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CYTOCHROMES IN THE RAT KIDNEY BRUSH BORDER MEMBRANE

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SUMMARY

Spectrophotometric studies of the brush border membrane fraction of the rat kidney as compared with those of its mitochondria and microsomes were carried out. Occurrence of cytochromes has been demonstrated in the brush border membranes. Either in the brush border membranes and in the mitochondria evidence for the presence of cytochromes of the types a, b and c was found, whereas in the microsomes only cytochrome b was demonstrated.

INTRODUCTION

The occurrence of cytochromes has been well documented in a wide variety of animal cell membranes such as the mitochondrial inner membrane, the microsomal membrane (1) and the nuclear membrane (2). However, no information is available regarding the presence of cytochromes in the brush border membrane of the renal tubule.

The results presented provide spectral evidence for the occurrence of cytochromes of the types a, b and c in the rat kidney brush border membranes.

MATERIAL AND METHODS

Animals

Adult male rats of the Wistar strain weighing 150-200g were used.

Isolation of brush border membranes

Luminal membranes derived from the proximal tubules were isolated by the method of Thuneberg and Rostgaard (3) as modified

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by Aronson and Sacktor (4) and Mitchell et al. (5). The isolation medium was 0.5M sucrose prepared with distilled- deionized water, which was previously filtered through Millipore filters (0.22μm) in order to avoid possible bacterial contamination.

Membranes prepared in 0.5M sucrose were suspended in ice-cold medium A, which consisted of 120mM KCl and 22mM potassium phosphate buffer (pH 7.4), and centrifuged at 27000 x g for 5min. The pellet was washed three times with the medium A by resuspension and centrifugation at 12000 x g for 5 min. The final pellet was resuspended in medium A.

Isolation of mitochondria

Mitochondria from the renal cortex were prepared by the method of Hogeboom (6) modified by us. The isolation medium contained 210mM mannitol, 70mM sucrose, 5mM Tris-HCl and 1mM EGTA, pH 7.4. After centrifugation at 800 x g for 10 min, mitochondria was centrifuged from the supernatant at 8200 x g for 10 min. The pellet was resuspended in 0.33M sucrose and centrifuged at 8200 x g for 10 min. The final pellet was resuspended in medium A.

Isolation of microsomes

The cortex kidney microsomal fraction was obtained essentially as described by Baginski et al. (7). After centrifugation at 16000 x g for 20 min, the mitochondria free supernatant obtained was centrifuged at 105000 x g for 1 hr. The final pellet was resuspended in medium A.

Spectrophotometric studies

Difference spectra of cytochromes were measured using a Hitachi Model 356 Spectrophotometer at room temperature. Cytochromes were reduced with sodium dithionite. Experiments with glucose plus glucose oxidase were carried out as described (8).

Determination of enzyme activities

Membrane marker enzymes were assayed as follows: alkaline phosphatase by the method of Walter and Schutt (9), maltase as described by Dalquist (10) and γ-glutamyl transpeptidase by direct spectrophotometric measurement as described by Orlowski and Meister (11). Glutamate dehydrogenase was determined as described by Schmidt (12). Succinate dehydrogenase was assayed according to the method of Porteous and Clark (13).

Protein determination

Protein was determined according to the method of Lowry et al. (14) using crystalline bovine serum albumin as standard.

Chemicals

Sucrose, glucose and dithionite were obtained from Merck. NADH was purchased from Boehringer Corp. All the substrates for enzyme assays, mannitol, EGTA, p-iodonitrotetrazolium violet and glucose oxidase from Aspergillus niger were obtained from Sigma Chemical Co. All common laboratory chemical were of reagent grade and were used without further purification.

RESULTS AND DISCUSSION

As shown in Table I, the brush border membrane fraction was of high purity as far as the values of the marker enzyme

TABLE I

SPECIFIC ACTIVITY* OF MARKER ENZYMES IN INITIAL HOMOGENATE, BRUSH BORDER MEMBRANES AND MITOCHONDRIA.

