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DISTRIBUTION OF NAD(P)H-DEPENDENT CYTOCHROME *P*-450 MIXED FUNCTION OXIDASE SYSTEM IN THE BRUSH BORDER MEMBRANE OF RABBIT KIDNEY CORTEX

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SUMMARY

Optical and magnetic studies were made on subfractions of rabbit kidney cortex. Cytochrome *P*-450 and cytochrome b_5-dependent mixed function oxidase systems were localized mainly in the brush border membranes and microsomes. Cytochrome *P*-450-dependent mixed function oxidases in the membranes comprised both an NADPH-dependent system and an NADH-dependent system.

INTRODUCTION

Cytochrome *P*-450 is known to be present in the cortical microsomes of mammalian kidney [1]. Various special kinds of cells are present in kidney cortex so it would be useful to know which kinds contain NAD(P)H-dependent cytochrome *P*-450 oxidase and related redox systems. Methods for isolation of brush border fragments of kidney cortex have been established on both morphological and enzymatic criteria [2, 3]. Using these methods, we investigated the distribution of cytochrome *P*-450 and cytochrome b_5 and related redox systems in subcellular membrane fractions of kidney cortex.

This paper reports that NADPH and NADH-dependent cytochrome *P*-450 mixed function oxidase systems are present in the brush border and microsomal membranes of kidney cortex. It is suggested that NAD(P)H-dependent cytochrome *P*-450 oxidase systems of brush border membranes are involved in the physiological role of detoxication of xenobiotics.

MATERIALS AND METHODS

Male New Zealand white rabbits weighing 2.5±0.2 kg were used. Rabbit kidney cortex was homogenized in a Teflon homogenizer (Glenco Scientific Inc.,

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Houston, Texas) equipped with a loose-fitting pestle. Glass fiber was obtained from the Corning glass works, Corning, New York. Nylon gauze, 0.1 mm pore size, was obtained from Sears-Roebuck and Co., Portland, Oreg. Centrifugation was carried out in a Beckman Spinco model L preparative ultracentrifuge with the rotor chamber and rotors cooled to 0 °C before use. An SW-25.1 Spinco rotor was used with cellulose nitrate tubes.

For assay of enzymatic activity, a Cary model 14 spectrophotometer with a low range slide wire (0.00–0.20 *A*: noise level, 0.001 *A*) and a high range slide wire (0.00–2.00 *A*: noise level, 0.001 *A*) equipped with a scattered transmission accessory and cuvettes of 1.0 cm light path were used. Wavelength measurements were standardized with a holmium oxide crystal. Optical spectra at low temperature were measured with a Cary model 14 recording spectrophotometer equipped with a scattered transmission accessory (helium dewar for operation between 1.2 °K and 300 °K, model DT, Janis Research Co. Inc., Stoneham, Mass., 02180) and an accessory for various low temperature measurements and cuvettes of 3.0 mm light path. Temperatures were measured with a germanium cryogenic temperature sensor, Cryo Cal, Inc., in the 1.5 °K–100 °K range.

An Ott compensating polar planimeter, sliding arm model (American Industrial and Scientific Co., L.A.) was used for the estimation of spin contents from EPR spectra.

Subcellular fractions

Fractionation was carried out as described below at 0–4 °C. Male New Zealand white rabbits were fasted for 24 h and then killed by air emboli introduced into the ear vein. The kidneys were removed rapidly, immersed in ice-cold 0.86 % (w/v) NaCl and washed very carefully to remove blood. Fat and surrounding connective tissues and capsules were trimmed away and then the kidney cortex was cut into small pieces. The pieces were washed with ice-cold saline, to remove as much contaminating blood as possible. The small pieces of kidney cortex were placed in 6 vols of 0.5 M sucrose, adjusted to pH 7.0 with 1.0 M Tris and homogenized with a Potter-Elvehjem homogenizer using 5 strokes of a loosely fitting Teflon pestle revolving at 1300 rev./min. The homogenate was filtered through a coarse glass fiber sieve and then through nylon gauze. The homogenate in 0.5 M sucrose was fractionated by layering it on a discontinuous gradient formed from 8.0 ml each of 1.4 M and 1.7 M sucrose, following the method of Berger and Sacktor [2]. The tubes were centrifuged at 25 000 rev./min (90 137 $\times g_{max}$) for 60 min using a type SW 25.1 rotor in a Beckman centrifuge. A pinkish fluffy layer containing the crude brush borders was located at the interface of the 0.5 M and 1.4 M sucrose zones. The bands of crude brush border fraction, crude mitochondrial fraction and nuclei and cell debris were carefully separated by a Pasteur pipette [2]. The crude brush border rich fraction and crude mitochondrial fraction were subjected to further purification by centrifugation. A centrifugation of the crude brush border fraction at $4000 \times g_{max}$ for 30 min separated the brush borders from the microsomal fraction. The microsomes was obtained from the post-brush border supernatant by centrifugation at 105 000 $\times$ g_{max} for 90 min. After the centrifugation of the crude mitochondrial fraction at $900 \times g_{max}$ for 10 min, the mitochondrial fraction was obtained from the supernatant by centrifuging at 12 000 $\times g$ for 5 min.

