Action of Liver Glutamine Transaminase and L-Amino Acid Oxidase on Several Glutamine Analogs

PREPARATION AND PROPERTIES OF THE 4-S, O, AND NH ANALOGS OF α-KETOGLUTARAMIC ACID*

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SUMMARY

The L-glutamine analogs, L-albizziin (L-α-amino-β-ureidopropionic acid), S-carbamyl-L-cysteine, and O-carbamyl-L-serine were found to be substrates for purified rat liver glutamine transaminase, and the α-keto acid product formed in each case was found to cyclize to a lactam analogous in structure to the cyclic form of α-ketoglutaramic acid (2-pyrrolidone-5-hydroxy-5-carboxylic acid). Evidence was obtained that the initial product of transamination of albizziin, S-carbamylcysteine, and O-carbamylserine are the corresponding α-keto acids, which were found to be converted by ω-amidase (followed by spontaneous decarboxylation) to β-aminopropionate, β-mercaptopyruvate, and β-hydroxypropionate, respectively. Incubation of the glutamine analogs with L-amino acid oxidase from Crotalus adamanteus venom gave the corresponding cyclic lactam forms; the products obtained from albizziin and S-carbamyl-cysteine were rapidly and irreversibly dehydrated in acid or base to yield 2-imidazoline-4-carboxylic acid and 2-thiazoline-4-carboxylic acid, respectively. Neither α-ketoglutaramate nor 2-oxazolone-4-hydroxy-4-carboxylic acid (which was isolated as the corresponding barium salt) was dehydrated under these conditions.

Previous studies on highly purified rat liver glutamine transaminase showed that this enzyme can catalyze transamination between a wide variety of α-keto acids and glutamine, glutamic acid γ-ethyl ester, γ-glutamyl methylamide, methionine, and ethionine (1). In the present studies we have examined the activity of the enzyme toward several analogs of glutamine in which the 4-methylene moiety of glutamine is replaced by S, O, or NH. Thus, we have found that L-albizziin (L-α-amino-β-ureidopropionic acid), S-carbamyl-L-cysteine, and O-carbamyl-L-serine can effectively replace L-glutamine in the transamination reaction. In earlier work (2, 3) it was shown that the α-keto analog of glutamine, α-ketoglutamic acid, exists predominantly in solution in an unreactive cyclic (lactam) form, 2-pyrrolidone-5-hydroxy-5-carboxylic acid. The α-keto acid analog of glutamine is not decarboxylated by 1 M hydrogen peroxide at pH 4 nor does it readily form a 2,4-dinitrophenyl hydrazone; however, it is hydrolyzed by ω-amidase at pH values of 7.5 or higher because the lactam form is rapidly converted to the open chain form under these conditions. In the present work we have obtained evidence that the α-keto analogs of albizziin, S carbamylcysteine, and O-carbamylserine are substrates of ω-amidase. These compounds, like α-ketoglutarate, are rapidly converted to the corresponding cyclic lactams. These compounds were also obtained by the action of L-amino acid oxidase on the several glutamine analogues. Two of the cyclic compounds (those derived from albizziin and S-carbamylcysteine) were found to undergo rapid and irreversible dehydration in acid or base. The present communication describes the preparation and some of the chemical and enzymatic properties of these analogs of α-ketoglutarate.

EXPERIMENTAL PROCEDURE

Materials—S-Carbamyl-L-cysteine was prepared as described by Revel et al. (4). O-Carbamyl-L-serine was obtained from Ceylo Chemical Corporation. L-Albizziin and O-phenylene diamine were obtained from Aldrich Chemical Company. γ-Cyano-L-L-aminobutyric acid was kindly donated by Dr. Charlotte Ressler. Ceric sulfate and ferric chloride were pur chased from Fisher Scientific Company. L-γ-Glutamyl hydrazide was obtained from Fox Chemical Co.

Deer liver catalase was obtained from Sigma. Purified Crotalus adamanteus L-amino acid oxidase (5) was donated by Mr. Edmund Hafner of this department. Crotalus adamanteus venom was obtained from Ross Allen's Reptile Institute. Rat liver glutamine transaminase was prepared as previously described (1). ω-Amidase was purified as described by Hersh (6), except that the final step of the purification procedure was omitted. In some of the experiments, purified ω-amidase kindly donated by Dr. Thomas Duffy, Department of Neurology, Cornell University Medical College, was used.

