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Regulation mechanism of the catalytic activity of bovine adrenal cytochrome $P\text{-}450_{11\beta}$

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Abstract

In our previous paper (Ikushiro et al. (1992) J. Biol. Chem. 267, 1464), two catalytic states were proposed for bovine adrenocortical $P\text{-}450_{11\beta}$ at 37°C: one in liposome membranes and the other in liposome membranes containing $P\text{-}450_{scc}$. Similar reaction characteristics were observed at 5°C and all the experiments in this study were performed at 5°C. $P\text{-}450_{11\beta}$-proteoliposomes had relatively low 11β-hydroxylase activity and could catalyze aldosterone formation from 11-deoxycorticosterone. Relatively high 11β-hydroxylase activity was observed in $P\text{-}450_{11\beta}$-proteoliposomes containing $P\text{-}450_{scc}$ and in Tween-20 solubilized $P\text{-}450_{11\beta}$, in which no aldosterone formation could be detected. Optical titration indicated binding of corticosterone to $P\text{-}450_{11\beta}$ to be much weaker in the Tween-20 solubilized state than in proteoliposomes. Corticosterone competitively inhibited 11β-hydroxylation reaction of $P\text{-}450_{11\beta}$-proteoliposomes, but neither in $P\text{-}450_{11\beta}$-proteoliposomes containing $P\text{-}450_{scc}$ nor in the Tween-20 solubilized system. The binding of corticosterone to $P\text{-}450_{11\beta}$ was concluded quite weak in proteoliposomes in the presence of $P\text{-}450_{scc}$ and in the Tween-20 solubilized state. Aldosterone formation thus was not possible in these systems. Inability of the bovine adrenocortical zonae fasciculata and reticularis to produce aldosterone may be due to the weak binding of corticosterone to $P\text{-}450_{11\beta}$ in these zones.

Key words: Cytochrome $P\text{-}450_{11\beta}$; Aldosterone synthesis; Liposome; Detergent effect; Cytochrome $P\text{-}450_{scc}$

1. Introduction

Aldosterone is synthesized in the mammalian adrenal cortex and plays a vital role in regulation of sodium and potassium ion excretion by the kidney. The adrenal cortex consists of three zones, zonae glomerulosa, fasciculata and reticularis. Only the zona glomerulosa can secrete aldosterone. In rat and mouse as well as in human adrenal, two forms of cytochrome *P*-450 are involved in the metabolism of 11-deoxycorticosterone (DOC) [1–8]. In these adrenals, one form of the cytochrome *P*-450 is present in the zonae fasciculata and reticularis and catalyzes the conversion of DOC to corticosterone, 18-hydroxycorticosterone and 18-hydroxy-DOC, but not to aldosterone. The other form is present only in the zona glomerulosa and catalyzes the conversion of DOC to corticosterone, 18-hydroxycorticosterone and 18-hydroxy-DOC and also to aldosterone. The specific localization of these cytochrome *P*-450s well explains the specific secretion of aldosterone in the adrenal cortex zona glomerulosa. Two distinct forms of $P\text{-}450_{11\beta}$ were also detected in the bovine adrenal cortex. Both were capable of producing aldosterone and distributed almost equally throughout the three zones of bovine adrenal cortex [9,10]. Zone specific aldosterone synthesis in bovine adrenal cortex cannot be due to the specific localization of two $P\text{-}450_{11\beta}$.

In the presence of Tween-20, a nonionic detergent, purified $P\text{-}450_{11\beta}$ from bovine adrenocortical mitochondria in zonae fasciculata and reticularis catalyzes

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Abbreviations: $P\text{-}450_{11\beta}$, cytochrome *P*-450 having steroid 11β-hydroxylase activity (*P*-450 11B1); $P\text{-}450_{scc}$, cytochrome *P*-450 having cholesterol side chain cleavage activity (*P*-450 11A1); $P\text{-}450_{arom}$, cytochrome *P*-450 having aromatase activity (*P*-450 19A1); $P\text{-}450_{17\alpha,lyase}$, cytochrome *P*-450 having steroid 17α-hydroxylase and C17-C20 lyase activity (*P*-450 17A1); HPLC, high performance liquid chromatography; DOC, 11-deoxycorticosterone.

