Phosphate-independent Glutaminase from Rat Kidney

PARTIAL PURIFICATION AND IDENTITY WITH \(\gamma\)-GLUTAMYLTRANSPEPTIDASE

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SUMMARY

Phosphate-independent glutaminase can be quantitatively solubilized from a microsomal preparation of rat kidney by treatment with papain. Subsequent gel filtration and chromatography on quaternary aminoethyl (QAE)-Sephadex and hydroxylapatite yield a 200-fold purified preparation of this glutaminase. The purified enzyme also hydrolyzes \(\gamma\)-glutamylhydroxamate and exhibits substrate inhibition at high concentrations of either glutamine or \(\gamma\)-glutamylhydroxamate, which is partially relieved by increasing concentrations of maleate. Rat kidney phosphate-independent glutaminase reaction is catalyzed by the same enzyme which catalyzes the \(\gamma\)-glutamyltranspeptidase reaction. The ratio of glutaminase to transpeptidase activities remained constant throughout a 200-fold purification of this enzyme. The observation that the phosphate-independent glutaminase and \(\gamma\)-glutamyltranspeptidase activities exhibit coincident mobilities during electrophoresis, both before and after extensive treatment with neuraminidase, strongly suggests that both reactions are catalyzed by the same enzyme. This conclusion is strengthened by the observation that maleate and various amino acids have reciprocal effects on the two activities. Maleate increases glutaminase activity and blocks transpeptidation, whereas amino acids activate the transpeptidase but inhibit glutaminase activity. In contrast, the addition of both maleate and alanine resulted in a strong inhibition of both activities. Both activities exhibit a similar distribution in the various regions of the kidney. Recovery of maximal activities in the outer stripe region of the medulla is consistent with previous quantitative microanalysis which indicated that this glutaminase activity is localized primarily in the proximal straight tubule cells. The glutaminase and transpeptidase activities have different pH optima. Examination of the product specificity suggests that decreasing pH also promotes glutaminase activity and that below pH 6.0, this enzyme functions strictly as a glutaminase. Because of the localization of this activity on the brush border membrane, these results are consistent with the possibility that the physiological conditions induced by metabolic acidosis

could convert this enzyme from a broad specificity transpeptidase to a glutaminase. Therefore, this enzyme could contribute to the increased renal synthesis of ammonia from glutamine which is observed during metabolic acidosis.

\(\gamma\)-Glutamyltranspeptidase has been extensively purified from hog kidney (1) and from beef kidney (2). It has been characterized as an enzyme of very broad specificity. It can use a variety of \(\gamma\)-glutamyl compounds, such as glutathione or glutamine, as substrates. It transfers a \(\gamma\)-glutamyl moiety to a large number of amino acids and peptides which serve as acceptors. Recent interest in this enzyme has been stimulated by its clinical use as an index of hepatic function (3) and its proposed roles in formation of mercapturic acids (4) and in amino acid transport (5, 6).

If histochemical characterization has indicated that in rat kidney the \(\gamma\)-glutamyltranspeptidase is localized primarily on the brush border membrane of the proximal tubule cells (7, 8). The rat kidney phosphate-independent glutaminase, originally described by Katunuma et al. (9), has recently been characterized as also being located in the brush border membrane (10). This finding prompted us to investigate the possibility that both of these reactions are catalyzed by the same enzyme.

EXPERIMENTAL PROCEDURE

Materials

White male Sprague-Dawley rats (200 to 400 g) were obtained from Zivic Miller and were maintained on Purina rat chow. Glutamic dehydrogenase in 50% glycerol was obtained from Boehringer. Sephadex G-200 and quaternary aminoethyl (QAE)-Sephadex were products of Pharmacia and hydroxylapatite and acrylamide were obtained from Bio-Rad. All other biochemicals were obtained from Sigma.

