The synthesis of $\gamma$-glutamylhydroxamic acid from glutamine and hydroxylamine is catalyzed by glutamine synthetase in the presence of adenosine diphosphate, inorganic phosphate, and magnesium ions (1), and also by other enzyme preparations (2, 3) that do not exhibit a requirement for nucleotides or metal ions. The synthesis of $\gamma$-glutamylhydroxamic acid from glutamic acid and hydroxylamine that is catalyzed by glutamine synthetase requires both magnesium (or manganese) ions and amounts of ATP that are stoichiometric with those of the $\gamma$-glutamylhydroxamic acid formed. In all these reactions, it appears that hydroxylamine reacts with the enzymatically activated $\gamma$-carboxyl group of glutamic acid; this conclusion follows from the important work of Lipmann and Tuttle (4), who first used the formation of hydroxamic acids as an indication and measure of carboxyl group activation.

The present communication describes the enzymatic synthesis of $\gamma$-glutamylhydroxamic acid from glutamic acid and hydroxylamine according to Equation 1.

$$\text{Glutamic acid} + \text{NH}_2\text{OH} = \gamma\text{-glutamylhydroxamic acid} + \text{H}_2\text{O} \quad (1)$$

The reaction requires neither ATP nor added metal ions. The enzyme that catalyzes this reaction has been purified from a bacterial organism; it also catalyzes the hydrolysis of several amides. The apparent equilibrium constant for Equation 1 is not far from unity, and the data indicate that the standard free energy of hydrolysis of $\gamma$-glutamylhydroxamic acid is considerably less than that of glutamine. This conclusion is consistent with studies on the synthesis of $\gamma$-glutamylhydroxamic acid by glutamine synthetase. Additional observations bearing on the properties and mechanism of action of this enzyme are also described in this paper.

**EXPERIMENTAL PROCEDURE**

**Materials**—Uniformly labeled $^{14}$C-l-glutamic acid was obtained from the New England Nuclear Corporation and purified by paper chromatography in butanol-acetic acid-water (4:1:1) and paper electrophoresis at pH 5.5 (5). L-Glutamic acid, L-glutamine, and streptomycin sulfate were obtained from Mann Research Laboratories, Inc. The other amino acids and amides were obtained as previously reported (2). The authors are indebted to Dr. John M. Buchanan for a supply of $\alpha$-amino-$\delta$-serine and $6\text{-diaz}-5\text{-oxo-}\text{l}$-norleucine.

The authors are indebted to the National Institutes of Health, Public Health Service, and the National Science Foundation for generous support of this research.

**Methods**—Protein was determined as described by Lowry et al. (6). Quantitative determinations of ammonia were performed after separation by aeration (7). Absorbance measurements were made with a Weston colorimeter.

**Determination of Enzyme Activity**—The formation of $\gamma$-glutamylhydroxamic acid from glutamine was determined in reaction mixtures consisting of $\gamma$-glutamine (5 $\mu$moles), hydroxylamine (500 $\mu$moles; prepared by neutralizing $\text{NH}_4\text{OH}\cdot\text{HCl}$ with $\text{NaOH}$), imidazole- HCl buffer (pH 7.2; 50 $\mu$moles), and enzyme in a final volume of 1.0 ml. After incubation for 10 minutes at $37^\circ$, 1.5 ml of a solution containing 0.37 $M$ ferric chloride, 0.67 $M$ HCl, and 0.2 $M$ trichloroacetic acid were added; after removal of the precipitated protein by centrifugation, the absorbance of the iron $\gamma$-glutamylhydroxamic acid complex was measured at 535 $\mu$m against a reference solution prepared in a similar manner from a reaction mixture lacking enzyme. An amount of enzyme was used that catalyzed the formation of no more than 2 $\mu$moles of $\gamma$-glutamylhydroxamic acid (4, 0.640) under the conditions of assay. A unit of enzyme is defined as the amount that catalyzes the formation of 1 $\mu$ mole of $\gamma$-glutamylhydroxamic acid under these conditions. Specific activity is given in units per mg of protein.

The formation of $\gamma$-glutamylhydroxamic acid from glutamic acid was determined under the same conditions except that l-glutamic acid (50 $\mu$moles) was used in place of l-glutamine.

