Biochimica et Biophysica Acta, 692 (1982) 371–376
Elsevier Biomedical Press

BBA 71415

TOPOLOGY OF MEMBRANE EXPOSURE IN THE RENAL CORTEX SLICE

STUDIES OF GLUTATHIONE AND MALTOSE CLEAVAGE

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(Received June 14th, 1982)

Key words: Membrane topology; Membrane exposure; Glutathione; Maltose; (Kidney cortex slice)

We measured glycine release from ([2-^{3}H]glycine)-labelled GSH and glucose formation from maltose incubated with rat kidney whole cortex homogenate, thin cortex slices or collagenase-treated tubule fragments. Liberation of glycine was inhibited (74–83%) by serine borate (20 mM), indicating a γ-glutamyltransferase-dependent hydrolysis of GSH. In whole cortex homogenate, the GSH cleavage activity was 17.4±0.6 nmol GSH degraded/mg protein per min (mean±S.D.); cleavage activity by intact slices was 3.5±0.7 ($P<0.001$ relative to whole cortex homogenate) and in tubule fragments 9.4±0.8 ($P<0.001$). Homogenizing the tissue preparation increased cleavage rate in slices about 4-fold (12.4±2.9; $P<0.005$ relative to intact slice) but did not change the rate in tubule fragments (9.8±0.5). Maltose cleavage activity in whole cortex homogenate was 512±22 nmol glucose formed/mg protein per min, in slices 162±12, and in tubules 884±48. These findings imply that substrate in the incubation medium has a limited access to the luminal membrane of cortex slices but not of tubule fragments. They further imply that basolateral membrane is preferentially exposed in the slice preparation.

Introduction

This paper poses the question: is the brush border membrane of the renal cortex slice accessible to solute in the incubation medium? Whereas the renal cortex slice has been used widely to study renal metabolism and transport of substrates [1–3], it has been the object of criticism in the latter context, because the specific functional orientation of brush border and basolateral membranes that serve transport in vivo is altered in the slice preparation. Whether solute has uniform access to brush border and basolateral membranes during incubation of the slice is a matter open to dispute [4,5]. The isolated renal tubule fragment preparation [6] is subject to a similar query. We studied this problem by measuring the cleavage of two substrates, glutathione (GSH) and maltose, in slice and tubule preparations, comparing cleavage activity with homogenates in which cytoarchitecture was disrupted. Glutathione (L-γ-glutamyl-L-cysteinylglycine) and maltose (4-*O*-D-glucopyranosyl-α-D-glucopyranose) are cleaved by γ-glutamyltransferase (EC 2.3.2.2) and maltase (α-D-glucosidase glucohydrolase, EC 3.2.1.20) respectively. Both enzymes are prominent in the renal brush border membrane and are absent from the renal basolateral membrane [7,8]. γ-Glutamyltransferase has an asymmetry of orientation in the brush border membrane that permits cleavage of substrate on the lumen side of the brush border membrane [9,10]. Our findings suggest that GSH and maltose have only limited access to the brush border membrane in the renal cortex slice whereas

the tubule fragment preparation appears to expose the brush border membrane.

This study was presented in part at the International Congress of Physiology, Budapest, 1980 and 13th Annual Meeting of the American Society of Nephrology, Washington, 1980.

Material and Methods

Renal tissue preparations. Kidneys were obtained from adult male Sprague-Dawley rats weighing 200–350 g (Canadian Breeding Farms, St-Eustache, Québec). Animals were fed standard rodent lab chow and had free access to tap water. After decapitation, the kidneys were removed immediately, decapsulated, hemisectioned and placed in chilled buffer (4°C). Thick cortex slices (0.2–0.3 mm) were used to prepare homogenate and tubule fragments; thin slices (< 0.2 mm) were used for all other experiments. Slices were obtained from the cortex portion only with a chilled Stadie-Riggs microtome.