Methods—Transamination between glutamine (or glutamine analogs) and α-keto acids was determined as previously described (1). The reaction mixtures contained 20 mM L-glutamine (or analog) and 20 mM sodium glyoxylate in 0.1 ml of 50 mM Tris-HCl buffer (final pH 8.4 at 37°). Transamination between glyoxylate and glutamine, albizziin, or γ-cyano-L-aminobutyrate was followed by determining the glycine formed by the method...
Table I

Transamination between glyoxylate and glutamine analogs catalyzed by rat liver glutamine transaminase in presence of \( \omega \)-amidase

| Experiment | Amino acid | Transamination | Ammonia formation |
|------------|------------|----------------|------------------|
| 1          | L-Glutamine| 1000           | 914              |
| 2          | \( \alpha \)-Carbamyl-L-cysteine| 510           | 262              |
| 3          | \( \alpha \)-Carbamyl-L-serine| 420           | 50               |
| 4          | \( \alpha \)-Albizziin| 580           | 150              |
| 5          | L-Albizziin| 850           | 42.5             |
| 6          | L-Albizziin| 850           | 87.5             |

*Fig. 1* Some enzymatic and nonenzymatic reactions of glutamine and glutamine analogs.

of Patton (7) as modified by Cooper and Meister (1). In one of the experiments transamination was followed by determining the disappearance of glyoxylate by the 2,4-dinitrophenylhydrazine procedure. Aliquots (5 \( \mu \)l) were withdrawn and added to 0.1 ml of 0.1% 2,4-dinitrophenylhydrazine in 2 \( \overline{\text{N}} \) HCl; after incubation at 37° for 20 min, 0.9 ml of 1 \( \overline{\text{N}} \) potassium hydroxide was added and the absorbance at 430 nm was read against the blank lacking keto acid.

\( \omega \)-Amidase was assayed as described by Hersh (6). The specific activity of the \( \omega \)-amidase used in these studies was 600 to 750 units per mg. A unit is defined as the amount of enzyme that converts 1 \( \mu \)mole of \( \alpha \)-ketoglutaramate to \( \alpha \)-ketoglutarate per hour under the conditions of assay. Ammonia was determined using a Conway (8) diffusion apparatus; color was developed with Nessler's reagent or by use of the indophenol procedure (9).

Paper chromatography was carried out by the ascending technique using the solvents given below. \( \alpha \)-Keto acids were detected by the \( \sigma \)-phenylenediamine technique (10). In this procedure, \( \alpha \)-keto acids may be located on paper chromatograms sprayed with \( \sigma \)-phenylenediamine in trichloroacetic acid by their fluorescence under ultraviolet light. The procedure is very sensitive for a number of \( \alpha \)-keto acids; however, glyoxylate does not fluoresce, but quenches under these conditions. The cyclic compounds were detected by the Rydon and Smith procedure (11) as modified by Pan and Dutcher (12). Since Tris reacts under these conditions, the experiments in which chromatographic procedures were used were carried out in bicarbonate or borate buffers.