Methods

Enzyme Assays—Phosphate-independent glutaminase was assayed by measuring the amount of glutamate formed (11). Except where indicated in the text the standard assay conditions (20 mM glutamine, 60 mM maleate, 0.2 mM EDTA, pH 6.6) were used. \(\gamma\)-Glutamyltranspeptidase was assayed (1) by following the appearance of \(p\)-nitroaniline at 25° from a solution containing 5 mM \(\gamma\)-glutamyl-\(p\)-nitroanilide, 0.2 mM EDTA, 30 mM imidazole, pH 7.2. This activity is optimal at slightly higher pH and is activated
by addition of amino acids which serve as acceptors. As described in the text, the assay conditions were modified for various experiments either to take advantage of this greater activity or to investigate the activation. The products formed from \( \gamma \)-glutamyl-p-nitroanilide were characterized by thin layer chromatography. Samples were spotted on silica gel plates (Merek), subjected to chromatography in CHCl \( \text{III} \) and MeOH, the solvent adjusted to pH 7.2 with concentrated NH\( \text{OH} \) and products were detected with ninhydrin. \( \gamma \)-Glutamyltransferase was also assayed by the procedure of Palekar et al. (12) which follows the disappearance of glutathiones using 40 mM alanine as an acceptor amino acid. Alkaline phosphatase was assayed using \( p \)-nitrophenyl phosphate as the substrate (13). The regional localization of the brush border membrane marker activities within the kidney was determined by the procedure of Waldman and Burch (14). For all experiments in this manuscript, enzyme units are expressed as micromoles min\( ^{-1} \). Protein was assayed by the procedure of Lowry et al. (15).

Partial Purification of Phosphate-independent Glutaminase—Except for the initial homogenization, all subsequent steps were carried out in a 50 mM imidazole buffer, pH 7.2. Kidneys from 50 rats (350 to 400 g) were homogenized in 8 volumes of 0.33 M sucrose, 25 mM Tris, 0.2 mM EDTA buffer, pH 7.5, with a Potter-Elvehjem homogenizer. Mitochondria were isolated by differential centrifugation and used as the starting material for purification of phosphate-dependent glutaminase. The post-mitochondrial supernatant was then centrifuged at 20,000 rpm in a Spinco type 21 rotor for 2 hours. The resulting microsomal pellet was resuspended in imidazole buffer and sufficient papain was added to make its concentration 0.25 mg per ml. After incubating at 37\( ^\circ \)C for 3 hours, the microsomal preparation was centrifuged at 45,000 rpm in a Ti 50 rotor for 1 hour. The phosphate-independent glutaminase was quantitatively recovered in the supernatant. The supernatant was then concentrated to 13 ml in a Diaflo apparatus using an XM-50 membrane and applied to a Sephadex G-200 column (4 \( \times \) 100 cm) which had previously been equilibrated with buffer. The fractions containing glutaminase activity were pooled (270 ml) and were applied directly to a QAE-Sephadex column (4 \( \times \) 50 cm). Part of the glutaminase activity washed through the column and the remainder was eluted with 50 mM imidazole-0.1 M KCl buffer, pH 7.2. Sufficient solid ammonium sulfate was then added to combined fractions (400 ml) to make it 65% saturated (39.8 g/100 ml). This was allowed to stand for 15 min at 3\( ^\circ \)C and was then centrifuged at 20,000 \( \times \) g for 10 min. Sufficient solid ammonium sulfate was then added to the supernatant solution to make it 95% saturated (20.5 g/100 ml). After standing for 15 min at 3\( ^\circ \)C, the solution was centrifuged at 20,000 \( \times \) g for 10 min. The resulting pellet was resuspended in 10 ml of buffer and dialyzed for 4 hours against two 1-liter changes of buffer. The sample was then applied to a hydroxylapatite column (2 \( \times \) 25 cm). The column was washed with 200 ml of imidazole buffer and then the glutaminase activity was eluted with a 300-ml linear gradient of potassium phosphate (0 to 0.15 M) in buffer. The phosphate-independent glutaminase was again concentrated by collecting the precipitate which formed between 65 to 95% saturation with ammonium sulfate, resuspending with 10 ml of buffer and dialyzing extensively.