**Isolation of Azobacter agile**—One gram of moist soil obtained from the Boston Public Garden was shaken at 26° for 2 days with 10 ml of the medium described below. Approximately 0.01 ml of this mixture was transferred to a flask containing 10 ml of medium which was shaken for 48 hours, and then two additional successive transfers were made under sterile conditions. The culture was plated in serial dilution on solid medium containing 1.5% agar. A pure culture of a gram-negative rod was obtained, which was identified by Sias Laboratories, Brooks Hospital, Brookline, Massachusetts, as Azobacter agile. The organism was maintained by weekly transfer and stored at 3° on solid medium. Large amounts of the organism were grown in 20-liter carboys containing 10 liters of medium inoculated with 100 ml of a 24-hour culture; growth was at 26° for 20 hours with vigorous aeration. The organisms were harvested with a Sharples centrifuge, washed three times with distilled water, and then lyophilized. The yield of dry cells from 40 liters of medium varied between 20 and 40 g.

The medium contained $\text{KH}_2\text{PO}_4$ (5.5 $\times 10^{-3} M$), $\text{Na}_2\text{HPO}_4$ (3.5 $\times 10^{-2} M$), $\text{MgSO}_4$ (6.1 $\times 10^{-4} M$), $\text{CaCl}_2$ (2.2 $\times 10^{-2} M$), $\text{FeCl}_3$ (1.5 $\times 10^{-9} M$), $\text{MnCl}_2$ (1.52 $\times 10^{-5} M$), $\text{Na}_2\text{MoO}_4$ (4.9 $\times 10^{-6} M$), $\text{NH}_2\text{NO}_3$ (0.03 M), l-glutamic acid (0.034 M), and yeast.
extract (0.01%); the medium was prepared in tap water and adjusted to pH 6.8 by addition of KOH.

**Purification of Enzyme—**Two grams of dried organisms were homogenized for 20 minutes in a Virtis "45" homogenizer at 0° with 30 g of acid-washed glass beads and 20 ml of 0.1 M 2-amino-2-(hydroxymethyl)-1,3-propanediol-HCl buffer (pH 6.8). The glass beads were allowed to settle, and the supernatant solution was separated by decantation; the glass beads were washed successively with 10–10 ml portions of 0.01 M buffer. The combined supernatant solution and washings from three such homogenates were centrifuged for 30 minutes at 27,000 × g. This and all subsequent steps were performed at 0–4°. The supernatant solution was treated dropwise with 1/16 of its volume of a 30% solution of streptomycin sulfate. The mixture was heated with stirring in a test tube (suspended in a water bath at 70°) until the temperature of the protein solution increased to 75% of saturation; after being stirred for 15 minutes, the precipitated protein was collected by centrifugation. The precipitate was dissolved in 2.0 ml of 0.02 M sodium phosphate buffer (pH 6.8), and this solution was successively dialyzed for 2-hour periods against two batches of 2 liters of the same buffer. This preparation of the enzyme was used in the studies reported here; it was stored at 0° or frozen at −20°. No significant loss of activity was observed with enzyme preparations that were stored for several months. A representative purification is described in Table I. A number of unsuccessful attempts were made to purify the enzyme further; in these efforts, conventional procedures involving the use of calcium phosphate gel, alumina C•Y gel, hydroxyapatite, diethylaminoethyl cellulose, and carboxymethyl cellulose were employed.

The enzyme preparation exhibited three components when examined in the analytical ultracentrifuge.1 Sedimentation of the enzyme in a sucrose gradient (8) indicated that enzymatic activity was associated only with one component, which was estimated to have a sedimentation coefficient in the range of 4.4 to 4.8 S.

**RESULTS**

**Formation of γ-Glutamylhydrazodic Acid from L-Glutamine—**Study of the enzymatic formation of 7-glutamylhydrazodic acid from L-glutamine and hydroxylamine indicated that the course of the reaction was linear with time until virtually all of the glutamine was converted to γ-glutamylhydrazodic acid. The initial velocity of the reaction was directly proportional to the concentration of enzyme. No significant differences were observed between studies carried out in imidazole-HCl and potassium phosphate buffers. The reaction velocity was approximately 25% greater in 1 M sodium chloride than in 0.05 M sodium fluoride, or 0.01 M ethylenediaminetetraacetic acid. The enzyme was not activated by 2-mercaptoethanol (0.001 to 0.025 M).

The pH optimum for the formation of γ-glutamylhydrazodic acid from L-glutamine and hydroxylamine was in the range of 0 to 7 (Fig. 1). The $K_m$ values for L-glutamine and hydroxylamine were, respectively, $1.0 \times 10^{-4}$ and 0.2 M. Studies with a large number of amides indicated that, under the conditions employed, only L-glutamine, D-glutamine, L-asparagine, D-asparagine, and succinamic acid were active in hydrazodic acid formation (Table II).