Enzyme	Homogenate	Brush border membranes	Mitochondria	$\frac{\text{Brush border membranes}}{\text{Mitochondria}}$ ratio
Alkaline phosphatase	0.324 ± 0.032	2.814 ± 0.330	0.374 ± 0.063	7.5
Maltase	0.134 ± 0.001	1.481 ± 0.070	-	-
γ-Glutamyl transpeptidase	0.080 ± 0.010	0.795 ± 0.041	-	-
Glutamate dehydrogenase	0.032 ± 0.005	0.017 ± 0.006	0.234 ± 0.061	0.07
Succinate dehydrogenase	0.004 ± 0.001	0.002 ± 0.001	0.021 ± 0.003	0.09

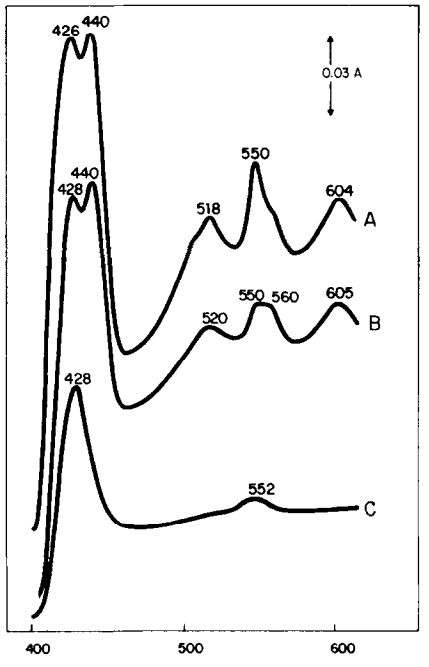
* Specific activities are means ± S.E.M. of at least four duplicate observations and are expressed as micromoles of product formed per mg of protein per min.

activities is concerned. The low glutamate dehydrogenase and succinate dehydrogenase activities in the brush border membranes demonstrate very litle contamination of this preparation by mitochondria.

The difference spectra of kidney mitochondria, brush-border membranes and microsomes are shown in Figs. 1 and 2 which demonstrate that the cytochromes found in the membranes were similar in their spectral properties, although different in their relative concentrations, from those found in mitochondria.

The alpha region of the spectra shows a reduced cytochrome a absorption with a maximum at 605 nm for the mitochondria and 604 nm for the membranes, a reduced cytochrome b absorption which appears as a slight shoulder at 560 and a reduced cytochrome c peak with an absorption maximum at 550. The Soret region of the spectra show a reduced cytochrome a shoulder at 440 nm and a reduced cytochrome b peak at 428 nm in both mitochondrial and membrane preparations. Difference spectrum of the microsomes shows only absorption peaks at 552 and 428 nm, characteristic of cytochrome b.

The results provide convincing evidence for the occurrence of cytochromes in the rat kidney brush border membrane. The



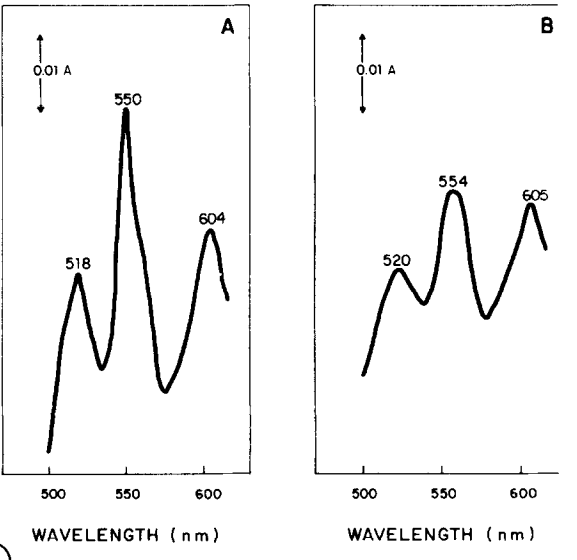


Fig. 1. Reduced versus oxidized difference spectra. A, mitochondria (6,5 mg of protein per ml); B, brush border membranes (7,1 mg/ml); C, microsomes (6,1 mg/ml).

Fig. 2. Detailed study of the α- and β-region of the reduced versus oxidized difference spectra. A, mitochondria (6,5 mg of protein per ml); B, brush border membranes (7,1 mg/ml).

possibility of an artifact due to contamination with mitochondria is unlikely since activities of marker enzymes demonstrated that membrane fraction contained less than 10% of mitochondrial contamination, in agreement with the results of Liang and Sacktor (15), for rabbit kidney brush border membrane preparations.

When glucose plus glucose-oxidase was added to the test cuvette in order to eliminate oxygen (16), peaks of reduced cytochromes a (605 and 440 nm) and c (550 nm) and only a slight shoulder of cytochrome b 560 nm were obtained (Fig.3). However, total reduction of cytochrome b was achieved only when NADH was added as indicated by the absoption peaks at 560 and 428 nm. Non-stable effect could be obtained when glucose plus glucose oxidase were added to the mitochondria.