Electrophoresis—Polyacrylamide gel electrophoresis was carried out by the procedure of Orban (16) and Davis (17) using 5% acrylamide gels. Protein was stained with Coomassie blue and a duplicate gel was assayed for enzymatic activities. In order to avoid artifacts due to distortion of the gel used for activity assays, this gel was first floated in water in a shallow tray. Then, the water was drained off slowly and the tray was frozen in a -60\( ^\circ \)C freezer. The frozen gel was then sliced into 1.3-mm slices with a gel slicer. Sequential gel slices were incubated in 100 \( \mu \)l of 50 mM imidazole, pH 7.2, overnight at 4\( ^\circ \)C. Aliquots of each sample were then assayed for enzymatic activities. About 50% of the activities applied to the gels were recovered. In order to remove sialic acid, 5 \( \mu \)g of neuraminidase (Worthington, 0.6 \( \mu \)mol min\( ^{-1} \) mg\( ^{-1} \)) were added to 0.5 mg of purified phosphate-independent glutaminase, and the mixture was dialyzed overnight against 200 ml of sodium acetate buffer, pH 5.0. Assays with thiobarbituric acid (18), showed that this treatment reduced the sialic acid content of this glutaminase preparation from 1.2% to less than 0.1%. This procedure had no effect on the total phosphate independent glutaminase activity.

RESULTS

Purification and Properties—Phosphate-independent glutaminase can be selectively released from intact kidney cells by treatment with papain (10). It was found that this procedure also solubilizes the enzyme from kidney microsomal preparations. The solubilized enzyme was relatively free of lipid and could be purified further without addition of detergents. The results of the purification scheme are shown in Table I. About 30 mg of 200-fold purified enzyme was obtained from 50 rats. This represents approximately 25% recovery of initial activity.

Katnuma et al. reports that the phosphate-independent glutaminase is strongly activated by maleate (9). With the purified glutaminase, the addition of saturating amounts of maleate causes a 7-fold increase in the rate of glutamate formation from glutamine (Fig. 1). The phosphate-independent glutaminase preparation hydrolyzes \( \gamma \)-glutamyl hydroxamate at about one-half the rate at which it hydrolyzes glutamine. This reaction is also activated strongly by maleate. The half-maximal activation of both reactions occurred at 12 mM maleate. The effect of increasing glutamine concentration at different maleate concentrations, is shown in Fig. 2. In the absence of maleate, the phosphate-independent glutaminase activity exhibited strong substrate inhibition. At all levels of maleate tested, substrate inhibition was still observed, but increasing concentrations of maleate delayed the onset of substrate inhibition.

### Table I

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume</th>
<th>Protein</th>
<th>Activity</th>
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</thead>
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<tr>
<td>Crude homogenate</td>
<td>1250</td>
<td>25,700</td>
<td>1620</td>
<td>0.063</td>
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<tr>
<td>Microsomes</td>
<td>155</td>
<td>4,050</td>
<td>990</td>
<td>0.24</td>
</tr>
<tr>
<td>Papain treatment</td>
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<td>1,060</td>
<td>980</td>
<td>0.92</td>
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<td>Diaflo concentrate</td>
<td>13</td>
<td>400</td>
<td>960</td>
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<tr>
<td>QAE-Sephadex column</td>
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<td>170</td>
<td>820</td>
<td>4.8</td>
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<td>Hydroxylapatite column</td>
<td>400</td>
<td>46</td>
<td>500</td>
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Fig. 1 (left). Maleate activation of phosphate-independent glutaminase activity. The assays were carried out at pH 7.2 in the presence of 5 mM glutamine (GLN) or 5 mM \( \gamma \)-glutamyl hydroxamate (GLUNHOF) as substrate. Glutaminase activity is expressed as micromoles min\( ^{-1} \) ml\( ^{-1} \).