The enzyme also catalyzed the hydrolysis of L-glutamine to complete. The initial rates of hydrolysis exhibited little change over the pH range 4.5 to 8 (Fig. 1). The $K_m$ value for L-glutamine was $1.0 \times 10^{-4}$ M. The rate of hydrolysis of L-glutamine was about the same as the rate of hydrazodic acid formation from this amide at pH 7.2. d-Glutamine, L-asparagine, and D-asparagine were hydrolyzed more slowly than L-glutamine (Table II).

**Formation of γ-Glutamylhydrazodic Acid from L-Glutamic Acid—**In the course of these studies, it was observed that incubation of the enzyme with L-glutamic acid and hydroxylamine led to significant synthesis of γ-glutamylhydrazodic acid. This reaction exhibited optimal velocity in the pH range 5.5 to 6.5 (Fig. 1). At pH 7.2, the rate of hydrazodic acid formation from L-glutamic acid was approximately 16% of that from L-glutamine (Table II). The $K_m$ values for L-glutamic acid were very much lower than those for L-glutamine (Table II).

1 We are indebted to Dr. Rudy H. Haschemeyer for these studies.
and hydroxylamine were, respectively, $8 \times 10^{-3}$ and $6 \times 10^{-2}$ M. The synthesis of $\gamma$-glutamylhydroxamic acid from $\lambda$-glutamic acid and hydroxylamine (in contrast to the comparable reaction with $\kappa$-glutamine) did not go to completion (Fig. 2B). Since

![Graph 1](image1.png)

**Fig. 1.** pH dependence of the formation of $\gamma$-glutamylhydroxamic acid and of the hydrolysis of glutamine. In Curve 1, the reaction mixtures consisted of $\lambda$-glutamine (5 $\mu$moles), hydroxylamine (500 $\mu$moles), potassium phosphate (100 $\mu$moles), and enzyme (1.7 units), in a final volume of 1.0 ml. In Curve 2, the reaction mixtures consisted of $\lambda$-glutamine (20 $\mu$moles), potassium phosphate (100 $\mu$moles), and enzyme (1.5 units), in a final volume of 1.0 ml. In Curve 3, the reaction mixtures consisted of $\lambda$-glutamic acid (50 $\mu$moles), hydroxylamine (500 $\mu$moles), potassium phosphate (100 $\mu$moles), and enzyme (4.2 units), in a final volume of 1.0 ml. The reaction mixtures were incubated at 37° for 10 minutes. The hydroxylamine and phosphate solutions were adjusted to the indicated values of pH, which were checked in the reaction mixtures before and after incubation with a glass electrode.

**TABLE II**

Relative rates of hydroxamic acid formation and of amide hydrolysis

<table>
<thead>
<tr>
<th>Substrate*</th>
<th>Hydroxamic acid formation</th>
<th>Hydrolysis of amide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$moles/10 min</td>
<td>$\mu$moles NH$_2$/10 min</td>
</tr>
<tr>
<td>$\lambda$-Glutamine</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>$\delta$-Glutamine</td>
<td>0.59</td>
<td>0.57</td>
</tr>
<tr>
<td>$\lambda$-Asparagine</td>
<td>0.61</td>
<td>0.70</td>
</tr>
<tr>
<td>$\delta$-Asparagine</td>
<td>0.20</td>
<td>0.24</td>
</tr>
<tr>
<td>Sucinate acid</td>
<td>0.17</td>
<td>0.15</td>
</tr>
<tr>
<td>$\lambda$-Glutamic acid</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>$\delta$-Glutamic acid</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$\lambda$-Aspartic acid</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*The following compounds were inactive in enzymatic hydroxamic acid formation: acetamide, propionamide, butyramide, isobutyramide, isovaleramide, caproamide, oxamic acid, malonamide acid, glutaramic acid, adipamic acid, oxamide, malonamide, succinamide, succinimide, glutaramide, adipamide, $\lambda$-glutamic acid $\gamma$-ethylamide, $\lambda$-glutamic acid $\gamma$-ethyl ester, $\lambda$-tyrosine amide, $\lambda$-phenylalanine amide, $\lambda$-leucine amide, glycine ethyl ester, $\lambda$-isoasparagine, and $\lambda$-homoglutamine.

