sephadex. This preparation interacts spectrally with and performs side-chain cleavage of cholesterol and 20α-hydroxycholesterol but does not react with substrates for 11β- or 18-hydroxylation. Absolute visible and ESR spectra indicate that this preparation is an equilibrium mixture of high- and low-spin states of P-450 Fe^{3+}.

Cholesterol binding increases the relative proportion of the high-spin form, and 20α-hydroxycholesterol increases the relative proportion of the low-spin form of the cytochrome. Kinetic studies of the enzyme's reducibility show that the rate of reduction of Fe^{3+} to Fe^{2+} is related to the proportion of high-spin P-450 present.

INTRODUCTION

The mixed function oxidase, cytochrome P-450, has been associated with hydroxylation of steroid substrates at C-11, 18, 20α and 22R in adrenal cortical mitochondria[1-3].

A number of adrenal mitochondrial preparations have been reported which oxidize cholesterol to pregnenolone[4-6]. The most extensive characterization of cholesterol oxidase activity has been reported by Mitani and Horie[7], Jefcoate *et al.*[8] and Schleyer *et al.*[9]. Mitani and Horie[7] and Schleyer *et al.*[9] isolated bovine adrenal mitochondrial P-450 which retained both side-chain cleavage and 11β-hydroxylase properties. Jefcoate *et al.*[8] separated these activities into two different ammonium sulfate fractions of isooctane-extracted submitochondrial particles. None of these isolations has markedly improved the heme to protein ratio over the starting material.

We report here the solubilization of a cytochrome P-450, from bovine adrenal submitochondrial particles, which catalyzes cholesterol side-chain cleavage. Sodium

deoxycholate and glycerol were used to solubilize this cholesterol oxidase in the presence of dithiothreitol. This material was chromatographed twice on DEAE-Sephadex. The visible and ESR spectra in the absence and presence of substrates, the kinetics of reduction and the side-chain cleavage activity of this cholesterol oxidase will be presented.

Following completion of these studies, Isaka and Hall[10] reported the solubilization of an adrenal mitochondrial cholesterol side-chain cleavage cytochrome P-450 without 11β-hydroxylation activity. However, no data regarding absolute visible, ESR spectra, P-450·CO or P-420·CO content, heme or protein concentration was presented.

METHODS

Preparation and solubilization of submitochondrial particles

Submitochondrial particles were prepared from beef adrenal cortex as described[11]. Solubilization was essentially the same as the procedure used by Lu and Coon[12]. To each 50 ml of particles in 0.25 M sucrose –10^{-3} M EDTA (containing 100 μM P-450, 30 μM P-420 and 60 mg protein per ml) was added 35 ml of glycerol, 18 mg of dithiothreitol, 11.5 ml of 1.0 M citrate (pH 7.4), 11.5 ml of 1.0 M KCl and 5.5 ml of 10% sodium deoxycholate. This mixture was stirred for 30 min at 5 °C and then centrifuged at 100 000 × g for 120 min. The precipitate was discarded.

Chromatography

DEAE-Sephadex (A-50-120 Pharmacia) was equilibrated in potassium phosphate buffer (0.16 M, pH 7.4) containing 10% ethylene glycol, 0.1 mM dithiothreitol, 1 mM EDTA and 0.05% sodium deoxycholate (column mix). The solubilized submitichondrial particles were diluted 50% with column mix and applied to a 5.0 cm × 67 cm column of the DEAE-Sephadex. P-450 was eluted with a linear gradient of KCl (0.5 M) in column mix. The P-450-containing fractions were concentrated in dialysis tubing under vacuum and dialyzed twice against 100 vol. of the column mix which did not contain EDTA or sodium deoxycholate (Fraction A). Rechromatography of the dialyzed material on DEAE-Sephadex was carried out in a similar manner on a 1.0 cm × 30 cm column. The P-450 fractions (10–20) were concentrated and dialyzed as before (Fraction B).

ESR and absorption spectroscopy

Different spectroscopy and ESR experiments were done as described previously[13]. Absolute visible spectra were obtained with phosphate buffer or a cholesterol–lecithin–albumin emulsion in the reference cuvette. The 1% lecithin–cholesterol emulsion was prepared as follows. 100 mg of L-α-lecithin (β,γ-dipalmitoyl, synthetic Grade A, Calbiochem) was added to 10 ml of water, sonicated for 5 min at 4 °C. 80 mg of cholesterol in chloroform was added to this sonicated preparation, chloroform removed with nitrogen followed by sonification for 10 min at 4 °C. The emulsion was then centrifuged at 10 000 × g for 10 min and the supernatant stored at 4 °C.