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the conversion of DOC to corticosterone, 18-hydroxycorticosterone and 18-hydroxy-DOC but not to aldosterone in the reconstituted system with adrenodoxin and NADPH–adrenodoxin reductase [11]. In the *P*-$450_{11\beta}$-proteoliposome system, *P*-$450_{11\beta}$ can synthesize aldosterone from DOC but the activity of the conversion from DOC to corticosterone (11β-hydroxylation) is considerably lower than in the Tween-20 solubilized system [12]. Since *P*-$450_{11\beta}$ purified from mitochondria of bovine adrenocortical zona fasciculata-reticularis produces aldosterone as well as corticosterone in the liposome membranes, the inherent aldosterone synthesizing activity of *P*-$450_{11\beta}$ must be suppressed by a certain factor in the zona fasciculata-reticularis. In the very recent studies on *P*-$450_{11\beta}$-proteoliposomes, we found that the addition of another mitochondrial cytochrome *P*-450, *P*-450_{scc}, enhanced 11β-hydroxylation activity but suppressed aldosterone synthesizing activity [13]. Kinetic analysis of this effect indicated the formation of an equimolar complex between *P*-$450_{11\beta}$ and *P*-450_{scc} in liposome membranes. Activity of the *P*-$450_{11\beta}$-*P*-450_{scc} complex in liposome membranes is quite similar to that of *P*-$450_{11\beta}$ in the Tween-20 system. The interrelationship between 11β-hydroxylation and aldosterone formation activity may possibly provide some clarification for regulation mechanism of the catalytic reactions of *P*-$450_{11\beta}$. Isotope dilution experiments using radioactive DOC and nonradioactive corticosterone or 18-hydroxycorticosterone showed *P*-$450_{11\beta}$-proteoliposomes to catalyze aldosterone production from DOC by a successive reaction mechanism where the intermediate metabolites, corticosterone and 18-hydroxycorticosterone, do not leave *P*-$450_{11\beta}$ [12].

Previous experiments had been performed at 37°C [12,13] and similar reaction characteristics were observed at 5°C. In order to compare the reaction mechanism of *P*-$450_{11\beta}$ in liposome membranes with that of unstable *P*-$450_{11\beta}$ in Tween-20 solubilized state, all the experiments were performed at 5°C in this study. The main purpose of this study is to answer a question why *P*-$450_{11\beta}$ cannot catalyze aldosterone production in the form of complex with *P*-450_{scc} and in the Tween-20 solubilized system.

2. Materials and methods

2.1. Chemicals

11-Deoxy[1,2-^{3}H]corticosterone, [1,2,3,6-^{3}H]corticosterone and [1,2-^{3}H]aldosterone were obtained from Amersham International (Amersham). 11-[4-^{14}C]Deoxycorticosterone and [1-^{14}C]L-α-dipalmitoylphosphatidylcholine were from NEN Research Products (Boston, MA). Phosphatidylcholine (egg yolk, Type III-E), cardiolipin (bovine heart), 11-deoxycorticosterone, corticosterone, sodium cholate, 20α-hydroxycholesterol and alumina C_γ were purchased from Sigma (St. Louis, MO). NADPH and dithiothereitol were from Boehringer-Yamanouchi (Tokyo). Phosphatidylethanolamine (egg yolk), metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone) and Tween-20 were from Lipid Product (Surrey), Aldrich (Milwaukee, WI) and Nakalai Tesque (Kyoto), respectively. All other chemicals were of the highest grade commercially available.