Method of isolation of renal brush border membranes. The purified brush border fraction was diluted and suspended with ice cold water in 5 vols to 0.25 M sucrose (adjusted to pH 7.4 with 1.0 M Tris solution) and treated for 10 min in a 9 kcycle sonic oscillator at 0 °C. The sonicate was centrifuged at 105 000 × *g* for 90 min. The pellet (brush border membranes) was resuspended in 0.15 M KCl (adjusted to pH 7.4 with 1.0 M Tris solution) to remove contaminating hemoglobin. This procedure was repeated.

For EPR spectra, each fraction obtained was transferred to an EPR tube using a Pasteur capillary pipette and frozen within 30 s in liquid nitrogen.

Electron paramagnetic resonance spectroscopy

Electron paramagnetic resonance (EPR) derivative absorption spectra were observed with a Varian X-band spectrometer, model 4502, and a Nippon-denshi X-band spectrometer, model JES-ME 2X, equipped with a 100 KHz field modulation. Liquid hydrogen was used for cooling. Quartz sample tubes with 3.0 mm internal diameter were used. Spectra were measured at an incident power of 3.0 mW at a field modulation amplitude of 12.5 G at 20 °K and the scanning speed was 200 G/min. This microwave power is below the level for saturation of cytochrome *P*-450 and cytochrome b_5 at 20 °K.

EPR experiments under strictly anaerobic conditions were carried out using EPR tubes of 3.0 mm internal diameter. Samples in the EPR tubes were made anaerobic for EPR spectral observations by repeated evacuation and flushing of the tubes with pure nitrogen.

For determination of *g* values, magnetic field strengths were measured with a Varian F-8 Flux meter and a Hewlett Packard 524 C electronic frequency counter. The microwave frequency (9.15 GHz) was measured with a Hewlett-Packard 532 B frequency meter.

Enzyme assays

The following enzymes were assayed by the methods given in the associated references. Succinate oxidase system [7], trehalase (EC 3. 2. 1. 28) [8], alkaline phosphatase (EC 3. 1. 3. 1) and acid phosphatase (EC 3. 1. 3. 2) were assayed with *p*-nitrophenylphosphate as substrate [9]. Glucose-6-phosphatase (EC 3. 1. 3. 9) was assayed in the presence of EDTA [10]. Cytochrome b_5 was determined, taking a value of 163 $mM^{-1}cm^{-1}$ as the molar extinction coefficient between 424 nm and 409 nm in the difference spectrum between the aerobic NADH reduced and the oxidized form [11]. Cytochrome *P*-450 was estimated from the difference spectrum of the dithionite reduced cytochrome *P*-450 · CO complex minus the oxidized form. The value of 115 $mM^{-1}cm^{-1}$ was used as the molar extinction coefficient between 450 nm and 500 nm in the difference spectrum [12]. Using this method spectral interference from contaminating hemoglobin can be avoided.

The contents of oxidized low- and high-spin cytochrome *P*-450 iron were estimated by comparison of the double integral of its first derivative EPR spectrum with that of a 1.0 mM cupric EDTA standard and metmyoglobin measured under exactly the same conditions.