Kinetics of P-450 reduction

The rate of P-450 reduction was measured by monitoring the rate of formation

of its reduced CO complex at 450–430 nm in a dual wavelength spectrophotometer, essentially as described by Gigon *et al.*[14]. Each cuvette contained P-450 (1.1 μM), NADPH (200 μM) and the flavoprotein–non-heme iron fraction of sonicated adrenal mitochondria (S_2'), prepared as described by Omura *et al.*[15]. The flavoprotein–non-heme iron fraction employed had NADPH–cytochrome *c* reductase activity of 51.0 nmoles of cytochrome *c* reduced per min per mg protein in sodium phosphate buffer (pH 7.4, 130 mM). The concentration of added cholesterol was 240 μM; and that of 20α-hydroxycholesterol, 80 μM.

Side-chain cleavage activity

Flavoprotein dehydrogenase and non-heme iron protein were partially purified from S_2' on a DEAE-cellulose column as described by Omura *et al.*[15]. Each flask contained Fraction B (1.9 μM P-450, 1.5 μM P-420, 0.20 mg of protein per ml); a mixture of the non-heme iron protein (NHI) and flavoprotein dehydrogenase, which had a NADPH–cytochrome *c* reductase activity of 1.9 μmoles cytochrome reduced per min; 300 μM NADPH, 2 units of glucose-6-phosphate dehydrogenase and 5 mM glucose 6-phosphate in 3.2 mM sodium phosphate–0.8 mM potassium phosphate; 3.2 mM $MgCl_2$, 24 mM KCl and 0.25% bovine serum albumin. The incubations were carried out at 20 °C and pH 7.4. For 22*R*-hydroxylation, the flask contained 50 μCi of 20α-[7α-^{3}H]hydroxycholesterol. Cholesterol and deoxycorticosterone incubations contained 0.10 μCi of [4-^{14}C]cholesterol, and [4-^{14}C]deoxycorticosterone. The radioactive steroids were purchased from New England Nuclear with the following specific activities: [4-^{14}C]cholesterol 143 mCi/g, 20α-[7α-^{3}H]hydroxycholesterol 230 Ci/g and [4-^{14}C]deoxycorticosterone 133 mCi/g. The extraction and chromatography of the steroid incubations and the enzyme assays have been described[13].

Protein and heme chromagen determination

Protein was determined by the method of Yonetani[16]. Heme chromagen was measured as described by Porra and Jones[17].

RESULTS

Chromatography

The elution pattern from successive DEAE-Sephadex columns for the solubilized submitochondrial particles is shown in Fig. 1. The CO-combining material comes off in two peaks from the first column; the majority of the P-450 was present in the peak which was eluted with greater than 0.3 M KCl (Fraction A). When this fraction was re-chromatographed, the P-450 was eluted just after the void volume (Fraction B). This difference in mobility of the P-450 between the two columns may be related to protein aggregation or to the difference in salt and detergent concentration of the applied material. This pattern of elution was invariable for five different preparations. Analyses of P-450 and P-420 were made on each fraction collected. The P-420/P-450 ratio increased with increase in fraction number from approximately 10% in the first fraction from the chromatography to greater than 80% in the final fractions in the second small DEAE-Sephadex column. The actual ratio of P-420 to P-450 in a given fraction varied from preparation to preparation. Concentration of P-420 in Fraction B ranged from 30 to 50% in different preparations. This change

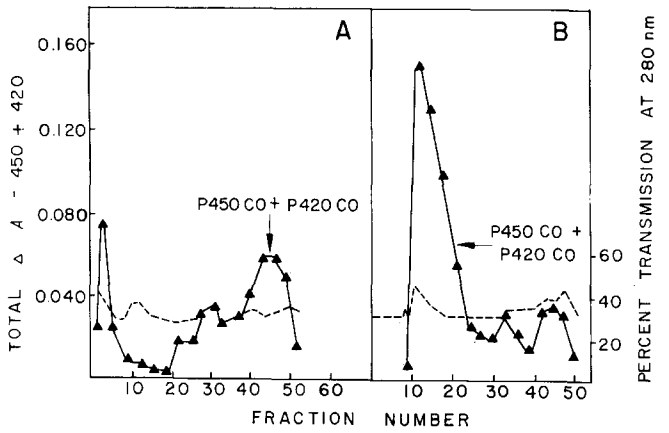

Fig. 1. Chromatography of solubilized submitochondrial particles on DEAE-Sephadex. Chromatography was carried out as described under Methods. The absorbance at 420 nm and 450 nm was measured after dithionite reduction gassing with CO and the total absorbance at these two wavelengths plotted.

in proportion of P-420 apparently had no effect on the mobility of the CO-binding pigments.