2.2. Preparation of enzymes

P-$450_{11\beta}$ and *P*-450_{scc} were purified from bovine adrenocortical mitochondria of the zona fasciculata-reticularis according to methods previously described [12,14]. Cytochrome *P*-450 concentration was determined from the dithionite-reduced CO difference spectrum using the difference absorption coefficient $\Delta\epsilon$ = 91 mM^{-1} cm^{-1} [15]. Adrenodoxin and NADPH–adrenodoxin reductase were purified from bovine adrenocortical mitochondria [16,17]. All these enzymes are homogeneous as determined by sodium dodecyl sulfate-polyacryamide gel electrophoresis.

2.3. Preparation of proteoliposomes

Incorporation of *P*-$450_{11\beta}$ into the liposome membranes was carried out basically by the method previously described [12] but with some improvements using a phospholipid mixture of phosphatidylcholine, phosphatidylethanolamine and cardiolipin at a weight ratio of 2:2:1. Phase transition temperature of these phospholipids are below 0°C. 30 mg of dry lipids were dispersed at 4°C in 1 ml of 10% sodium cholate solution under a stream of Ar with a sonicator (Heat system W-225) equipped with a cup-horn. After the solution became transparent, ^{14}C-dipalmitoylphosphatidylcholine (300 kcpm) was added for determination of the lipid recovery. To the lipid solution, NaCl, dithiothreitol, EDTA, glycerol, DOC and potassium phosphate buffer (pH 7.8) were added at final concentrations of 0.5 M, 0.1 mM, 0.1 mM, 20% (v/v), 10 μM and 50 mM, respectively. *P*-$450_{11\beta}$ was subsequently added at a molar ratio of 1:2000 to the phospholipids and dialyzed for 12 h against 50 mM potassium phosphate buffer (pH 7.2) containing 0.1 mM EDTA, 0.1 mM dithiothreitol, 10 μM DOC, 500 mM NaCl, 30 μM 2,6-di-*tert*-butyl-*p*-crezol (BHT) and 20% (v/v) glycerol. The dialysis buffer was deoxygenated with Ar-babbling before use. To remove unincorporated *P*-$450_{11\beta}$, the dialyzed solution was centrifuged at 24000 × *g* for 45 min and passed through a filter of pore size, 0.45 lm (Milipore). To remove DOC from the proteoliposomes, the filtrate was applied onto a TSK-PWH column (7.5 mm × 15 cm, Tosoh) using a HPLC system (CCPM pump, UV-8011, Tosoh) and

eluted with 50 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA and 200 mM NaCl. The proteoliposome solution was laid on 50 mM Tris-HCl buffer (pH 7.2) containing 40% (v/v) glycerol and centrifuged at 200000 × *g* for 90 min. The red layer just above the 40% glycerol solution was collected and dialyzed against 50 mM potassium phosphate buffer (pH 7.2) containing 0.1 mM EDTA and 200 mM NaCl. The *P*-$450_{11\beta}$-proteoliposomes thus prepared contained *P*-$450_{11\beta}$ at a weight ratio of about 1:40 to phospholipids and less than 0.05 mol DOC per 1 mol *P*-$450_{11\beta}$. The proteoliposome solution was mixed with glycerol at the final concentration of 20% (v/v) and frozen at 77 K. *P*-450_{scc} was incorporated into liposome membranes by incubation with *P*-$450_{11\beta}$-proteoliposomes [13].

2.4. Assay of enzyme activity of *P*-$450_{11\beta}$

Steroid metabolizing activity of *P*-$450_{11\beta}$ was measured at 5°C under aerobic conditions in 50 mM potassium phosphate buffer (pH 7.1) containing 0.1 mM EDTA and 20% (v/v) glycerol which will be referred to as the basal buffer, hereafter. The reaction solution (1 ml) contained 20 pmol *P*-$450_{11\beta}$, 5 nmol adrenodoxin, 0.5 nmol NADPH–adrenodoxin reductase and a certain amount of substrate (DOC or corticosterone) with the corresponding ^{3}H-labeled steroid (1 μCi) [12]. Following preincubation at 5°C for 2 min, reaction was initiated by the addition of 100 nmol NADPH and terminated by vigorous mixing with 1 ml chloroform. The extracted metabolites were separated with a HPLC system (CCPM, UV-8000, AS-48; Tosoh, Gilson 202) using a reverse phase column (RP-18, Kanto Chemicals) with a concentration gradient elution program from 63:37 (v/v) of water/acetonitril to 100% acetonitril [18]. Radioactivity of the separated metabolites was measured with a liquid scintillation spectrometer (LSC-701, Aloka).