NAD(P)H-ferricyanide reductase and NADPH-cytochrome *c* reductase [13] and NADH-cytochrome *c* reductase in the presence and absence of 1.5 μM

rotenone [14] were measured at 25 °C. The oxidations of β-NADH or NADPH were followed by observing the decrease of absorbancy at 340 nm, taking the extinction efficient as 6.22 $mM^{-1}cm^{-1}$ [15].

Hydroxylation rate at the para-position of *o*-chloroaniline was assayed by the method of Brodie and Axelrod [16] in the presence or absence of 0.5 mM KCN, since cytochrome *c* plus cytochrome oxidase strongly inhibits hydroxylation and demethylation activities. Demethylation rate for *p*-nitroanisole was measured by the method of Netter and Seidal [17]. The amount of nitrophenol produced by the demethylation of *p*-nitroanisole was determined spectrophotometrically from the absorbance at 420 nm to exclude as far as possible interference from the absorption of the *p*-nitroanisole used as substrate. Reactions were carried out at 37 °C for 30 min with mechanical shaking (110 times/min) in air and stopped by addition of 0.5 ml of 8.0 % (w/v) trichloroacetic acid to 1.0 ml of test solution. After centrifugation for 30 min remove denatured proteins, aliquots of the supernatant were taken for measurements by the methods described above.

Protein estimation

Protein content was determined by the biuret reaction to avoid interference from nucleic acids, although this reaction is not as sensitive as that with Folin-Ciocalteu reagent. Samples were taken after addition of 1 % (w/v) sodium cholate to the solution to remove turbidity. Values were corrected for heme absorption at 540 nm and crystalline bovine serum albumin was used as a standard. Samples containing sucrose were diluted with water before protein estimation, so that the absorbance difference between the sample and blank due to sucrose and phosphate became negligible compared to the absorbance due to protein.

Chemicals

Water was distilled before use. Ultra-pure, special enzyme grade sucrose from Schwartz/Mann, Cat. No. 3568 was used. β-NAD^+, $NADP^+$ and their reduced forms were purchased from Sigma Chemical Co. Cytochrome *c* (bovine heart type V) was obtained from Sigma Chemical Co. Crystalline phenylisocyanide was synthesized by the method of Prager et al. [18] All other reagents used were of the best commercial grade available and were used without further purification.

Electron microscopy

Samples were fixed in collidine-buffered 2 % osmium tetroxide, dehydrated in alcohols through propylene oxide and embedded in Epon 812 [19]. Ultrathin sections stained with uranyl acetate and lead citrate, were examined with a Hitachi 11-B electron microscope operating at 75 kV.

RESULTS

Table I shows the distributions of several enzymatic activities in the subcellular fractions of rabbit kidney cortex. The specific activities of specific organelle marker enzymes in the homogenate, mitochondria, brush borders and microsomes were compared. The activities of alkaline phosphatase and trehalase are highest in the brush borders. The microsomes and brush borders isolated from rabbit kidney

TABLE I

MARKER ENZYME ACTIVITIES IN SUBCELLULAR FRACTIONS OF RABBIT KIDNEY CORTEX

All enzymatic activities were measured at 25° C. Values are from 5–10 experiments with individual rabbits, and are given as mean ± S.D. Succinate oxidase activity is expressed as μl O_2/min per mg protein. All phosphatase activities are expressed as nmol Pi released/min per mg protein. Trehalase activity is expressed as nmol glucose formed/min per mg protein.

	Homogenate	Mitochondria	Brush borders	Microsomes
Succinate oxidase	0.47 ± 0.10	2.30 ± 0.41	0.03 ± 0.01	0.01
Glucose-6-phosphatase	8.5 ± 2.5	4.3 ± 2.0	1.2 ± 0.5	135.2 ± 27.7
Acid phosphatase	10.6 ± 5.5	25.4 ± 4.4	0.2 ± 0.1	0.1
Alkaline phosphatase	28.4 ± 5.5	22.5 ± 7.5	304.5 ± 25.5	35.7 ± 7.5
Trehalase	4.7 ± 1.5	2.4 ± 0.5	65.5 ± 5.5	5.5 ± 2.5

cortex were also confirmed microscopically (not shown). The marker enzymes of the other fractions used were glucose-6-phosphatase for microsomes [20], succinate oxidase for mitochondria [7] and acid phosphatase for lysosomes [20]. Values in the table represent the means and standard errors of values in five to ten independent experiments. The low level of succinate oxidase activity in the microsomes and brush borders indicates that they are not significantly contaminated with mitochondria.