Fig. 2 (right). The effect of maleate on the substrate inhibition of phosphate-independent glutaminase activity. The assays were carried out at pH 7.2 in the presence of the indicated maleate concentrations. Glutaminase activity is expressed as micromoles min\( ^{-1} \) ml\( ^{-1} \).
Comparison of rates of ammonia and glutamate synthesis from glutamine by purified phosphate-independent glutaminase

The rate of glutamate formation was determined by adding enzyme to 100 μL of a solution containing 50 mM imidazole, 20 mM α-ketoglutarate, 0.2 mM EDTA, 0.25 mM ADP, and the indicated amounts of glutamine and maleate adjusted to pH 7.2 and incubated at 20°C. After 10 min, the reaction was stopped by addition of 10 μL of 2 N HCl, and then glutamate was determined as described previously (9). The rate of ammonia formation was determined under the same conditions except that the assay mixture also contained 0.15 mM DPNH and 250 μg per ml of glutamic dehydrogenase. The complete mixture was preincubated for 10 min to remove endogenous ammonia. Then enzyme was added and the linear decrease in absorbance at 340 nm was recorded. Data was corrected for a slight, nonenzymatic ammonia formation that occurred in a sample in which glutaminase was omitted.

Table II. Comparison of rates of ammonia and glutamate synthesis from glutamine by purified phosphate-independent glutaminase

<table>
<thead>
<tr>
<th>Condition</th>
<th>Rate of product formation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glutamate</td>
</tr>
<tr>
<td>1. 5 mM glutamine</td>
<td>4.3</td>
</tr>
<tr>
<td>2. 5 mM glutamine + 60 mM maleate</td>
<td>22.4</td>
</tr>
<tr>
<td>3. 20 mM glutamine</td>
<td>3.3</td>
</tr>
<tr>
<td>4. 20 mM glutamine + 60 mM maleate</td>
<td>24.6</td>
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</table>

Comparison of phosphate-independent glutaminase and γ-glutamyltranspeptidase activities

<table>
<thead>
<tr>
<th>Step</th>
<th>Phosphate-independent glutaminase</th>
<th>γ-glutamyltranspeptidase assayed with γ-glutamyl-p-nitroanilide</th>
<th>γ-Glutamyltranspeptidase assayed with glutathione</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity</td>
<td>Specific activity</td>
<td>Specific activity</td>
</tr>
<tr>
<td></td>
<td>μmol min⁻¹ mg⁻¹</td>
<td>μmol min⁻¹ mg⁻¹</td>
<td>μmol min⁻¹ mg⁻¹</td>
</tr>
<tr>
<td>Crude homogenate</td>
<td>0.063</td>
<td>0.084 (1.3)*</td>
<td>1.3 (20)</td>
</tr>
<tr>
<td>Heated microsomes</td>
<td>0.32</td>
<td>0.41 (1.3)</td>
<td>0.6 (19)</td>
</tr>
<tr>
<td>Purified preparation</td>
<td>12.7</td>
<td>16.5 (1.3)</td>
<td>203 (16)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are the ratio of γ-glutamyltranspeptidase to glutaminase activity.

Fig. 3. Elution of phosphate independent glutaminase and γ-glutamyltranspeptidase activities from polyacrylamide gels. The purified preparation of phosphate-independent glutaminase was subjected to polyacrylamide gel electrophoresis before (A) and after (B) extensive treatment with neuraminidase. One gel was stained for protein and a duplicate gel was sliced and assayed for both glutaminase and γ-glutamyltranspeptidase activities. γ-Glutamyltranspeptidase was assayed with 5 mM γ-glutamyl-p-nitroanilide in the presence of 40 mM methionine at pH 8.4. Both activities are expressed as 10⁴ μmol m⁻¹ min⁻¹ slice⁻¹.

As shown in Fig. 3A, polyacrylamide gel electrophoresis of the purified phosphate-independent glutaminase yields one major protein band, preceded by a region of diffuse staining. Analysis of an identical gel indicated that the phosphate-independent glutaminase and γ-glutamyltranspeptidase activities migrated as a single coincident band, which corresponded to the region which stained diffusely for protein. Following treatment with neuraminidase to extensively remove sialic acid, the phosphate-independent glutaminase preparation was again subjected to polyacrylamide gel electrophoresis (Fig. 3B). The major protein

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N. P. Curthoys, unpublished results.
band migrated slightly slower, but at this time the diffusely stained region was replaced by three bands of significantly slower mobility. Again the phosphate-independent glutaminase and $\gamma$-glutamyltranspeptidase activities exhibited a coincident migration but now they peaked in the region of the three slowly moving bands.