![Graph 2](image2.png)

**Fig. 2 (above).** Reversible synthesis of $\gamma$-glutamylhydroxamic acid from $\lambda$-glutamic acid and hydroxylamine. In Curve A, the reaction mixtures consisted of $\lambda$-$\gamma$-glutamylhydroxamic acid (2 $\mu$moles), hydroxylamine (500 $\mu$moles), and enzyme (10 units), in a final volume of 1.0 ml; temperature, 37°; pH 7.2. In Curve B, the reaction mixtures consisted of $\lambda$-glutamic acid (5 $\mu$moles), hydroxylamine (300 $\mu$moles), imidazole-HCl buffer (50 $\mu$moles), and enzyme (10 units) in a final volume of 1.0 ml; temperature, 37°; pH 7.2.

![Graph 3](image3.png)

**Fig. 3 (below).** Enzymatic reaction of $\lambda$-glutamine with hydroxylamine. The reaction mixtures consisted of $\lambda$-glutamine (2 $\mu$moles), hydroxylamine (800 $\mu$moles), imidazole-HCl buffer (50 $\mu$moles), and enzyme (8 units) in a final volume of 1.0 ml; temperature, 37°; pH 7.2.

the enzyme also catalyzed the hydrolysis of $\lambda$-$\gamma$-glutamylhydroxamic acid (Fig. 2A), it was possible to approach equilibrium from both directions. The experimental data (Table III) lead to an apparent equilibrium constant

$$K = \frac{[\text{glutamic acid}][\text{hydroxylamine}]}{[\text{glutamylhydroxamic acid}]}$$

at pH 7.2 and 37° of between 0.24 and 0.43 liter per mole. From these data it may be calculated that the apparent free energy change associated with the reaction is about 700 calories per mole.

When the synthesis of $\gamma$-glutamylhydroxamic acid from $\lambda$-glutamine and hydroxylamine was performed with a relatively large quantity of enzyme and followed over a period of time, it was observed that the initial rapid formation of $\gamma$-glutamylhydroxamic acid is followed by its slower hydrolysis (Fig. 3). The reaction reached equilibrium, and calculation of an apparent equilibrium constant from these data gave a value of 0.3 liter per mole in good agreement with the values given in Table III.

**Evidence for Binding of Glutamic Acid to Enzyme—** An attempt was made to obtain evidence for the binding of glutamic acid...
TABLE III

Equilibrium of formation of γ-glutamylhydroxamic acid from L-glutamic acid and hydroxylamine

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Glutamic acid</th>
<th>NH₄OH</th>
<th>γ-Glutamylhydroxamic acid</th>
<th>Glutamic acid</th>
<th>NH₄OH</th>
<th>γ-Glutamylhydroxamic acid</th>
<th>Kₑq</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol</td>
<td>mmol</td>
<td>mmol</td>
<td>mmol</td>
<td>mmol</td>
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</tr>
<tr>
<td>1</td>
<td>5</td>
<td>300</td>
<td>0</td>
<td>4.55</td>
<td>299.5</td>
<td>0.450</td>
<td>0.33</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>500</td>
<td>0</td>
<td>4.36</td>
<td>499.4</td>
<td>0.645</td>
<td>0.30</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>800</td>
<td>0</td>
<td>4.15</td>
<td>799.1</td>
<td>0.855</td>
<td>0.26</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>800</td>
<td>0</td>
<td>1.67</td>
<td>790.7</td>
<td>0.323</td>
<td>0.24</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>500</td>
<td>2</td>
<td>1.69</td>
<td>501.7</td>
<td>0.313</td>
<td>0.37</td>
</tr>
<tr>
<td>6</td>
<td>400</td>
<td>0</td>
<td>2</td>
<td>401.7</td>
<td>1.7</td>
<td>0.296</td>
<td>0.43</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>200</td>
<td>2</td>
<td>6.48</td>
<td>201.5</td>
<td>0.823</td>
<td>0.40</td>
</tr>
</tbody>
</table>