2.5. Other methods

Optical absorption spectra were measured with a Beckman DU-7 spectrophotometer using the basal buffer. Phospholipid concentration was determined from the radioactivity of ^{14}C-dipalmitoylphosphatidylcholine.

3. Results

3.1. Characteristics of *P*-$450_{11\beta}$ in liposome membranes and in the Tween-20 solubilized state

Incorporation of *P*-$450_{11\beta}$ into liposome membranes was confirmed by density gradient centrifugation using 1–10% (w/v) of Ficoll [12]. The prepared proteoliposomes were unilamellar of about 40 nm in average diameter [12]. More than 90% of *P*-$450_{11\beta}$ was reduced by the external addition of NADPH and the electron transfer system composed of adrenodoxin and NADPH–adrenodoxin reductase, indicating most of the *P*-$450_{11\beta}$ to be located on the external side of the liposome membranes. *P*-$450_{11\beta}$ in liposome membranes was very stable even without the substrates and still possessed 80% of its binding ability with DOC after storage at 0°C for 20 h. 50% of Tween-20 solubilized *P*-$450_{11\beta}$ lost its binding ability in 2 h at 0°C [12]. *P*-$450_{11\beta}$ in liposome membranes expressed molecular activity (nmol/min per nmol *P*-$450_{11\beta}$) of 20, 1.0, 4.0 and 2.0 at 37°C for metabolism from DOC to corticosterone, 18-hydroxy-DOC, 18-hydroxycorticosterone and aldosterone, respectively. In the Tween 20 solubilized system, *P*-$450_{11\beta}$ metabolized DOC mainly to corticosterone but not to aldosterone [11]. The 11β-hydroxylase activity of *P*-$450_{11\beta}$ was dependent on Tween-20 concentration and maximum at 1% (v/v) at 5°C. 1% of Tween-20 (v/v) was used to solubilize the liposomes in this study. Solubilized *P*-$450_{11\beta}$ had 11β-hydroxylase activity of 100 nmol/min per nmol *P*-$450_{11\beta}$ at 37°C. The stability of Tween-20 solubilized *P*-$450_{11\beta}$ was measured in the absence of DOC. The incubation of solubilized *P*-$450_{11\beta}$ at 37°C, 10°C and 5°C for 30 min decreased 11β-hydroxylase activity, respectively, to 3%, 70% and 85% the original activity of nonincubated *P*-$450_{11\beta}$. All experiments in this study were performed at 5°C within 20 min after the solubilization.

3.2. Optical titration with DOC and corticosterone

The prepared *P*-$450_{11\beta}$-proteoliposomes showed a typical low spin absorption spectrum of cytochrome *P*-450. The addition of DOC or corticosterone to *P*-$450_{11\beta}$-proteoliposomes induced typical type I difference spectra at 5°C in the basal buffer as shown in Fig. 1 [19]. The insert shows the dependence of the amplitude of the difference spectrum on DOC concentration. The dissociation constant of *P*-$450_{11\beta}$-DOC complex can be estimated assuming ΔA to be proportional to the concentration of the complex. The solid line is a theoretical curve drawn based on a dissociation constant obtained by non-linear least square fitting for observed points. Optical titration was conducted at 5°C for Tween-20 solubilized *P*-$450_{11\beta}$. The type I difference spectrum was also observed upon the addition of DOC but no spectral changes were detected with corticosterone. The apparent dissociation constants were calculated from optical titrations and are listed in Table 1. The apparent dissociation constants of *P*-$450_{11\beta}$-steroid complexes were much larger in the solubilized state than in liposome membranes. Optical titration of *P*-$450_{11\beta}$-proteoliposomes was conducted in