Table I presents recovery and specific activity figures for one of these preparations. The ratio of P-450 to protein is as high as any reported for other mammalian P-450 preparations[4,7-9]. Although the CO-pigment/heme ratio remained constant between the first and second DEAE-Sephadex columns (Fractions A and B) and the amount of P-420 increased approximately 8%, the second column produced better than a 2-fold increase in P-450/protein ratio. The decreasing P-450 + P-420 heme ratio between solubilized particles and Fraction A could occur if the CO-pigments were degraded to heme protein unable to bind CO but still contaminating native P-450. Such a possibility could also account for the degraded inability to achieve very high P-450/ protein ratios.

TABLE I

PROPERTIES OF CHOLESTEROL OXIDASE

	Total P-450 (nmoles)	*Total P-420 (nmoles)*	*Total protein (mg)*	*P-450/ protein*	*Total heme (nmoles)*	*(P-450+P-420)/ heme*
Submitochondrial particles	3200	1130	2500	1.3	—	—
Solubilized particles	2700	950	1250	2.2	1000	3.6
Fraction A	490	250	123	4.0	660	1.1
Fraction B	196	143	23	8.5	294	1.1

Visible spectroscopy

The absolute visible spectra for oxidized P-450, as well as the dithionite reduced, and CO-combined reduced cytochrome are presented in Fig. 2. The oxidized material (solid line) has 418-, 535- and 565-nm peaks and a shoulder at 640 nm. Upon

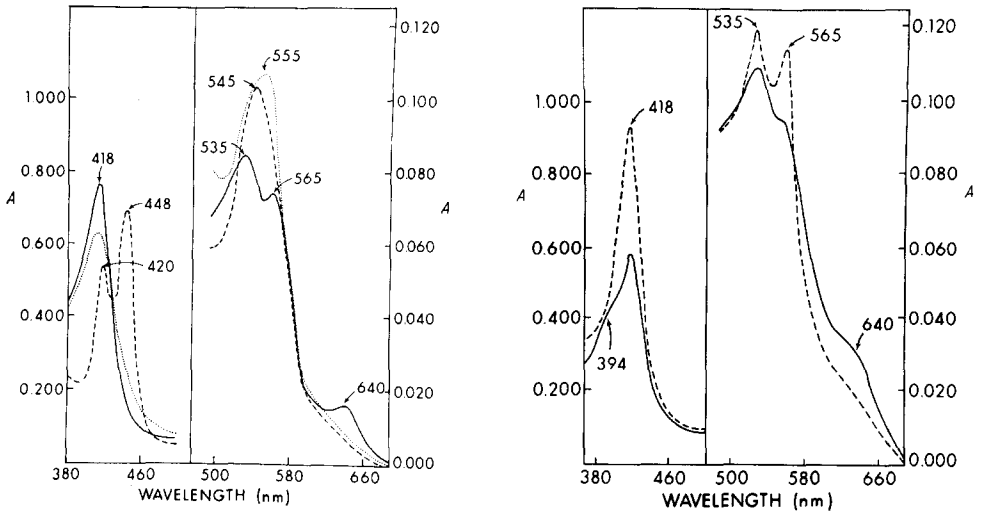

Fig. 2. Absolute spectra for oxidized (———), reduced (· · · · ·), and CO-reduced (— — —) forms of cholesterol-oxidase spectra were determined with a Cary model 14. The reference cell contained water and the sample cell contained Fraction B (4.0 μM P-450, 2.9 μM P-420, 0.5 mg protein) in phosphate buffer.