TABLE IV

Evidence for binding of glutamic acid to enzyme

In Column A, the enzyme (1.8 to 2.1 mg; 500 units; 630 units in Experiments 2 and 3) was incubated with 14C-L-glutamic acid (0.1 to 0.30 μmoles) and imidazole-β-Cl buffer (25 μmoles) in a final volume of 0.44 to 0.65 ml for 2 minutes at 37° and pH 7.2. Then 0.17 ml of a solution at pH 7.2 containing NH₄OH (800 μmoles; 1300 μmoles in Experiment 4) and 14C-L-glutamic acid (55 μmoles) was added, and after 15 seconds, 0.1 ml of 4 M HClO₄ was added. After removal of the protein by centrifugation, an aliquot of the supernatant solution was adjusted to pH 7.0 with KOH and the precipitated potassium perchlorate was removed by centrifugation. The solution was then placed at 100° for 15 minutes. Under these conditions, γ-glutamylhydroxamic acid is quantitatively converted to pyrrolidonecarboxylic acid while glutamic acid is not significantly cyclized. After heating, the 14C-pyrrolidonecarboxylic acid was separated by paper chromatography and its radioactivity was determined as previously described (5). In Experiments 2, 3, and 4, the reaction mixtures were not deproteinized with HClO₄, but were heated directly at 100° for 15 minutes, and the denatured protein was subsequently centrifuged. In Column B, the same amounts of 14C- and 12C-glutamic acid as in A were added together initially; otherwise, the conditions were the same as in Column A.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Total 14C-glutamic acid added</th>
<th>Enzyme + 14C-glutamic acid; + NH₄OH</th>
<th>Enzyme + 14C- and 12C-glutamic acid; + NH₄OH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c.p.m. x 10⁻⁶</td>
<td>c.p.m.</td>
<td>c.p.m.</td>
</tr>
<tr>
<td>1</td>
<td>1.0</td>
<td>15,000</td>
<td>9,000</td>
</tr>
<tr>
<td>2</td>
<td>1.3</td>
<td>32,000</td>
<td>30,000</td>
</tr>
<tr>
<td>3</td>
<td>2.6</td>
<td>93,500</td>
<td>65,000</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>31,500</td>
<td>12,000</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>20,000</td>
<td>10,000</td>
</tr>
</tbody>
</table>

Inhibition by L-Asparagine and 6-Diazo-5-oxo-L-norleucine—The formation of γ-glutamylhydroxamic acid from glutamine and from glutamic acid, as well as the hydrolysis of glutamine (studied under the conditions given in Table II), were inhibited by 6-diazo-5-oxo-L-norleucine and to a lesser extent by L-asparagine. Both inhibitors were active only if preincubated with the enzyme prior to addition of substrates. The reactions were inhibited approximately 25 and 95% when the enzyme was preincubated for 10 minutes with 0.001 M L-asparagine and 0.001 M 6-diazo-5-

(5); the glutamine was found to be devoid of radioactivity. The reaction mixtures were freed from ammonia by exhaustive aeration at pH 10 to 11, and the glutamine was hydrolyzed in 2 N hydrochloric acid at 100°; the resulting ammonia was aminated into hydrochloric acid traps, the quantity of ammonia present was determined, and 66 μmoles of carrier ammonium chloride were added. The ammonia was converted to nitrogen by treatment with hypobromite, and the 15N content was determined. Analysis indicated that the amide nitrogen of the glutamine after incubation contained 3.94 atom % excess of 15N, indicating about 7% of the theoretically maximal equilibrium exchange. Controls in which enzyme was omitted gave values between 0.4 and 0.7 atom % excess. In an experiment in which 33% of the glutamine was hydrolyzed, the amide nitrogen contained 2.08 atom % excess 15N.

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The formation of a hydroxamic acid from a carboxylic acid in an enzymatic system is often considered as evidence for the formation of an activated carboxyl group. The ability of the enzyme studied here to catalyze γ-glutamylhydroxamic acid formation from glutamine and hydroxylamine indicates that the enzyme catalyzes activation of the γ-glutamyl carboxyl group and is consistent with the formation of a γ-glutamyl-enzyme complex. Such a complex may react with water or with hydroxylamine. However, it appears that glutamic acid itself can react with the enzyme to yield a γ-glutamyl-enzyme complex which may be the same as that formed from glutamine. Thus, the enzyme catalyzes γ-glutamylhydroxamic acid formation from glutamic acid and hydroxylamine, and the experiments in which a pulse-labeling technique was employed (Table IV) are in accord with the belief that glutamic acid is bound to the enzyme prior to reaction of hydroxylamine. Although activated carboxyl groups react rapidly with hydroxylamine, appreciable hydroxamic acid formation can occur by nonenzymatic reaction of carboxylic acids with hydroxylamine; the available data indicate that the equilibrium constant for this type of reaction is not very far from unity. Previous studies showed the nonenzymatic formation of hydroxamic acids from glutamic, glutamic, and aspartic acids (2). The present findings indicate that the standard free energy of hydrolysis of γ-glutamylhydroxamic acid is relatively small compared to that of the corresponding amide.