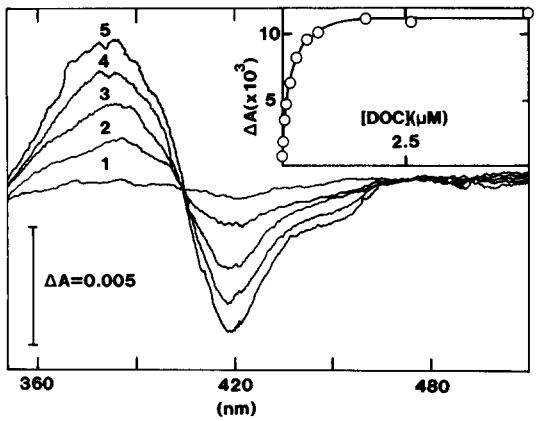

Fig. 1. Optical titration of $P\text{-}450_{11\beta}$-proteoliposomes using type I difference spectra induced by DOC. Proteoliposomes containing 0.2 μM $P\text{-}450_{11\beta}$ were titrated in the basal buffer at 5°C. Curves 1–5 are the difference spectra obtained upon the addition of 0.005 μM, 0.025 μM, 0.170 μM, 0.510 μM and 2.5 μM DOC, respectively. Amplitudes of the difference spectra are plotted against DOC concentration. The solid line is a best fit-curve with K_d of 0.06 μM.

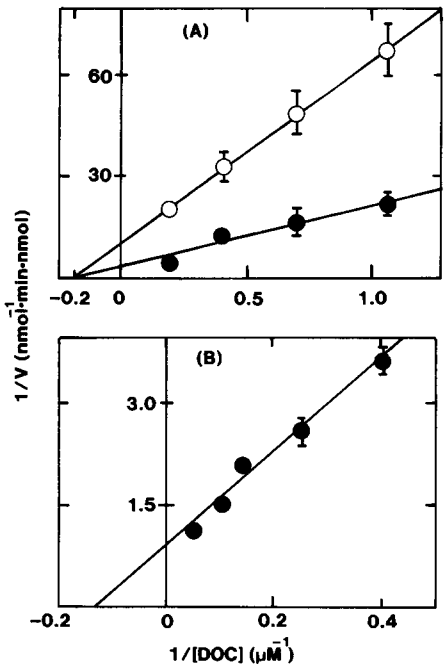

Fig. 2. Lineweaver-Burk plots of activity of $P\text{-}450_{11\beta}$. (A) Activity of $P\text{-}450_{11\beta}$-proteoliposomes. Open and closed circles show activity of the conversion of DOC to aldosterone and to corticosterone, respectively. (B) Activity of $P\text{-}450_{11\beta}$ of the conversion of DOC to corticosterone in the Tween-20 solubilized system. Aldosterone formation was not detected in the Tween-20 solubilized system. Assay conditions are described in Materials and methods.

the presence of $P\text{-}450_{scc}$, but the data obtained were not reliable since DOC and corticosterone caused spectral change in $P\text{-}450_{scc}$ itself.