The chromatogram shown in Fig. 4 indicates that maleate also affects the product specificity with $\gamma$-glutamyl-$p$-nitroanilide as a substrate. In the absence of maleate, the major product formed migrates more slowly than $\gamma$-glutamyl-$p$-nitroanilide and is probably $\gamma$-glutamyl-$\gamma$-glutamyl-$p$-nitroanilide. But, in the presence of maleate, the major product is glutamate. Addition of maleate has the same effect if the reaction is carried out in the presence of methionine or alanine, it inhibits the formation of dipeptides and promotes glutamate formation. Therefore, maleate binds to this enzyme and blocks transpeptidation, making it a specific glutaminase.

If maleate promotes glutaminase activity by blocking transpeptidation then maleate should also inhibit amino acid activation of the transpeptidase reaction. As shown in Fig. 5, the addition of increasing concentrations of amino acids results in increasing $\gamma$-glutamyltranspeptidase activity of this enzyme preparation. The addition of 100 mM alanine or glycine causes approximately a 2-fold activation, whereas the presence of 100 mM methionine causes a 5-fold activation. In the absence of any amino acids, the addition of 60 mM maleate caused a slight increase in the rate of $p$-nitroaniline formation. As shown in the previous figure, the other product formed under the latter conditions was glutamate. The addition of increasing concentrations of methionine in the presence of maleate still resulted in a slight activation. But, the presence of maleate greatly reduced the degree of activation observed. In contrast, the presence of maleate not only blocked activation by glycine or alanine, the addition of increasing concentrations of these two amino acids resulted in a decrease in the rate of $p$-nitroaniline formation.

As shown in Fig. 6, the addition of amino acids has a reciprocal effect on glutaminase activity (glutamate formation from glutamine). In the absence of maleate, the phosphate-independent glutaminase is inhibited by increasing concentrations of either alanine, glycine, or methionine. The effect of increasing amino acids is probably similar to that of increasing glutamine concentration. In the presence of 60 mM maleate, the three amino acids still act as inhibitors of glutaminase activity and the degree of inhibition is similar to that observed with $\gamma$-glutamyl-$p$-nitroanilide as substrate. Increasing concentrations of methionine result in only a slight inhibition. Glycine produces a greater inhibition, but alanine is a very potent inhibitor of glutaminase activity. The fact that these amino acids show the same order of potency as inhibitors with either glutamine or $\gamma$-glutamyl-$p$-nitroanilide as substrate supports the conclusion that both reactions are catalyzed by the same enzyme. However, an understanding of why glycine and alanine are such potent inhibitors in the presence of maleate will require further investigation.

Previous reports (7, 8), using histological staining have indicated that the $\gamma$-glutamyltranspeptidase is localized in the brush border membrane of the proximal convoluted tubules in the rat kidney. In contrast, the quantitative microanalysis of Curthoys and Lowry (19) has shown that the phosphate-independent glutaminase is localized primarily in the proximal straight tubules. This represents the only inconsistency, which we could find in the literature, suggesting that the two reactions are not catalyzed by the same enzyme. To investigate this apparent difference in localization, activities of various brush border marker enzymes were assayed in the various regions of kidney tissue (Fig. 7). A close correlation between phosphate inde-
FIG. 7. Regional distribution of brush border membrane marker activities in rat kidney. A cone of kidney tissue was cut in such a way that its base consisted solely of cortical tissue and its apex consisted solely of papillary region. Progression from the base of the cone to its apex was associated with progression from cortex, through outer stripe and inner stripe regions of medulla, and finally into papillary region. Consecutive slices were then cut from the base of the cone, homogenized in 0.33 M sucrose, 25 mM Tris, and 0.2 mM EDTA buffer, pH 7.5, and assayed for phosphate-independent glutaminase (PIG), γ-glutamyltranspeptidase (γGT), and alkaline phosphatase (Al. Phos) activities, and for protein concentration. Specific activities are expressed as micromoles min⁻¹ mg⁻¹.