**Results**

Transamination between Glyoxylate and Glutamine Analogs—

Rat liver glutamine transaminase was found to be active when glutamine was replaced by certain glutamine analogs. Thus, as indicated in Table I, substantial transamination was observed when the enzyme was incubated with glyoxylate and \( \omega \)-carbamyl-L-cysteine, \( \omega \)-carbamyl-L-serine, or L-albizziin. Earlier studies (2, 3, 13) showed that transamination of glutamine with \( \alpha \)-keto acids yields the corresponding amino acids and the \( \alpha \)-keto analog of glutamine, \( \alpha \)-ketoglutarate, which spontaneously cyclizes to form the keto lactam, 2-pyruvlyl-5-hydroxy-5-carboxylate. At pH 7.0 more than 99% of the \( \alpha \)-keto analog of glutamine exists in the cyclic form, but, as stated above, the compound is a good substrate for \( \omega \)-amidase at values of pH above 7.5 because the rate of interconversion between the open chain and cyclic forms is rapid. Thus, as demonstrated previously (3, 13) and in Table I, incubation of glutamine, glyoxylate, and rat liver glutamine transaminase in the presence of purified \( \omega \)-amidase leads to ammonia formation which is almost equivalent to the extent of transamination. In comparable experiments in which \( \omega \)-carbamyl-L-cysteine and glyoxylate were incubated with both the transaminase and the \( \omega \)-amidase, the formation of ammonia was appreciable but considerably less than the extent of transamination. (Relatively short incubation periods were used in experiments with \( \omega \)-carbamylcysteine because this amino acid readily decomposes to cysteine and cyanate at values of pH above 8.0 (14).) The findings suggest that the \( \alpha \)-keto analog of \( \omega \)-carbamyl-L-cysteine expected to be formed by transamination under these conditions is deamidated to the corresponding \( \alpha \)-carboxy derivative, which would undergo spontaneous decarboxylation to yield \( \beta \)-mercaptoxyvurate. In agreement with these expectations, paper chromatographic studies of reaction mixtures identical to those described in Experiment 2 (Table I) demonstrated the formation of \( \beta \)-mercaptoxyvurate (Fig. 1; \( X = S \)).

In studies in which \( \omega \)-carbamyl-L-serine was incubated with glyoxylate in the presence of the transaminase and \( \omega \)-amidase, a small but significant amount of ammonia was formed (Experiment 3, Table I); paper chromatographic study of such reaction mixtures revealed the formation of \( \beta \)-hydroxyxyvurate. The latter compound would be expected to be produced by decarboxylation of the product formed by enzymatic deamidation of the \( \alpha \)-keto analog of \( \omega \)-carbamylserine.

In analogous studies on transamination between glyoxylate and albizziin in the presence of \( \omega \)-amidase (Experiments 4 to 6; Table I), small but significant amounts of ammonia were formed. It is notable that the formation of ammonia was increased when larger amounts of \( \omega \)-amidase were added to the reaction mixture. Paper chromatographic studies revealed the formation of \( \beta \)-aminoxyvurate. Deamidation of the \( \alpha \)-keto analog of albizziin would be expected to yield the corresponding \( \beta \)-N-carboxy derivative, which on spontaneous decarboxylation would give
β-aminopyruvate. While the latter compound has apparently not been previously described, the available data are consistent with its formation under these conditions. The new compound formed in reaction mixtures containing albizziin, glyoxylate, glutamine transaminase, and ω-amidase (but not in controls in which any one of these was omitted) gave a brown spot on paper chromatograms after treatment with the o-phenylenediamine reagent when viewed under ultraviolet light. Additional evidence for the formation of β-aminopyruvate was obtained in studies in which albizziin was incubated with pyruvate, glutamine transaminase, and ω-amidase under conditions similar to those given in Table I except that 50 units of ω-amidase were added and the reaction mixture was incubated for 3 hours. After incubation, the reaction mixture was adjusted to pH 4.0 by addition of 1 N HCl. The acidified reaction mixture was then treated with 1 N hydrogen peroxide, and after incubation for 10 min at 37° an aliquot was subjected to paper chromatography. These studies revealed the presence of glycine (the expected product of the reaction between β-aminopyruvate and hydrogen peroxide); no glycine was formed in control studies in which reaction mixtures lacking ω-amidase were treated with hydrogen peroxide.

The findings described above are consistent with the occurrence of Reactions 1, 5, and 6 indicated in Fig. 1. While the data indicate that the 3 analogs of glutamine studied undergo enzymatic and nonenzymatic reactions analogous to those previously observed with glutamine (2, 3, 13), it is evident that the analogs are less effectively transaminated than is glutamine, and that the corresponding α-keto acid analogs are less susceptible to deamination by ω-amidase than is α-ketoglutarate. These considerations suggest that the α-keto analogs of S-carbamylcysteine, O-carbamylserine, and albizziin, like that of glutamine, undergo spontaneous cyclization. Studies described below demonstrate that this is the case.