Fig. 3. Absolute spectra for substrate interaction with cholesterol oxidase. Spectra were done on a Cary model 14. Fraction B (4.0 μM P-450 and 2.9 μM P-420; 0.5 mg protein) and either 20α-hydroxycholesterol (— — —, 160 μM) or cholesterol (———, 870 μM) in lecithin were added to the sample cell. The 20α-hydroxycholesterol-treated sample was read against water. Because of the turbidity of the cholesterol–lecithin mixture in water, the cholesterol-treated sample was read against cholesterol–lecithin in bovine serum albumin (1%). The absorbance of this reference protein–phospholipid mixture was flat through the region 700–350 nm.

reduction by dithionite (dotted line), the 418-nm peak decreases in intensity and broadens, the 535- and 565-nm peaks coalesce into a single 555-nm peak, and the 640-nm shoulder is lost. The reduced, CO-combined cytochrome (dashed line) has 420-, 448- and 545-nm peaks. These are similar to the spectra of Mitani and Horie[7] and Schleyer *et al.*[9], except for the absence of a 390-nm shoulder in the oxidized spectra found by Mitani and Horie[7]. In addition, this preparation is contaminated with a greater amount of P-420 than the preparation of Schleyer *et al.*[9] since the reduced CO spectrum has a large 420-nm peak.

It was found that, although the solubilized starting material gave a Type I (high spin) difference spectrum with peaks at 390–395 nm and troughs at 420 nm with deoxycorticosterone, Fraction A gave small 420-nm peaks and Fraction B no longer interacted with this 11β-hydroxylase substrate. This sudden loss of reactivity upon exposure to the DEAE-Sephadex columns was also associated with loss of 11β-hydroxylase activity. A number of unsuccessful attempts to recover the capacity for reaction with substrates for 11β-hydroxylation were made. These included recombination of the DEAE-Sephadex column eluates, exhaustive high-salt extraction of the DEAE-Sephadex followed by concentration of these extracts and recombination with different column eluates, addition of phospholipids such as lecithin and asolectin to the various fractions, and finally complete preparation of the fraction in the presence of 100 μM deoxycorticosterone.

Fig. 3 shows the "absolute" visible spectra for the interaction of Fraction B with cholesterol and 20α-hydroxycholesterol. Fraction B interacts with cholesterol, only if lecithin is present in the sample. Because the turbidity of the solution of cholesterol, lecithin and protein distorts the absolute spectrum of the enzyme, an attempt was made to cancel this turbidity by using a bovine serum albumin reference with cholesterol and lecithin present in the same concentration as the sample. 20α-Hydroxycholesterol (dashed line) eliminates the 640-nm shoulder, and increases the 418-, 535- and 565-nm peaks relative to oxidized P-450. Cholesterol (solid line) intensifies the 640-nm shoulder and 535-nm peak, and decreases the 418- and 565-nm peaks. In addition, it induces a shoulder at 394 nm (see oxidized P-450, Fig. 2). The cholesterol spectra differs from that reported by Mitani and Horie[7] for the high-spin substrate, deoxycorticosterone, because of the much less clear-cut shift of the 418-nm peak to 393 nm. This difference may be attributed to either the difference in the two enzymes (cholesterol side-chain cleaving *versus* 11β-hydroxylation), differences in the two preparations or to problems with differences in the absorbance of the cholesterol–lecithin emulsions in the sample and reference cuvettes. The latter possibility seems the more likely (not shown) since in difference spectroscopy with enzyme in both cuvettes, addition of cholesterol induces a typical type I spectrum. However, there is a clear increase in both the charge transfer absorption bands (535 and 640 nm) and evidence of a shift of the Soret band (418 nm) to shorter wavelengths. This is an incomplete spectral shift for that expected in the transition of heme iron from low spin ($S = 1/2$) to high spin ($S = 5/2$).

TABLE II

EFFECT OF PURIFICATION OF P-450 ON K_s' FOR 20α-HYDROXYCHOLESTEROL AND CHOLESTEROL

Preparations of P-450 (1.0 μM) were titrated with 20α-hydroxycholesterol in ethanol or cholesterol in lecithin in the sample cuvette and ethanol or lecithin, respectively, in the reference cuvette. The difference in absorbance between 450 and 420 nm was plotted and K_s' calculated, as described previously[9].