FIG. 8. pH profiles of glutaminase and γ-glutamyltranspeptidase activities. Glutaminase activities were determined by measuring glutamate formation from 20 mM glutamine either in the absence (open figures) or presence (half-shaded figures) of 60 mM maleate. γ-Glutamyltranspeptidase activity was determined by measuring p-nitroaniline formation from 50 mM γ-glutamyl-p-nitroanilide in the presence of 40 mM methionine (shaded figures). pH was maintained by using the following buffers: 50 mM imidazole (circles), 50 mM piperazine (triangles), 50 mM Tris (squares), or 50 mM bicarbonate (X). Enzyme activities are expressed as micromoles min⁻¹ ml⁻¹.

FIG. 9. Effect of pH on product specificity of purified phosphate-independent glutaminase using γ-glutamyl-p-nitroanilide as substrate. Sufficient enzyme was added to 100 μl of 5 mM γ-glutamyl-p-nitroanilide at each of the various pH values so that when the reaction was stopped with acid about 50% of the substrate was converted to product. pH was maintained with either 50 mM imidazole (pH 8.0 to 6.5) or 50 mM piperazine (pH 6.5 to 5.0) buffers. Unreacted γ-glutamyl-p-nitroanilide (γGpNA) and 5 mM glutamate (GLU) were spotted as standards. In all positions, 10 μl of sample were spotted. Chromatography was carried out as described under “Experimental Procedure.”

In contrast, the γ-glutamyltranspeptidase activity, assayed with γ-glutamyl-p-nitroanilide in the presence of 40 mM methionine, exhibits maximal activity at pH 8.6. If an amino acid is not added, a similar profile, but with the activity reduced 3-fold, is observed. Using γ-glutamyl-p-nitroanilide as a substrate, and following only the appearance of p-nitroaniline, one cannot distinguish whether the enzyme is catalyzing a glutaminase- or transpeptidase-type reaction. Comparison of the pH profiles suggests that a decrease in pH may promote glutaminase activity over transpeptidation. Chromatographic analysis of products formed from γ-glutamyl-p-nitroanilide as a function of pH is shown in Fig. 9. From pH 8.0 to 7.0, the enzyme functions primarily as a transpeptidase (the major product formed is probably γ-glutamyl-γ-glutamyl-p-nitroanilide). With decreasing pH the percent formation of this product decreases and an increasing amount of glutamate is formed. Below pH 6.0, this enzyme preparation functions exclusively as a glutaminase.

DISCUSSION

The phosphate-independent glutaminase activity is extremely stable. A dilute preparation (less than 1 mg per ml) of the purified phosphate-independent glutaminase has been stored at 4°C for 6 months without any loss of activity. All of the chromatographic steps during the purification are conducted at room temperature without encountering any sizable loss of activity. The enzyme is also extremely resistant to inactivation by papain. Rat kidney microsomal preparations can be incubated up to 16 hours in a solution containing 0.25 mg per ml of papain.
without any loss of activity. The enzyme solubilized by this procedure exhibits the same kinetic properties in terms of glutamine saturation and maleate activation as the particulate enzyme. These observations suggest that papain does not cause any proteolytic degradation in the active site region of the phosphate-independent glutaminase.

Polyacrylamide gel electrophoresis shows that the preparation of phosphate-independent glutaminase is not pure. But, neuraminidase treatment of the glutaminase preparation decreases its mobility on polyacrylamide gels from an $R_f$ of 0.25 to one of 0.10. This is consistent with the removal of negatively charged sialic acid residues and strongly suggests that this enzyme is a glycoprotein. Staining of duplicate gels for protein with Coomassie blue and for carbohydrate with periodic-acid Schiff reagent (20) produces identical banding patterns; this result suggests that the major contaminating protein is also a glycoprotein.

The finding that the phosphate-independent glutaminase exhibits substrate inhibition at high glutamine concentrations suggests that this enzyme contains more than one site which binds glutamine. It appears that maleate increases glutaminase activity by preventing substrate inhibition and by altering the product specificity. These observations, along with the ability of this glutaminase to hydrolyze $\gamma$-glutamylhydroxamate and its subcellular localization (10), suggested to us that the phosphate-independent glutaminase could be a partial reaction catalyzed by $\gamma$-glutamyltranspeptidase.