Homogenates were prepared from whole cortex or from 3–5 freshly cut unincubated slices in Hanks' medium (10% w/v) in a chilled Potter-Elvehjem teflon-glass homogenizer (clearance 0.10–0.15 mm) with three strokes, then centrifuged at $800 \times g$ for 10 min at 4°C. Supernatant was used immediately or frozen at −20°C; freezing did not impair enzyme activity.

Tubules were prepared by incubating 30–50 mg thick slices for 45 min in 10 ml of collagenase solution (4 mg/ml) at pH 7.4, 37°C under 95% O_2/5% CO_2 gas mixture followed by light centrifugation ($50 \times g$ for 30 s at 4°C). The pellet was washed with buffer and respun three times, then filtered through nylon stocking mesh to separate debris and glomeruli from tubules (filtrate). Tubules (20–60 mg/ml) were suspended in buffer for incubation after inspection for purity under an inverted microscope; preparations were less than 5% contaminated with glomeruli. Protein was determined by the method of Lowry et al. [11].

Thin cortex slices were trimmed with a scalpel blade to 5 mm squares (3–5 mg wet wt).

Incubations were performed with approximately 40 mg tissue of each preparation in 2 ml medium pH 7.4 at 37°C under 95% O_2/5% CO_2 gas mixture.

Assay of cleavage activities. Glutathione cleavage was measured in tissues incubated at pH 7.4, 37°C in Hanks' medium containing reduced dithiothreitol (1 mM) and L-glutahione substrate labelled with tritium in the glycine residue. The reaction was terminated by boiling (homogenate and tubule fragments) or by removing tissue (slices). The solution was applied to a resin column (Dowex AG1 X2, BioRad) in the acetate form according to the technique of Furano [12]. The column was washed with distilled water to elute labelled cysteinyl-glycine and free glycine; the retained glutathione was then eluted with acetic acid (2 M). Radioactivity in the eluates was counted in scintillation fluid with a Beckman Scintillation Counter Model LS-2000. L-Serine in sodium borate was used as a selective inhibitor of γ-glutamyltransferase activity [13] to study specificity of the cleavage activity. Incubation without renal tissue was used as the blank.

Maltose cleavage was measured in tissues incubated at pH 6.0, 37°C in sodium maleate buffer [14] containing maltose substrate free of D-glucose contamination (less than 0.5%). Glucose released into the medium was measured by the glucose oxidase reaction after centrifugation of the incubation medium ($3000 \times g$ for 10 min at 4°C). Incubation without renal tissue was used as the blank.

Chemicals. Hanks' medium concentrate without sodium bicarbonate or phenol red (Grand Island Biological Co., New York) was diluted 10-fold with double-distilled water, adjusted to pH 7.4 with a 5% sodium bicarbonate solution and gassed with 5% CO_2 in O_2. Reagent grade chemicals were used. We obtained collagenase (grade II) from Worthington Biochem. Corp. with a specific activity of 161 I.U./mg; maltose containing less than 0.5% glucose (Cat. M-5885) from Sigma (St. Louis, MO); L-glutathione from Boehringer Mannheim (Cat. 1277436); L-glutathione ^{3}H-labeled in the glycine residue (1.41 Ci/mmol) from New England Nuclear (Boston, MA).

Results

Glutathione cleavage

Cleavage activity measured in whole cortex homogenate (control) was directly proportional to