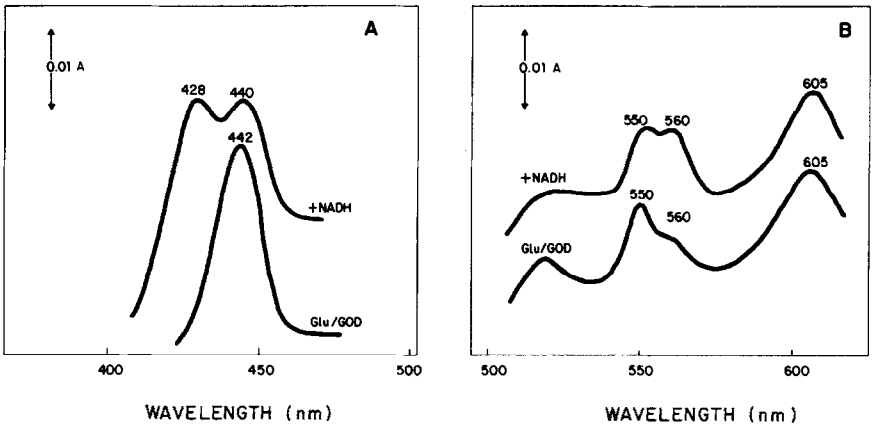


Fig. 3. NADH reduced versus oxidized difference spectra of the brush border membranes. A, Soret region; B, α- and β-region of spectra. Reduction with NADH was carried out after treatment of the test cuvette but not the reference one with glucose plus glucose oxidase (6,3 mg of protein per ml).

In order to better show the effect of NADH on the cytochrome spectra of the membrane fraction difference spectra were obtained by adding NADH to the test cuvette after reduction of both test and reference cuvettes with glucose plus glucose oxidase. Only a reduced cytochrome b peak was observable in the difference spectra (Fig. 4).

The effect of glucose plus glucose oxidase on the cytochrome system of the membrane could be explained as reduction via flavoprotein in the membrane, because glucose oxidase from Aspergillus, which is also a flavoprotein, was able to reduce the FAD added to the cuvette in the presence of glucose (data not shown).

On the other hand, reduction due to some endogenous substrate is unlikely because the membrane vesicles were completely empty of contents.

Since a non-permanent effect could be obtained with glucose plus glucose oxidase on the mitochondrial fraction, this effect distinguishes the brush border membrane cytochrome system from that of the mitochondrial preparation.

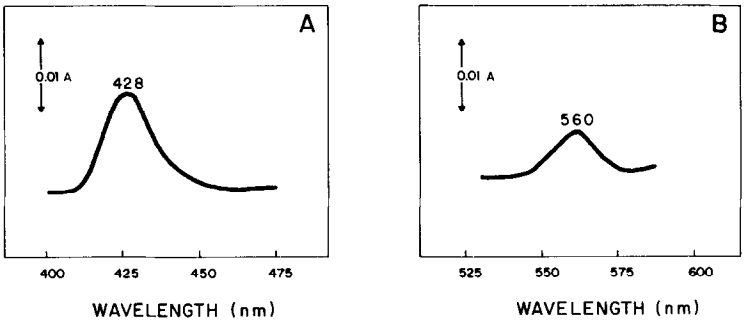


Fig. 4. NADH reduced versus glucose plus glucose oxidase reduced difference spectra of the brush border membranes. A, Soret region; B, α- and β-region of spectra. Reduction with NADH in the test cuvette was carried out after treatment of both the test and reference cuvettes with glucose plus glucose oxidase (6,3 mg of protein per ml).

The two steps in the reduction of the cytochrome system with glucose/glucose oxidase and NADH, that is, the absolute requirement of NADH for complete reduction suggests that two fragments of a respiratory chain are present in the brush border membrane, one with cytochromes of the types a and c, and the other one with cytochrome b being unequally accesible to the reducing equivalents. The possibility exists, however, that these two fragments of respiratory-chain have been uncoupled during the preparation of the membranes.

The existence of NADH dehydrogenase activity (data not shown) together with the cytochromes strongly suggests the presence of an electron transport system in the brush border membranes. The present finding may be relevant to mechanisms of active transport since it would permit the generation of a membrane potential. Thus, under conditions where the electrogenic sodium ion pump is inoperative, active transport could be energized by other electrogenic systems as suggested by Lever (17) for fibroblast plasma membranes. Furthermore, as demonstrated by MacDonald et al. in membrane vesicles of E. coli, the sodium gradient can be created by a protonmotive force via a H^+ -Na^+ antiport (18) which would be generated by the cytochrome system (19). This hypothesis is consistent with the efective transmembrane electrochemical potential (20) and the Na^+-H^+ antiport system (21) found in brush-border.

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