γ-Cyano-L-α-aminobutyrate was also found to effectively replace L-glutamine in the L-glutamine-glyoxylate transamination system. The rate of transamination (determined by measurement of the rate of glycine formation) under the conditions given in Table I was about 85% of that observed with glutamine. γ-Cyano-α-ketobutyrate was formed as determined by the 2,4-dinitrophenylhydrazine procedure. Identification of the α-keto acid by paper chromatography of the corresponding 2,4-dinitrophenylhydrazone was hindered by difficulty in separating the 2,4-dinitrophenylhydrazones of glyoxylate and γ-cyano-α-ketobutyrate using the solvent systems previously described (15). However, these α-keto acids can easily be separated by paper chromatography of the corresponding L-γ-glutamyl hydrazones (16). Thus, after incubation, the reaction mixtures were placed at 100° for 30 s and then cooled to 37°; 20 μl of 0.1 M L-γ-glutamyl hydrazide were added and the mixture was incubated at 37° for 30 min. Paper chromatography of the L-γ-glutamyl hydrazones led to separation of the derivatives of glyoxylic and γ-cyano-α-ketobutyrate (see below); authentic γ-cyano-α-ketobutyrate was obtained by oxidation of the corresponding L-amino acid with L-amino acid oxidase in the presence of catalase by the general procedure previously described (17).

Oxidation of Glutamine Analog by L-Amino Acid Oxidase—As indicated in Table II, the glutamine analogs discussed above are substrates of L-amino acid oxidase. When the enzymatic oxidation was carried out in the presence of added ω-amidase, no additional ammonia was formed in the studies with albizziin, S-carbamylcysteine, and O-carbamylserine, but (in confirmation of earlier studies (3)), additional ammonia was formed in experiments with glutamine. These observations indicate that the α-keto analog of glutamine is formed and hydrolyzed by ω-amidase, but that the α-keto analogs of albizziin, S-carbamylserine and O-carbamylserine are not formed to a detectable extent under these conditions.

Hafner and Wellner (18) have shown that the initial product formed in the oxidation of an L-amino acid by L-amino acid oxidase is the corresponding imino acid; this species has a half-life of several seconds at pH 8.0 and is extremely reactive. According to Hafner and Wellner, imino acids react with semicarbazide at pH 8.0 at rates that are several thousand times greater than the rates of reaction of semicarbazide with the analogous α-keto acids. These workers have found that more than 95% of the imino acid formed can be trapped as the corresponding α-keto acid semicarbazone in the presence of catalase by the general procedure previously described (17).

Oxidation of various glutamine analogs by L-amino acid oxidase in presence of ω-amidase

The reaction mixture (final volume, 0.10 ml) contained 20 mm amino acid, 100 mm Tris-HCl buffer (pH 8.4), 40 μg of L-amino acid oxidase, 100 units of catalase, and 6 units of ω-amidase (as indicated). After shaking in air for 10 min at 37°, the formation of ammonia was determined.

| Amino acid                | Ammonia formed |
|---------------------------|----------------|
|                           | Without ω-amidase | With ω-amidase |
| L-Glutamine               | 560             | 1200          |
| L-Albizziin               | 160             | 170           |
| S-Carbamyl-L-cysteine     | 300             | 260           |
| O-Carbamyl-L-serine       | 280             | 260           |
| L-γ-Cyano-α-aminobutyric  | 450             | 450           |

Table II

Action of L-amino acid oxidase on glutamine, O-carbamylserine, and albizziin in presence of semicarbazide

The reaction mixtures contained 10 mm amino acid, 100 mm sodium borate buffer (pH 8.4), 100 mm semicarbazide hydrochloride (adjusted to pH 8.4 by addition of 1 N NaOH), 100 μg of L-amino acid oxidase, and 100 units of catalase in a final volume of 1 ml. The reactions were carried out at 25° and the increase in absorbance at 248 nm was determined against a blank lacking amino acid. The formation of α-keto acid semicarbazone was determined from the increase in absorbance at 248 nm, using an extinction coefficient of 10,000. The formation of ammonia was determined with Nessler’s reagent in a parallel experiment.

| Amino acid                | Semicarbazone formation | Ammonia formation |
|---------------------------|------------------------|------------------|
|                           | μmole/minute           | μmole/minute     |
| L-Glutamine               | 130                    | 8                |
| O-Carbamyl-L-serine       | 8                      | 22               |
| L-Albizziin               | 1                      | 20               |