3.3. Steady-state reactions

The steroid metabolizing activity of $P\text{-}450_{11\beta}$ in the steady state was measured for the conversion from DOC to corticosterone and to aldosterone at 5°C in the basal buffer. The dependence of activity on DOC concentration was measured in proteoliposomes as well as in the solubilized state and is illustrated by Lineweaver-Burk plots in Fig. 2. The lines for the conversion of DOC to corticosterone and to aldosterone in proteoliposome system intersect almost at the same point on the horizontal axis, showing K_m for two reactions to be essentially the same (Fig. 2(A)). Maximum activity was about 2.5-times higher for the conversion to corticosterone than to aldosterone in proteoliposomes. 18-Hydroxycorticosterone and 18-hydroxy-DOC were formed in the $P\text{-}450_{11\beta}$-proteoliposome system from DOC in amounts twice and half that of aldosterone, respectively. 18-Hydroxy-DOC and 18-hydroxycorticosterone formation was essentially proportional to that of corticosterone and aldosterone, respectively [13] and thus attention was directed to formation of corticosterone and aldosterone in this study. At high DOC concentration, 11β-hydroxylase activity in Tween-20 solubilized $P\text{-}450_{11\beta}$ greatly exceeded that in $P\text{-}450_{11\beta}$-proteoliposomes, but aldosterone formation was not detected in the solubilized state (Fig. 2(B)). Steady-state reactions of $P\text{-}450_{11\beta}$-proteoliposomes were also carried out in the presence of $P\text{-}450_{scc}$ at a molar ratio of 12.5 : 1 to $P\text{-}450_{11\beta}$, in which almost all $P\text{-}450_{11\beta}$ is in the form of complex with $P\text{-}450_{scc}$ as estimated from K_d in our previous work [13]. Even in liposome membranes, $P\text{-}450_{11\beta}$ complexed with $P\text{-}450_{scc}$ showed high activity for the conversion from DOC to corticosterone while essentially none to aldosterone. The K_m and V_{max} values are listed in Table 2.

Table 1
Dissociation constants of the $P\text{-}450_{11\beta}$-steroid complex

Steroids	K_d (μM) [a]	
	Proteoliposomes	Tween-20 system [b]
DOC	0.05 ± 0.01	4.0 ± 1.0
Corticosterone	13 ± 2	> 2500 [c]

[a] Dissociation constants are the average of three individual experiments.
[b] $P\text{-}450_{11\beta}$-proteoliposomes were solubilized in 1% (v/v) Tween-20.
[c] Value estimated from the inhibitory effect of corticosterone on the binding of DOC.

3.4. Competitive inhibition of 11β-hydroxylase activity

Corticosterone competitively inhibited reactions of $P\text{-}450_{11\beta}$-proteoliposomes in the formation of corticosterone from DOC as shown in Fig. 3(A). Aldosterone formation from DOC was also competitively inhibited

Table 2
K_m and V_{max} for reactions of $P\text{-}450_{11\beta}$

Steroids	K_m (μM) [a]		
	proteo-liposomes	proteo-liposomes with $P\text{-}450_{scc}$	Tween-20 system
DOC	4 ±1 [b]	17±4	7.4±0.2
Corticosterone	8.8±0.5	n.d.	n.d.
Reactions	V_{max} (nmol/min/nmol P-450) [a]		
	proteo-liposomes	proteo-liposomes with $P\text{-}450_{scc}$	Tween-20 system
DOC → corticosterone	0.24 ±0.02	1.1±0.2	1.1±0.03
DOC → aldosterone	0.11 ±0.04	n.d.	n.d.
Corticosterone → aldosterone	0.023±0.005	n.d.	n.d.

[a] Values are the average of three individual experiments. n.d., product formation under the detection limit.
[b] K_m of DOC for the conversion to corticosterone is essentially the same as that to aldosterone.

by corticosterone. In the Tween-20 solubilized system, 500 μM of corticosterone did not inhibit the reaction of $P\text{-}450_{11\beta}$ in forming corticosterone from DOC. No inhibitory effect of corticosterone was observed for reactions of $P\text{-}450_{11\beta}$-proteoliposomes in the presence