Table II shows the distributions of cytochrome *P*-450 and cytochrome b_5 mixed function oxidase systems in the subcellular fractions of rabbit kidney cortex. These systems are localized in the brush border membranes and microsomal fraction. Their presence in the latter has been reported previously [1] but it is very interesting that these mixed function oxidase systems are also present in the brush border membranes of rabbit kidney cortex. In addition, high activities for hydroxylation

TABLE II

COMPARISON OF ELECTRON TRANSPORT COMPONENTS OF SUBCELLULAR FRACTIONS OF RABBIT KIDNEY CORTEX

Values are from 5–10 experiments with individual rabbits, and are given as mean ± S.D. Content of cytochromes is expressed as nmol/mg protein. Enzyme activities are expressed as nmol/min per mg protein.

	Homogenate	Mitochondria	Brush border membranes	Microsomes
NADPH-ferricyanide reductase	7.5 ± 2.5	2.0 ± 1.0	34.5 ± 4.5	25.5 ± 4.5
NADH-ferricyanide reductase	100.0 ± 24.5	42.5 ± 17.5	250.4 ± 34.5	150.1 ± 25.2
NADPH-cytochrome *c* reductase	2.0 ± 0.5	1.5 ± 0.5	20.0 ± 2.5	15.0 ± 2.5
NADH-cytochrome *c* reductase				
rotenone sensitive	3.5 ± 1.5	2.4 ± 1.0	1.0	1.2
rotenone insensitive	45.0 ± 15.5	34.5 ± 7.5	150.5 ± 32.5	100.4 ± 27.5
Cytochrome b_5		0.02	0.17 ± 0.05	0.12 ± 0.04
Cytochrome *P*-450		0.00	0.28 ± 0.05	0.07 ± 0.02
Hydroxylation of *o*-chloroaniline	0.05 ± 0.01	0.01	0.35 ± 0.05	0.10 ± 0.04
Demethylation of *p*-nitroanisole	0.04 ± 0.01	0.01	0.30 ± 0.05	0.10 ± 0.02

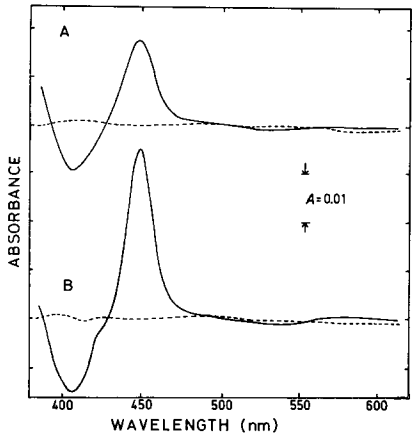

Fig. 1. Difference spectra of dithionite-reduced cytochrome *P*-450 · CO complexes, minus the dithionite reduced form of the brush border and microsomal membranes of rabbit kidney cortex. The sample and reference cuvettes contained suspensions of brush border membranes or microsomes (3.5 mg protein/ml, 0.1 M potassium phosphate, pH 7.4, 25 °C) in a total volume of 3.0 ml. Carbon monoxide gas was passed through the contents of the sample cuvette and then solid dithionite was added to the sample and reference cuvettes. Differences spectra were measured 2 min later. (A) Microsomes ,(B) brush border membranes.

and demethylation of aniline, *o*-chloroaniline and *p*-nitroanisole are mainly found in the brush border membranes.

Fig. 1 shows the difference spectra of the dithionite-reduced cytochrome *P*-450 · CO complexes minus the dithionite-reduced of the brush border and microsomal membranes of rabbit kidney cortex. Other hemoprotein complexes with CO, such as those of cytochromes *a* and a_3 [21], hemoglobin and cytochrome *P*-420 do not have absorption around 450 nm. It was confirmed that the cytochrome · CO complex of the brush border membranes and microsomes of kidney cortex was that of cytochrome *P*-450, not cytochrome *P*-454 [22]. It was important to exclude contaminating hemoglobin completely from the brush border membranes and microsomes to prevent a shift of the optical absorption spectra of dithionite-reduced cytochrome *P*-450 · CO complexes minus dithionite-reduced cytochrome *P*-450 to longer wavelengths. The levels of these cytochromes in the brush border membranes of rabbit kidney cortex are about one fifth of these in the smooth or rough endoplasmic reticulum of normal rabbit liver.