Table III

1 E. Hafner and D. Wellner, 9th International Congress of Biochemistry, Stockholm, Sweden, July 1 to 7, 1973, Abstract 2d 44.
Oxidation of amino acids by L-amino acid oxidase in absence of catalase and in presence of added hydrogen peroxide

| Amino acid       | Product formation | With catalase | Without catalase | Without catalase + 0.1 M HCl |
|------------------|-------------------|--------------|-----------------|----------------------------|
| L-Glutaminea     | 0.56              | 0.47         | 0.06            |
| L-Albizziia      | 0.16              | 0.16         | 0.16            |
| O-Carbamyl-L-serineb | 0.30         | 0.25         | 0.05            |
| S-Carbamyl-L-cysteinec | 0.30         | 0.30         | 0.30            |
| L-Methioninee    | 2.60              | 0.13         | 0.0              |

a The reaction mixtures (final volume, 0.10 ml) contained 20 mM amino acid, 100 mM Tris-HCl buffer (pH 8.4), 40 μg of L-amino acid oxidase, and 100 units of catalase (except where indicated). After 10 min the extent of the reaction was determined as indicated.

b a-Ketoglutaramate was determined with ω-amidase (6).

c Determined from the absorbance of 2-imidazolinone-4-carboxylate in alkali at 280 nm.

d Estimated from the intensities of the spots found for 2-oxazolone-4-carboxylate after paper chromatography and application of the modified Rydon and Smith procedure (11, 12).

e The α-keto acid was determined as the 2,4-dinitrophenylhydrazone (1).

Table IV

The oxidation of the several glutamine analogs by L-amino acid oxidase was also studied in the absence of catalase and in the presence of added hydrogen peroxide (Table IV). The findings indicate that the formation of product from glutamine and the 3 glutamine analogs was not substantially decreased by omission of catalase. In contrast, α-keto acid formation from L-methionine in the absence of catalase was only 5% of that observed in the presence of catalase. The studies in which hydrogen peroxide was added, there was a marked decrease in the formation of product from L-glutamine, O-carbamyl-L-serine, and L-methionine; in contrast, addition of hydrogen peroxide did not destroy the product obtained in the oxidation of L-albizziin and S-carbamyl-L-cysteine. Since it would be expected that hydrogen peroxide decarboxylates the α-imino acid (as well as the α-keto acid), these findings are in accord with the view that the α-imino acids formed from L-albizziin and S-carbamyl-L-cysteine undergo cyclization very rapidly, more rapidly than do the α-amino derivatives derived from glutamine and O-carbamylserine. This conclusion is consistent with the observations on the trapping of the α-imino acids by semicarbazide (Table III).

Preparation and Properties of α-Keto Analogs of Albizziin, S-Carbamylcysteine, and O-Carbamylserine—The findings described above indicate that the α-keto analog of albizzin, which is formed in the transaminase reaction exhibits a marked tendency to undergo conversion to a form, presumably a cyclic derivative, which does not possess a reactive α-keto group. The data also indicate that the α-imino acid formed by the action of L-amino acid oxidase on L-albizziin undergoes conversion to a cyclic form, apparently without prior conversion to the corresponding α-keto acid. No evidence for the conversion of the cyclic form to the open chain form was obtained. However, the open chain form of the α-keto analog of albizzin is probably formed transiently in the transamination reaction; thus, in the studies described above in which L-albizziin was incubated with transaminase, ω-amidase, and glycylxylate, ammonia was formed and evidence for β-aminopyruvate formation was obtained. Similar conclusions appear applicable to the α-keto analogs of S-carbamyleysteine and O-carbamylserine.

In order to further investigate the properties of these α-keto acids, the products formed by oxidation of the several glutamine analogs with L-amino acid oxidase in the presence of catalase were obtained in protein-free solution (pH 7 to 8) by ultrafiltration of the reaction mixtures. Studies on these solutions indicated that the initial product of oxidation is in each case analogous in structure to the lactam form of α-ketoglutarate (see below). Attempts to isolate the oxidation products as the free acids led in two cases to dehydration of the cyclic products.

Thus, upon acidification of the products obtained by oxidation of S-carbamyl-L-cysteine and L-albizziin, dehydration occurred leading to the formation of 2-thiazolinone-4-carboxylic acid and 2-imidazolinone-4-carboxylic acid, respectively.