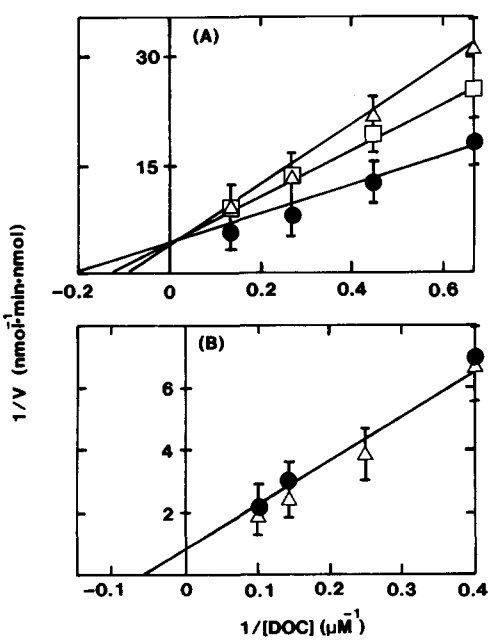

Fig. 3. Inhibitory effect of corticosterone on activity of $P\text{-}450_{11\beta}$-proteoliposomes for the conversion of DOC to corticosterone. (A) Activity was measured without $P\text{-}450_{scc}$. Closed circles, open squares and open triangles show activity in the presence of 0, 20 μM and 30 μM of corticosterone, respectively. (B) Activity was measured in the presence of $P\text{-}450_{scc}$. Closed circles and open triangles show activity in the presence of 0 and 30 μM of corticosterone, respectively. Assay conditions are described in Materials and methods.

Table 3
Inhibition constants of corticosterone in reactions of $P\text{-}450_{11\beta}$

Reactions	K_i (μM) [a]	
	proteoliposomes	proteoliposomes with $P\text{-}450_{scc}$
DOC → corticosterone	18±3	> 550 [b]
DOC → aldosterone	14±2	n.d.

[a] The values are the average of three individual experiments.
[b] Corticosterone production was not inhibited by 550 μM corticosterone.

of $P\text{-}450_{scc}$ as shown in Fig. 3(B). The inhibition constants are listed in Table 3.

4. Discussion

In our previous work, possibility of two alternative states of $P\text{-}450_{11\beta}$ was proposed at 37°C, one for $P\text{-}450_{11\beta}$ in liposome membranes with low 11β-hydroxylase activity and relatively high aldosterone formation activity for DOC, and the other, a complex with $P\text{-}450_{scc}$ which has high 11β-hydroxylase activity but virtually no aldosterone formation activity [13]. Similar reaction characteristics were observed at 5°C in this study. V_{max} in Table 2 shows solubilized $P\text{-}450_{11\beta}$ in Tween-20 to have about 4 times more 11β-hydroxylase activity than $P\text{-}450_{11\beta}$-proteoliposomes but no aldosterone production activity. The same was noted for $P\text{-}450_{11\beta}$ complexed with $P\text{-}450_{scc}$ in liposome membranes. The state of $P\text{-}450_{11\beta}$ complexed with $P\text{-}450_{scc}$ may be similar to that in the Tween-20 solubilized state. K_m for DOC is somewhat larger in $P\text{-}450_{11\beta}$ complexed with $P\text{-}450_{scc}$ in liposome membranes than in the Tween-20 solubilized system, possibly due to the binding of DOC to $P\text{-}450_{scc}$ [20].

Since the aldosterone producing activity of $P\text{-}450_{11\beta}$-proteoliposomes is 5-times higher from DOC than from corticosterone and K_m for DOC is about half that for corticosterone, DOC is a better substrate for aldosterone production than corticosterone. In the conversion of DOC to aldosterone by $P\text{-}450_{11\beta}$-proteoliposomes in the steady state, aldosterone has been shown not to be produced from accumulated intermediates such as corticosterone or 18-hydroxycorticosterone in the reaction solution but produced by the successive reactions without the intermediates leaving $P\text{-}450_{11\beta}$ [12]. It is not necessary for corticosterone to be a good substrate for aldosterone production in the successive reactions. V_{max} in Table 2 shows the successive reactions for aldosterone production to occur neither in $P\text{-}450_{11\beta}$ complexed with $P\text{-}450_{scc}$ nor in the Tween-20 solubilized state. K_d of the corticosterone-$P\text{-}450_{11\beta}$ complex is much higher in the Tween-20 solubilized system than in liposomal membranes, suggesting

binding of corticosterone to $P\text{-}450_{11\beta}$ to be very weak in the former. In the metabolism of DOC in the Tween-20 solubilized system, produced corticosterone easily dissociates from the catalytic site of $P\text{-}450_{11\beta}$ and the successive reactions cannot continue. This may be the reason why the successive reactions were not observed in Tween-20 solubilized $P\text{-}450_{11\beta}$.