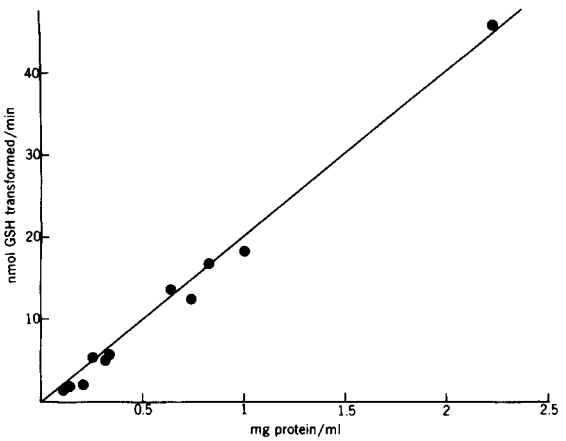

Fig. 1. Cleavage activity of GSH by rat renal cortex homogenate. The 2 ml assay mixture contained 1 mM ([^{3}H]glycine)-labelled GSH and 1 mM reduced dithiothreitol in Hanks' medium. Cleavage activity was assessed by measuring the radioactive dipeptide cysteinyl-glycine and glycine. L-Serine borate inhibited the reaction.

protein content (Fig. 1); activity was also linear with time in the first 30 min; γ-glutamyltransferase activity was 17.4 ± 0.6 (mean and S.D.) nmol substrate hydrolyzed per mg protein per min in our preparation. L-Serine borate inhibited cleavage activity: 75% at 10 mM inhibitor concentration, 92% at 50 mM and 95% at 100 mM. Boiling of tissue also obliterated activity.

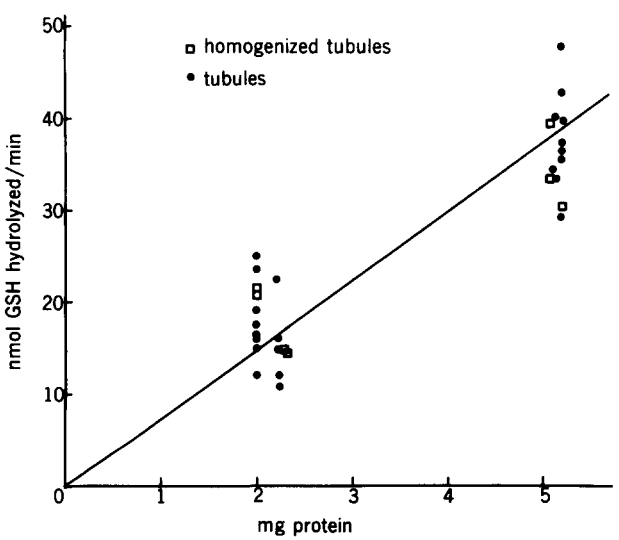

Fig. 2. Cleavage activity of GSH by homogenized tubules and tubule fragments. Conditions as in Fig. 1.

Cleavage activity was proportional to protein content when cortex tubule fragments were used, either intact or as homogenates (Fig. 2); it was also inhibited by L-serine borate (84% at 20 mM). Activity was similar in tubule fragments whether their cytoarchitecture was intact or disrupted by homogenization (Fig. 2, Table I). Cleavage activity in the collagenase-treated tubule preparations was lower than activity in homogenates of whole cortex or slices (Table I) implying that preparation of tubule fragments may impair enzyme activity.

Cleavage activity in cortex slice homogenates was about 30% lower than in whole cortex homogenates, implying that the technique of slice preparation again impairs enzyme activity. Cleavage of GSH during incubation of intact slices was only 28% of that in the homogenized slices with disrupted architecture and was not directly proportional to protein content of the slices themselves. These findings imply restriction of substrate access to γ-glutamyltransferase enzyme in the intact slice.

Maltose cleavage

Cleavage activity measured in whole cortex ho-

TABLE I

GLUTATHIONE CLEAVAGE ACTIVITY IN VARIOUS PREPARATIONS OF RAT RENAL CORTEX

Preparation	*n* [a]	nmol GSH hydrolyzed/mg protein per min [b]	*P*
Whole cortex homogenate	7	17.4±0.6	
Tubule fragments			
Homogenized	7	9.4±0.8	<0.001 [c]
Intact	27	9.8±0.5	N.S. [d]
Thin slices			
Homogenized	6	12.4±2.9	<0.001 [c]
Intact	24	3.5±0.7	<0.005 [d]

[a] Number of experiments; in each experiment at least three replicate observations were obtained.