P-450 preparation	K_s' (μM)	
	20α-Hydroxycholesterol	*Cholesterol*
Soluble submitochondrial particles	2.3	1200
Fraction A	10.0	750
Fraction B	15.0	410

ESR spectra

Fig. 4 shows the low-field region, 500–1200 G and the $g = 2.26$ signal in the high-field region of our P-450 preparation. The complete high-field spectrum of this P-450–P-420 preparation at liquid nitrogen temperatures with g values of 2.42, 2.26 and 1.91, was identical to that reported previously for adrenal submitochondrial particles[13]. Scrupulous attention to the considerations discussed under Methods was necessary to resolve the signals in the low-field region and to quantitate the changes in the intensities in all of the signals. Nevertheless, some ESR spectra were unaccountably associated with high noise to signal ratios and shifts in the baselines. Exhaustive cleaning and drying of the cavity in the Dewar system did not always remove these problems. The $g = 7.9$ signal previously described in submitochondrial

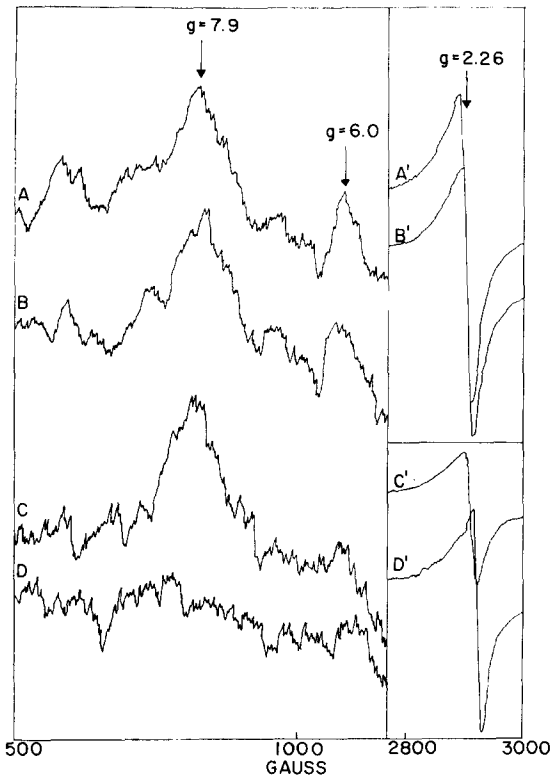

Fig. 4. Effects of cholesterol and 20α-hydroxycholesterol on the high- and low-field ESR spectrum of submitochondrial particles. Each ESR sample contained Fraction B (10 mg protein equivalent to 52 μM P-450, 52 μM P-420). Cholesterol in lecithin (B–B′; C–C′; 725 μM) and 20α-hydroxycholesterol (D–D′, 200 μM) were reacted with cholesterol oxidase for 30 min at room temperature. The cholesterol oxidase spectra (A–A′) contained lecithin at the same concentration as in the other samples. A, B, C, and D were recorded at a receiver gain of 10^4 and time-averaged over 8 scans on a Fabri-Tek 1074 computer. The receiver gain for A′ and B′ was 630, and for C′ and D′ was 320. The modulation amplitude for all scans was 16 G and power 200 mW.

particles[7,13], and later detected in bacterial P-450 (ref. 18), as well as in rabbit liver microsomes[19] is shown in the main spectrum (A) of our cholesterol side-chain cleaving preparation. In addition, a $g = 6.0$ signal is present which, from the observations of Murakami and Mason[20] and Whysner *et al.*[13], is presumably due to the P-420 which contaminates this preparation. Addition of 725 μM cholesterol to this preparation (Spectrum B, B′; Spectrum C, C′) does not change the intensity or line widths of either the $g = 7.9$ or $g = 2.26$ signals significantly. On the other hand, addition of 200 μM 20α-hydroxycholesterol (Spectrum D, D′) completely eliminates the $g = 7.9$ signal and increases the 2.26 signal almost 2-fold. The $g = 6.0$ signal present in Spectrum A and B is not invariably present as can be noted by examination of Spectrum C. We have no explanation for the variable appearance of this $g = 6.0$ signal. If it is due to P-420, one might expect that it would be present in all samples. However, a number of P-420 species have been reported, some of which have shown no detectable ESR signals[20]. While these studies must certainly remain inconclusive with respect to establishing the effect of substrates on the spin state of our P-450

preparation, they are compatible with the hypothesis that cholesterol induces the low-spin to high-spin transition in the heme iron, while 20α-hydroxycholesterol induces a high-spin to low-spin transition. The failure to observe the transition upon addition of cholesterol may be related to the higher concentration of cholesterol in the ESR cuvette resulting from the very high concentration of enzyme, presumably still contaminated with cholesterol, required for the ESR studies. In these studies at liquid nitrogen temperatures, we were unable to observe the $g = 4.0$ and $g = 1.8$ components of the $g = 7.9$ signal which Tsai and co-workers[18] and Peisach and Blumberg[19] have observed at liquid helium temperatures for P-450$_{CAM}$ and liver microsomal P-450 in the presence of their substrates (+)-camphor and methylcholanthrene, respectively.