The effects of $P\text{-}450_{scc}$ on $P\text{-}450_{11\beta}$-proteoliposomes could be derived from the same mechanism as that of $P\text{-}450_{11\beta}$ in the Tween-20 solubilized system. The weaker binding of corticosterone to $P\text{-}450_{11\beta}$ should thus be expected in the presence of $P\text{-}450_{scc}$. The optical titration of $P\text{-}450_{11\beta}$-proteoliposomes could not be carried out in the presence of $P\text{-}450_{scc}$, since $P\text{-}450_{scc}$ itself showed spectral changes upon the addition of corticosterone or DOC. $P\text{-}450_{scc}$ has been reported to metabolize DOC to 6β-hydroxy-DOC [20]. K_i of an inhibitor in the competitive inhibition mechanism is equal to K_d of the inhibitor [21]. In $P\text{-}450_{11\beta}$-proteoliposomes, corticosterone inhibited the metabolism of DOC to corticosterone and to aldosterone with similar inhibition constants (Table 3), both nearly equal to the dissociation constant of the corticosterone-$P\text{-}450_{11\beta}$ complex in liposome membranes (Table 1). In the presence of $P\text{-}450_{scc}$, 550 μM corticosterone did not inhibit the conversion of DOC to corticosterone. No inhibition by corticosterone was observed also in the Tween-20 solubilized system. The dissociation constant of the corticosterone-$P\text{-}450_{11\beta}$ complex may be assumed larger than 550 μM in the Tween-20 solubilized system and in the complex with $P\text{-}450_{scc}$. The large dissociation constant of the corticosterone-$P\text{-}450_{11\beta}$ complex accounts for the high 11β-hydroxylase activity of $P\text{-}450_{11\beta}$ in the complex with $P\text{-}450_{scc}$ and in the Tween-20 solubilized system.

Successive monooxygenase reactions without the intermediates leaving cytochrome P-450 have been reported in $P\text{-}450_{scc}$ [22,23], $P\text{-}450_{arom}$ [24,25], $P\text{-}450_{17\alpha,lyase}$ [26–30], and $P\text{-}450_{11\beta}$ [12]. In the first two cytochrome P-450s, dissociation of the reaction intermediates from cytochromes has not been reported and there are no physiological requirements for the dissociation of the intermediates. In the last two cytochrome P-450s, the dissociation of the intermediates is physiologically quite important. In the reaction of progesterone to androstenedione by $P\text{-}450_{17\alpha,lyase}$, 17$\alpha$-hydroxyprogesterone must dissociate to produce cortisol. Corticosterone is an important glucocorticoid and must be released from $P\text{-}450_{11\beta}$ in the metabolism of DOC.

The results of the present study clearly indicate the binding of corticosterone to $P\text{-}450_{11\beta}$ to be very weak in the $P\text{-}450_{11\beta}$-$P\text{-}450_{scc}$ complex. The zone specific aldosterone production in bovine adrenal cortex may possibly be due to the weak binding of corticosterone with $P\text{-}450_{11\beta}$ in the zona fasciculata-reticularis. The effects of anti-$P\text{-}450_{scc}$ IgG on DOC metabolism to corticosterone were examined using mitochondria from different zones of bovine adrenal cortex [13]. The IgG decreased 11β-hydroxylase activity more in the zona fasciculata-reticularis than zona glomerulosa, suggesting stronger interaction between $P\text{-}450_{11\beta}$ and $P\text{-}450_{scc}$ in the zona fasciculata-reticularis. The weak binding of corticosterone to the $P\text{-}450_{11\beta}$-$P\text{-}450_{scc}$ complex in the mitochondria of zona fasciculata-reticularis may reasonably be concluded to hinder the successive reactions from DOC to aldosterone. Experiments to determine why interaction between $P\text{-}450_{11\beta}$ and $P\text{-}450_{scc}$ is stronger in the zona fasciculata-reticularis than in the zona glomerulosa will be carried out in the near future.

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