[b] Cleavage activity was inhibited by L-serine borate (a specific inhibitor of γ-glutamyltransferase activity). It was measured by release, during 30 min incubation, of labelled glycine from L-glutathione substrate (^{3}H-labelled glycine residue) (see Methods).

[c] Compared with whole cortex homogenate

[d] Compared with homogenate of tissue preparation.

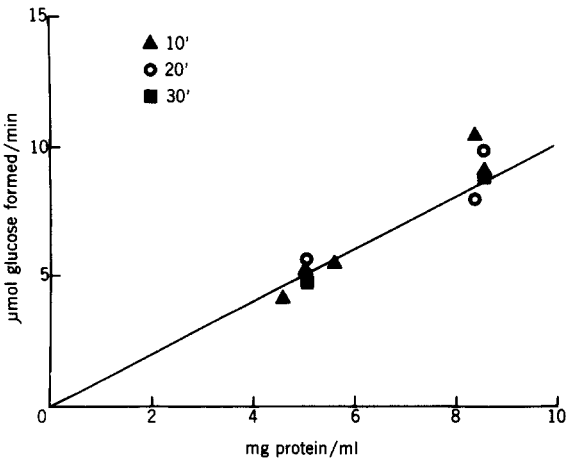

Fig. 3. Maltose cleavage activity by rat renal cortex homogenates at different times and protein concentrations. Incubation was carried out at pH 6 with maltose (0.056 mM); glucose formed was measured using the glucose oxidase reaction.

mogenates was directly proportional to protein content (Fig. 3); activity was also linear with time in the first 30 min; maltase activity was 512 ± 22 nmol glucose formed per mg protein per min. Less than 5% cleavage activity was recovered in supernatant after centrifugation of the homogenate for 60 min at 105000 × g at 4°C.

Cleavage activity was proportional to protein and time in tubule fragments; activity was 884 ± 48 nmol substrate hydrolyzed per mg protein per min (Table II). Thus, activity was 1.7 higher relative to cortex homogenate but protein content (mg/wet weight) of tubule fragments was correspondingly decreased by collagenase treatment; accordingly, increased maltose cleavage by tubule fragments

TABLE II

MALTOSE CLEAVAGE ACTIVITY IN RENAL TISSUE PREPARATION

Preparation	n	nmol maltose hydrolyzed/mg protein per min	P
Whole cortex homogenate	11	512±22	
Tubule fragments	18	884±48	<0.005 [a]
Thin slices	28	162±12	<0.005 [a]

[a] Compared with whole cortex homogenate.

TABLE III

MALTOSE CLEAVAGE ACTIVITY RELEASED INTO MEDIUM DURING SLICE INCUBATION

Preincubation time [a] (min)	Activity in medium (nmol maltose hydrolyzed/mg protein per min) [b]
35	43± 6
50	52±24
65	110±15 [a]

[a] Slices were preincubated for specific time in buffer, removed and activity measured in medium.

[b] Control activity in the intact slice was 162±12 nmol/mg protein per min.

apparently reflects partial purification of maltose activity in the preparation.

Maltose cleavage by the intact slice was low (162 ± 12 nmol glucose formed per mg protein per min) relative to activity in whole cortex homogenate and tubule fragment. Cleavage activity was measurable only after 30 min incubation of the slice and was not proportional to protein content. To test for loss of maltase from the slice into the medium we have incubated intact slices for various intervals at 37°C, then removed them and measured maltose cleavage in the residual medium (Table III). We found 27% of the cleavage activity, initially present in the slice, in the medium at 35 min and 68% at 65 min (assuming control activity to be 162 nmol per mg of protein per min in the intact slice). Accordingly, we presume that the low cleavage activity observed in the intact slice reflects both leakage of enzyme from slice to medium and failure of substrate to penetrate freely to in situ enzyme in brush border.