The observation that the ratio of $\gamma$-glutamyltranspeptidase to phosphate-independent glutaminase activities remains constant throughout a 200-fold purification and that these two activities exhibit coincident banding on polyacrylamide gels, both before and after extensive treatment with neuraminidase, strongly suggests that these two activities are catalyzed by the same enzyme. This conclusion is strengthened by the observation that maleate and various amino acids have reciprocal effects on the two activities. Maleate increases glutaminase activity and blocks transpeptidation, whereas, amino acids activate the transpeptidase but inhibit glutaminase activity.

The regional distribution for alkaline phosphatase activity in both cortical and outer stripe regions is consistent with histological staining for this activity (21); this observation suggests that it is localized in the brush border membrane of both the proximal convoluted and proximal straight tubule cells. In contrast, the results of the regional distribution analysis of the $\gamma$-glutamyltranspeptidase are not consistent with its reported histological localization in the proximal convoluted tubule brush border membrane (7, 8). The specific activity of both the phosphate-independent glutaminase and the $\gamma$-glutamyltranspeptidase are greatest in the outer stripe region. This observation is consistent with the quantitative microanalysis of the distribution of this glutaminase activity (19). When assayed in individually dissected tubular structures, the phosphate-independent glutaminase activity was found to be 10-fold greater in the proximal straight tubule cells than in any of the other structures of the kidney nephron.

In addition to the numerous functions already proposed (3–6), the phosphate-independent glutaminase-$\gamma$-glutamyltranspeptidase could also contribute to increased renal ammonia synthesis during metabolic acidosis. In the rat kidney, the largest proportion of the acidification of the urine occurs in the proximal convoluted tubule cell (22). Micropuncture studies in normal rats indicate that the fluid in the lumen at the end of the proximal convoluted tubule cell has a pH of about 6.8 (23). During metabolic acidosis, the decreased bicarbonate concentration in the glomerular filtrate greatly facilitates acidification of the fluid in the proximal convoluted tubule. Under these conditions, the fluid entering the lumen of the proximal straight tubule is close to pH 6.0. The phosphate-independent glutaminase appears to be localized on the external surface of the brush border membrane of these cells (10). Therefore, this fluid constitutes the physiological medium in which this enzyme functions. Examination of the products formed from $\gamma$-glutamyl-p-nitroanilide indicates that pH values in this range promote glutaminase activity, and that below pH 6.0 this enzyme functions strictly as a glutaminase. Therefore, acidification of the fluid in the tubular lumen during acidosis may convert this enzyme from a transpeptidase to a glutaminase.

Maleate also appears to alter the product specificity of this enzyme; it increases glutaminase activity and blocks transpeptidation. In contrast, alanine activates transpeptidation but inhibits glutaminase activity. The addition of both maleate and alanine causes a dramatic inhibition of both glutaminase and transpeptidase activities; this finding indicates that the enzyme has separated binding sites for both of these modulators. If maleate had affected the enzyme activity by only competing with amino acid binding at the acceptor site, it would be difficult to explain why addition of both of these modulators results in an effect different from that obtained by the addition of either separately. It is unlikely that maleate is a physiological activator of this glutaminase activity. But, if maleate binding occurs at a distinct site on the enzyme, this would suggest the possibility of a physiological counterpart to maleate. Katunuma et al. (24) have reported the extraction of a kidney factor which stimulates rat kidney phosphate-independent glutaminase activity and recently Alleyne and Roobol (25) have demonstrated the presence of a factor in serum of acutely acidotic rats which stimulates ammonia synthesis from glutamine in kidney slices. The appearance of such a factor in the tubular lumen in response to acidosis could convert $\gamma$-glutamyltranspeptidase into a specific glutaminase. Therefore, the possibility that the phosphate-independent glutaminase-$\gamma$-glutamyltranspeptidase enzyme may contribute to renal ammonia synthesis in response to metabolic acidosis warrants further investigation.

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Phosphate-independent glutaminase from rat kidney. Partial purification and identity with gamma-glutamyltranspeptidase.

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