The data are consistent with the scheme given in Fig. 4 in which the enzyme may react with glutamic acid, glutamine, or γ-glutamylhydroxamic acid to yield the same acyl-enzyme. Since hydrolysis of glutamine and formation of γ-glutamylhydroxamic acid from glutamine are more rapid than the hydrolysis of γ-glutamylhydroxamic acid or the formation of γ-glutamylhydroxamic acid from glutamic acid, it may be concluded that glutamine combines more rapidly with the enzyme (Reaction 1a) than do either glutamic acid (Reaction 7) or γ-glutamylhydroxamic acid (Reaction 6). The reactivity of hydroxylamine in this system is apparently favored by its ability to serve as an enzymatically active analogue of ammonia. The scheme permits an explanation for the observation that 14N-ammonia was incorporated into glutamine in the absence of detectable 14C-glutamic acid incorporation; thus, reaction of glutamic acid with the enzyme (Reactions 7 and 8) must be slower than the slowest step in the conversion of glutamyl-enzyme to free glutamine. It is of interest that Klingman and Handler (13) found that pig kidney phosphate-activated glutaminase catalyzed incorporation of 15NH3, but not of 14C-glutamic acid, into glutamine.

Although the present enzyme may be considered as a glutaminase, its activity toward other amides suggests that it is an amino acid ω-amidase of somewhat lower specificity than the

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Fig. 4. Tentative scheme for amide and hydroxamic acid formation and hydrolysis. Enz, enzyme.
Enzymatic Synthesis of \(\gamma\)-Glutamylhydroxamic Acid

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glutaminases and asparaginases previously studied. However, the possibility that more than one enzyme protein is present cannot be definitely excluded. Glutaminases previously described vary considerably in their ability to catalyze hydroxamic acid formation from glutamine. Thus, *Escherichia coli* glutaminase catalyzes formation of \(\gamma\)-glutamylhydroxamic acid from glutamine at a relatively low rate compared to that of amide hydrolysis (2), while the present enzyme catalyzes both reactions at about the same rate. Rat liver glutaminase was found to be inactive in catalyzing the formation of \(\gamma\)-glutamylhydroxamic acid from glutamine (2). The low optical specificity of the enzyme studied here contrasts with rat liver and *E. coli* glutaminases, which exhibit a high degree of optical specificity; on the other hand, glutamine synthetase (14) and \(\gamma\)-glutamyltranspeptidase (15) exhibit appreciable activity with both isomers of glutamic acid. There are other significant differences among the various enzymes that catalyze the hydrolysis of glutamine and the formation of \(\gamma\)-glutamylhydroxamic acid from glutamic acid and \(\gamma\)-glutamyl derivatives, and the available data therefore indicate the existence of several types of enzyme-bound glutamyl derivatives.

**SUMMARY**

A purified bacterial enzyme preparation has been obtained that catalyzes the synthesis of L-\(\gamma\)-glutamylhydroxamic acid from L-glutamic acid and hydroxylamine; the reaction requires neither added nucleotide nor metal ions. The enzyme preparation also catalyzes hydrolysis of both isomers of glutamine and asparagine as well as the formation of hydroxamic acids from these amides. The apparent equilibrium constant at pH 7.2 for the synthesis of \(\gamma\)-glutamylhydroxamic acid from glutamic acid and hydroxylamine is near unity (0.24 to 0.43 liter per mole), indicating that the standard free energy of hydrolysis of \(\gamma\)-glutamylhydroxamic acid is small compared to that of glutamine. This conclusion is consistent with the observation that the synthesis of \(\gamma\)-glutamylhydroxamic acid from 0.01 M concentrations of glutamic acid, hydroxylamine, and adenosine triphosphate catalyzed by glutamine synthetase goes virtually to completion in contrast to the synthesis of glutamine, which stops at about 90% of completion.

The data are in accord with a proposed tentative scheme in which glutamic acid reacts with the enzyme to yield a glutamyl-enzyme complex, which may also be formed from glutamine and \(\gamma\)-glutamylhydroxamic acid. The scheme also permits explanation of the observation that the enzyme catalyzes incorporation of \(^{15}\)N into the amide group of glutamine, in the absence of detectable \(^{14}\)C-glutamic acid incorporation.

**REFERENCES**

Enzymatic Synthesis of γ-Glutamylhydroxamic Acid from Glutamic Acid and Hydroxylamine

Elvera Ehrenfeld, Sara Jo Marble and Alton Meister


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