Fig. 2 shows the optical absorption spectra of cytochrome b_5 of the brush border membranes and microsomes of kidney cortex at 25 °C. The difference spectra of cytochrome b_5 reduced with NADH in air, minus its oxidized forms, in the brush border membranes and microsomes showed absorption peaks at 424, 526 and 556 nm in the visible region and the absorption spectrum of the reduced α-band had a shoulder at 560 nm at 25 °C. Furthermore, the absorption spectra of cytochrome b_5 reduced with NADH in air in the brush border membranes and microsomes showed splitting of the α-band at 20 °K. These results show that the absorption spectra of reduced cytochrome b_5 of brush border membranes and microsomes are identical with those of the reduced forms of the cytochromes b_5 in the smooth and rough endoplasmic reticulum and Golgi apparatus of rabbit livers [20]. This might be because the

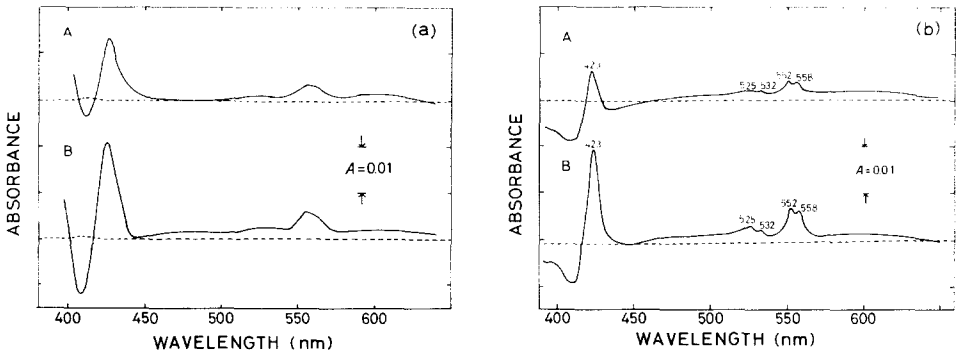

Fig. 2. (a) Difference spectra of aerobic NADH-reduced minus oxidized forms of cytochrome b_5 of brush border membranes and microsomes of rabbit kidney cortex at 25 °C. Protein concentration. 1.5 mg/ml, 0.1 M potassium phosphate, pH 7.4. Final concentration of NADH, 10 μM. (—) aerobic NADH-reduced minus oxidized forms, (- - -) base lines. (A) Microsomes, (B) brush border membranes. (b) Difference spectra of aerobic NADH-reduced minus oxidized forms of cytochrome b_5 of brush border membranes and microsomes of rabbit kidney cortex at 20 °K. Experimental conditions were as for Fig. 2a. (—) NADH-reduced minus oxidized forms, (- - -) base lines. (A) Microsomes. (B) brush border membranes.

brush border membranes are contaminated with microsomes, but this is unlikely because there was little glucose-6-phosphatase activity in the brush border membranes of rabbit kidney cortex.

Fig. 3 shows the EPR absorption spectra of oxidized and anaerobic NAD(P)H-reduced brush border membranes and microsomes of rabbit kidney cortex at 20 °K. The g values of oxidized cytochromes P-450 of the subcellular fractions of rabbit kidney cortex were observed to be $g_x = 1.91$, $g_y = 2.25$ and $g_z = 2.41$ in the