Discussion

A carrier-mediated concentrative form of amino acid and monosaccharide uptake has long been demonstrated in renal cortex slices [3,15,16]. Whether such uptake occurs predominantly at the basolateral or brush border membrane or both together in the slice is a relevant issue. We used a tripeptide and a disaccharide, whose cleavage to their constituent monomers requires renal brush border enzymes oriented toward the lumen, to

examine the problem of access to the brush border membrane from the peritubular space in vitro. We found substantially less cleavage activity in the intact cortex slice relative to the tubule fragment preparation and homogenate of renal cortex. This finding could indicate that the substrate in the medium has limited access to the luminal membrane in the intact slice and would indirectly imply that basolateral membrane is preferentially exposed. This conclusion would be in keeping with that obtained by the quick-freeze radioautography technique by Wedeen and Weiner [5].

Our findings could also mean only that enzyme (cleavage) activity was impaired by the method of slice preparation. While the latter seems to occur it is nonetheless insufficient to explain the attenuated cleavage of substrate in the intact slice. Homogenization of slices exposes substantial GSH cleavage activity, that is, when slice architecture is disrupted. Slices can also lose brush border located enzyme activity to the medium presumably in shed cells or membranes. In other studies [17] we have demonstrated, by electron microscopy, that brush border membrane integrity deteriorates with vesicle formation during incubation of renal cortex slices; we suggest that these vesicles of detached membrane could be a source of the cleavage activity in the medium in our experiments.

Isolated tubule fragments relative to the slice preparation yield higher cleavage activity toward glutathione and maltose. Moreover, activity is similar in the intact and in homogenized tubule fragments. These findings imply that substrate can gain access to the luminal space in tubule fragments and that brush border and basolateral membranes are both exposed. This conclusion is compatible with morphologic evidence [18,19] that the lumen is patent in proximal tubule fragments.

In the aggregate our studies with glutathione and maltose indicate that the brush border membrane is relatively occluded in the slice preparation; they further imply that the basolateral membrane is preferentially exposed in the slice preparation. Studies with isolated basolateral membrane vesicles indicate that L-amino acids and D-glucose [20] are transported by Na^+-independent mediations in this membrane. However such vesicles do not have a single major orientation and it is moot whether it is the uptake vector (into the cell) or the outward vector that is sodium independent. On the other hand, net concentrative uptake of amino acids and monosaccharides is Na^+-dependent in slices [3,21]; 3-*O*-methyl D-glucose, which is not accumulated by renal cells in situ or in vivo from the basolateral surface [22], is also taken up by the renal cortex slice [21]. From such evidence it could be argued that molecules such as neutral amino acids and D-glucose whose Stokes radii are small relative to glutathione and maltose, might gain access to the occluded lumen of the slice [5] by diffusion through the lateral intercellular space. Certain evidence argues that this explanation is not applicable to all small molecules. Uptake of L-cystine is not impaired in renal cortex slices obtained from cystinuric patients [23]; this Mendelian disorder of cystine transport is clearly identifiable to the brush border membrane [24]. An impairment of phosphate transport, in the murine Mendelian disorder of renal phosphate reabsorption, is confined to the brush border membrane and is expressed in vivo and in vitro in brush border membrane vesicles but not in the slice preparation [25]. Sulfate transport exhibits different characteristics in brush border and basolateral membrane vesicles [26], and those associated with the basolateral membrane predominantly in the slice preparation. Finally, a selective disorder of taurine reabsorption in vivo in a hypertaurinuric strain of mouse is identifiable in the slice preparation but not in brush border membrane vesicles [27]. These various findings in association with the present observations pertaining to glutathione and maltose cleavage imply that slices predominantly expose the basolateral membrane in vitro.

Acknowledgements

The authors wish to thank C. Manzini and C. Laurier for their technical assistance. The authors are also indebted to Drs. P. Vinay and R. Chesney for their advice on tubule preparation. This work was supported by grant MT-2862 (MB) and the Genetics Group Award (CRS) from the Medical Research Council of Canada.

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