P-450 reducibility

Fig. 5 shows the rate of reduction of P-450 in the presence of substrate and electron donor, employing the formation of the $Fe^{2+}\cdot CO$ complex as a measure of this rate. The increase in the initial rate of reduction produced by cholesterol, and the decrease in this rate produced by 20α-hydroxycholesterol, confirms the conclusions of Gigon *et al.*[14] and others[21,22] that substrates inducing Type I spectra increase, while those inducing Type II spectra decrease its rate of reduction. The biphasic rate in these studies may result from the presence of a certain amount of enzyme already bound by endogenous cholesterol substrate, therefore, rapidly reducible by electron donors. After 3–5 s, this endogenous substrate might be expected to be metabolized and a new rate appear which reflects a limiting diffusion rate of the added substrate from the aqueous media or its micellar location. Previous

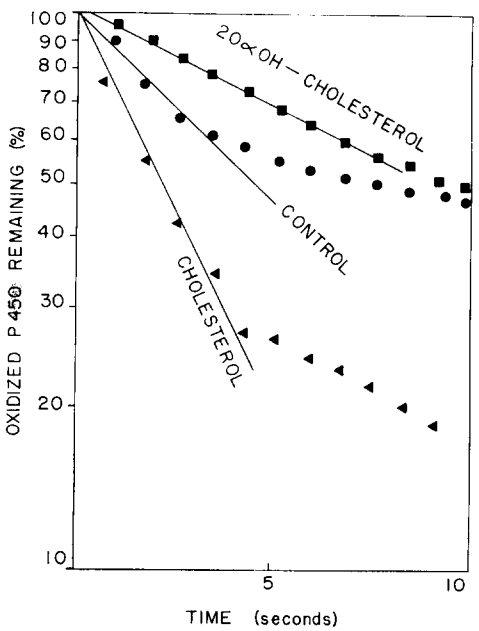

Fig. 5. Effect of substrate on the reduction rate of cholesterol oxidase. This study was performed as described under Methods.

observations by Harding *et al.*[23], support the possibility that endogenous substrates effect the spin states and, therefore, the spectral properties of P-450 preparations. These studies employing acetone–ether-extracted P-450 preparations demonstrated that the extracted particles were reduced at a much slower rate than the non-extracted preparations and that cholesterol addition, which originally produced only a small increase in rate and a small Type I or high-spin transition, produced a large rate increase and a marked high-spin transition following the ether–acetone extraction of the enzyme preparations. These observations were interpreted to indicate that endogenous substrates normally bind to many enzyme preparations and, therefore, prevent the spin state transitions which might otherwise be observed. More recent observations by Simpson *et al.*[24], Brownie *et al.*[25], and by Bell *et al.*[26], have amply demonstrated the existence of endogenous substrates binding P-450 and effecting the possible spin state transitions. Removal of these substrates by prior hydroxylation of the bound intermediate is followed by the ability to show characteristic high-spin spectral changes upon addition of appropriate substrates.

Side-chain cleavage studies

Saturation experiments for electron donor and the substrates cholesterol and 20α-hydroxycholesterol are shown in Table III. This P-450 preparation is able to

TABLE III

HYDROXYLATION ACTIVITY

Substrate	*Amount (nmoles)*	*NH1 + flavoprotein (nmoles cytochrome c reduced/min)*	*Activity per unit P-450 (nmoles/min)*
Cholesterol (0.1 μCi ^{14}C)	50	1.9	0.8
	125	1.9	1.3
	125	3.8	1.3
	250	1.9	1.8
20α-Hydroxycholesterol (50 μCi ^{3}H)	50	1.9	2.3
	125	1.9	4.0
	250	1.9	3.7
	250	0.6	1.1
Deoxycorticosterone (0.1 μCi ^{14}C)	1	1.9	0

cleave the side chain of cholesterol and 20α-hydroxycholesterol when it is reconstituted with the electron transport system. Since both compounds can serve as substrates, the preparation may either be a combination of 20α- and 22R-hydroxylases as well as the side-chain lyase, or it may be a single enzyme which has all these catalytic activities. It has no Δ^5-isomerase or 3β-hydroxydehydrogenase activity since pregnenolone, and not progesterone, is the product of side-chain cleavage of each substrate. Carrier-free 17α-hydroxydeoxycorticosterone (0.1 μCi) is not hydroxylated by either Fraction A or Fraction B.