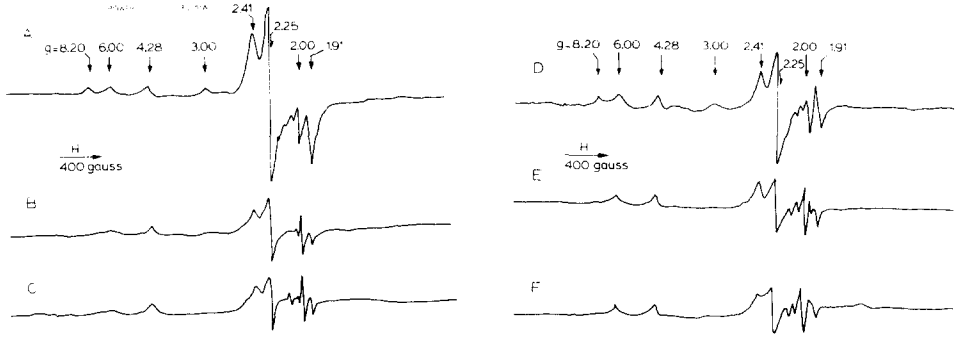

Fig. 3. EPR absorption spectra of oxidized and anaerobic NAD(P)H-reduced cytochromes P-450 of brush border membranes or microsomes of rabbit kidney cortex. The protein concentration was 85 mg/ml of 0.02 M potassium phosphate, pH 7.4. 0.3 ml of sample was used for the measurements. 0.5 mM NAD(P)H was used to reduce the samples under strictly anearobic conditions. Modulation amplitude, 12.5 G; microwave power, 3 mW; microwave frequency, 9.15 GHz; scanning rate, 200 G/min; magnetic field, 200–5200 G; temperature, 20 °K; magnetic field increase from low to high field. (A) Oxidized brush border membranes, (B) NADH-reduced brush border membranes 3 min after addition of 0.5 mM NADH, (C) NADPH-reduced brush border membranes under the same conditions as for B, (D) oxidized microsomes, (E) NADH-reduced microsomes under the same conditions as for B, (F) NADPH-reduced microsomes under the same conditions as for B.

TABLE III

SPIN CONTENTS OF HEME IRON OF OXIDIZED CYTOCHROME *P*-450 OF BRUSH BORDER MEMBRANES AND MICROSOMES AT 20 °K

Values are from 5–10 experiments with individual rabbit kidneys. Spin contents of high- and low-spin forms of oxidized cytochrome *P*-450 iron were obtained from the EPR spectra of observed g values at 20 °K. Values are given as mean ±S.D.

	Brush border membranes (nmol/mg protein)	Microsomes (nmol/mg protein)
High-spin form ($g_x = 8.20$, $g_y = 3.65$ and $g_z = 1.72$)	0.03±0.01	<0.02
Low-spin form ($g_x = 1.91$, $g_y = 2.25$ and $g_z = 2.41$)	0.25±0.03	0.05±0.02

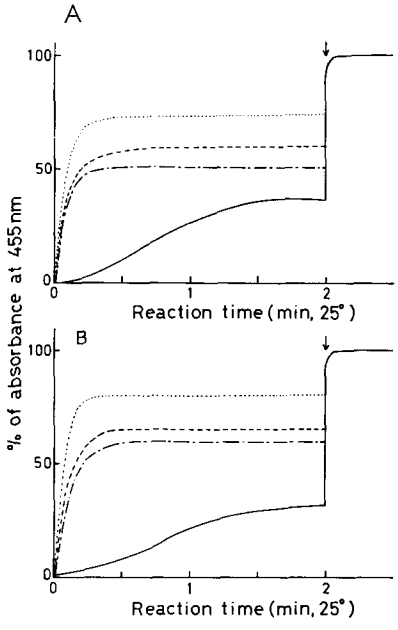

Fig. 4. Kinetics of reduction of cytochromes *P*-450 phenylisocyanide complexes of brush border membranes and microsomes of rabbit kidney cortex. Solutions containing brush border membranes or microsomes (3.0 mg protein/ml of 0.1 M potassium phosphate, pH 7.4) and 70 μM phenylisocyanide in the presence and absence of 5 mM $NADP^+$ were placed in two Thunberg cuvettes. The time courses of changes in absorbancy at 455 nm in the difference spectra were determined after addition of 10 μM NADH or 10 μM NADPH to the sample cuvettes under anaerobic conditions. The increments of absorbancy at 455 nm of reduced complexes of cytochrome *P*-450 with phenylisocyanide are given as percentage of those of dithionite reduced complexes of cytochrome *P*-450 with phenylisocyanide. Arrows indicate the points of addition of a little solid dithionite. (— · —) NADH-reduction of the cytochrome *P*-450 complex with phenylisocyanide in the presence of $NADP^+$, (—) NADPH-reduction of the cytochrome *P*-450 complex with phenylisocyanide in the presence of $NADP^+$, (- - -) NADH-reduction of cytochrome *P*-450 complex with phenylisocyanide in the absence of $NADP^+$, (. . .) NADPH-reduction of the cytochrome *P*-450 complex with phenylisocyanide in the absence of $NADP^+$. (A) Microsomes (B) Brush border membranes.