DISCUSSION

A number of cholesterol side-chain cleaving preparations have been described[4-9] and the presence or absence of 11β-hydroxylase activity in these materials has been studied. Isolation of adrenal mitochondrial P-450 from other mitochondrial components commonly results in loss of 11β-hydroxylase activity[27]. The loss of 11β-hydroxylase activity from our mitochondrial extracts may be accounted for in several ways. First, if the activity resides in a specific P-450, the cytochrome could have been separated upon chromatography. If this were the case, it seems likely that it must have either become irreversibly bound to the DEAE-Sephadex, since the column was exhaustively washed and the eluants analyzed, or become denatured and lost both its CO-binding properties and activity. Second, the cytochrome could have been preferentially degraded to P-420 and this inactive enzyme then followed the cholesterol oxidase through the isolation procedure. Third, an 11β-hydroxylase substrate binding site on one P-450 with multiple binding sites for different substrates could have been inactivated during the isolation; and, finally, a substrate-specific protein which binds each substrate to cytochrome P-450 may have been separated or destroyed. This latter explanation would make adrenal P-450 quite different from the highly purified P-450$_{CAM}$ from *Pseudomonas putida* which Katagiri *et al.*[28] have shown binds its substrate camphor, apparently in the absence of any other proteins. The observations of Jefcoate *et al.*[8], who separated the P-450 cytochromes of isooctane-extracted adrenal submitochondrial particles into two fractions by ammonium sulfate precipitation supports the possibility of at least two separate mitochondrial P-450 cytochromes. One of their fractions had predominantly side-chain cleaving activity. Each fraction reacted spectrally with its own substrate, 20α-hydroxycholesterol inducing a peak at 420 nm and deoxycorticosterone inducing a trough at 420 nm.

Isaka and Hall[10] have recently arrived at somewhat similar conclusions regarding mitochondrial cholesterol side-chain cleaving cytochrome P-450. However, insufficient data was presented by them to compare either the catalytic or the spectral properties of the two preparations. The minor spectral change produced by cholesterol in their enzyme may also suggest that their preparation contains sufficient cholesterol to obscure the appearance of additional high-spin P-450 upon addition of cholesterol.

The spectral reducibility studies of cholesterol oxidase are compatible with an equilibrium of Fe^{3+} spin states; the relative proportion of high or low spin being influenced by substrate interaction. Tsai *et al.*[18], employing liquid helium temperature (15 °K) first observed $g = 4$ and $g = 1.7$ components of a $g = 8$ signal of P-450$_{CAM}$. Our failure to observe these additional signals upon cholesterol interaction are related to the fact that our spectra were obtained at 100 °K.

Jefcoate *et al.*[8] believe they have isolated the two "spin forms" of adrenal P-450; the high-spin form is thought to be cholesterol oxidase, and the low-spin form, 11β-hydroxylase. An alternative explanation is that their cholesterol oxidase preparation results from its contamination with its substrate, cholesterol, a high-spin inducer. Such an explanation is supported by the fact that Jefcoate's preparation gives 420-nm peaks with 20α-hydroxycholesterol and our earlier observations that in acetone-extracted preparations of P-450, 20α-hydroxycholesterol induces the low-spin (420-nm peaks) form of cytochrome only after cholesterol is added back to the

preparation[23]. A similar explanation probably applies to the preparation of Isaka and Hall[10].

Cholesterol oxidase, in common with other mammalian P-450 cytochromes[4,7–9] has not been greatly purified in terms of P-450 to protein ratios. It is possible that this material represents an integral component of the adrenal mitochondrial membrane containing small amounts of contaminating cholesterol. Further dispersion and purification of this lipophilic unit may result in the conversion of P-450 to P-420 with an associated loss of membrane structure and activity.

This study of cholesterol oxidase indicates the existence of at least two separate P-450 cytochromes and suggests that one P-450 or group of P-450 cytochromes is responsible for side-chain cleavage of both cholesterol and 20α-hydroxycholesterol. It further supports the hypothesis that substrates for adrenal mitochondrial P-450 induce a high-spin form which may be essential for the rapid reduction of P-450.

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