low spin form and g_x 8.20, g_y 3.65 and g_z 1.72 in the high spin form. These g values were identical with those of liver endoplasmic reticulum and Golgi apparatus [23]. The amount of oxidized, high-spin cytochrome *P*-450 was less than 7 % of the total amount of oxidized, low- plus high-spin cytochrome *P*-450 at liquid hydrogen temperature (Table III). This shows that the cytochromes *P*-450 of the brush border membranes are rapidly reduced by either NADPH or NADH, as in the endoplasmic reticulum of rabbit liver [22]. Moreover, this finding indicates that these membranes isolated from kidney cortex contain an NADH-dependent cytochrome *P*-450 reductase system as well as an NADPH-dependent cytochrome *P*-450 reductase system. Similar results were obtained from smooth and rough rabbit liver microsomes [25].

Fig. 4 shows that oxidized cytochrome *P*-450-phenylisocyanide complexes of the brush border membranes and microsomes are rapidly reduced by either NADPH or NADH under anaerobic conditions, like the complex of cytochrome *P*-450 with phenylisocyanide in rabbit liver microsomes [24]. In addition, reduction of cytochrome *P*-450-phenylisocyanide complexes with NADPH was inhibited by $NADP^+$, but not by NAD^+, while an NADH reducible cytochrome *P*-450-phenylisocyanide complex was not inhibited by $NADP^+$ and NAD^+. This shows that NADH- and NADPH-reducible cytochromes *P*-450 are involved physiologically in the brush border membranes and microsomes of rabbit kidney cortex.

DISCUSSION

Methods for the isolation of brush borders of rabbit kidney cortex have been described. A method for isolation of brush border fragments was first described by Miller and Crane [4] and this method was modified for isolation of renal brush borders by Berger and Sacktor [2] and Wilfong and Neville [3]. The former used a high concentration of sucrose and the latter 4–20 mM sodium bicarbonate at pH 8.1. A high concentration of sucrose is known to inhibit some enzymatic activities, but sucrose does not affect the hydroxylation and demethylation activities of cytochrome *P*-450-dependent mixed function oxidase systems and stabilizes the optical absorption of cytochrome *P*-450 [5], while cytochrome *P*-450 is unstable in solutions at above pH 8.0 or below pH 6.0 [6]. Accordingly, we used the method of Berger and Sacktor [2] to isolate the brush border membranes.

It is known that the mixed function oxidase systems of cytochrome *P*-450 and cytochrome b_5 are present in various cellular membranes of various tissues [1, 20]. This paper shows that these microsomal electron transport systems are present in the brush border membranes and microsomes of rabbit kidney cortex. This is interesting in relation to the function of the kidney cortex and the origin of the brush border membranes. It is well known that the brush border membranes are present in the proximal tubules of epithelial cells of kidney cortex. Previous papers reported that the electron transport systems of cytochrome *P*-450 and cytochrome b_5 are present in microsomal fraction [1, 26]. However, it is possible that the microsomal fraction might be contaminated by the fragments of brush border membranes. In disagreement with previous papers [22, 26], we could not detect cytochrome *P*-453-4 in the microsomes and brush border membranes of rabbit kidney. The optical and magnetic properties of cytochrome *P*-450 of the membranes of

rabbit kidneys were identical to those of cytochrome *P*-450 of the microsomes of normal or phenobarbital treated rabbit liver. This discrepancy may be due to a species or strain difference in the animal used.

The cytochrome *P*-450 oxidase of brush border membranes and microsomes [26] contain an NADH- and an NADPH-dependent system. NADPH-cytochrome *P*-450-dependent hydroxylation and demethylation activities towards *o*-chloroaniline and *p*-nitroanisole are involved in their mixed function oxidase systems, unlike the case of cytochrome *P*-450 oxidase of beef adrenocortical microsomes [6, 25].

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