Some of the properties of the α-keto acid analogs of S-carbamyleysteine, O-carbamylserine, and albizzin are summarized in Table V; paper chromatographic data are summarized in Table VI. The products obtained in solution after oxidation of these glutamine analogs by L-amino acid oxidase, like that obtained in the oxidation of L-glutamine, did not readily form a 2,4-dinitrophenylhydrazone. However, these compounds are dephosphorylated by hydrogen peroxide under conditions in which pyruvic and glyoxylic acids as well as a great many other α-keto acids are decarboxylated (3). The products obtained by enzymatic oxidation of glutamine and the three glutamine analogs did not react with o-phenylenediamine in a manner typical of α-keto acids that possess a reactive α-keto acid moiety. In contrast, α-ketoglutaric acid and the compounds obtained by oxidation of S-carbamyl-L-cysteine, O-carbamyl-L-serine, and L-albizziin all formed a yellow complex on treatment with ferric chloride.

The product obtained by oxidation of O-carbamyl-L-serine, like α-ketoglutaric acid, exhibited only end absorption in the ultraviolet (Fig. 2). Similar end absorption was observed with the initial products of oxidation of S-carbamyleysteine and albizzin. However, on treatment of these products with alkali or acid, or after passage through a column of Dowex 50 (H+), the compounds derived from S-carbamyleysteine and albizzin ex-
Properties of oxidation products of glutamine analogs and other amino acids

| Compound                          | Formation of 2,4-dinitrophenylhydrazonoe | Decarboxylation by HClf | Reaction with o-phenylendiamineg | Formation of complex with FeIII-|  |
|-----------------------------------|----------------------------------------|------------------------|---------------------------------|-------------------------------|---|
| Fyruvic acid                     | +                                      | +                      | +                               | +                             |  |
| Glyoxylic acid                    | +                                      | +                      | +                               | +                             |  |
| 2-Pyrrolidone-5-hydroxy-5-carboxylic acid | -                                      | -                      | -                               | -                             |  |
| 2-Thiazolidone-4-hydroxy-4-carboxylic acid | -                                      | -                      | -                               | -                             |  |
| 2-Imidazolidone-4-hydroxy-4-carboxylic acid | -                                      | -                      | -                               | -                             |  |
| 2-Oxazolidone-4-hydroxy-4-carboxylic acid | -                                      | -                      | -                               | -                             |  |

* a, positive reaction; - , no reaction.
* b, Ability to form 2,4-dinitrophenylhydrazones in 2 N HCl.
* c, Oxidative decarboxylation by 1 M hydrogen peroxide in 0.1 M sodium acetate buffer (pH 4.0), as determined by CO2 formation (3).
* d, Production of a fluorescent spot on a paper chromatogram under ultraviolet light, after treating the paper with 0.1% o-phenylenediamine in 20% trichloroacetic acid; glyoxylate quenches under these conditions.
* e, Formation of a yellow FeIII+complex on addition of 0.05% aqueous ferric chloride.
* f, No reaction is given by the corresponding dehydrated compounds.

hibited characteristic absorption in the ultraviolet as indicated in Fig. 2. No such changes in absorbance on acidification or on treatment with alkali were observed with the oxidation products obtained from O-carbamyleserine and glutamine. After the products obtained from S-carbamyleserine and albizziin were acidified they failed to form a colored complex when treated with ferric chloride. Ultraviolet spectral studies of reaction mixtures containing the several glutamine analogs, glyoxylate, and glutamine transaminase indicate that the compounds formed from the glutamine analogs by transamination do not exhibit characteristic ultraviolet absorbance; on acidification of such reaction mixtures ultraviolet absorbance similar to that shown in Fig. 2 was observed only in the experiments with S-carbamyl-L-cysteine and L-albizzin.

The isolation of 2-thiazolinone-4-carboxylic acid, 2-imidazolone-4-carboxylic acid, and of barium 2-oxazolidone-4-hydroxy-4-carboxylate is described below.

2-Thiazolinone-4-carboxylic Acid—S-Carbamyl-L-cysteine (164 mg; 1 mmole) was dissolved in 5 ml of water containing 200 mg of dialyzed Crotalus adamanteus venom and 1000 units of beef liver catalase. The solution was aerated at 37° for 24 hours and then acidified with 0.05 M HCl. The protein was then removed by ultrafiltration with a Diaflow UM-2 membrane. The protein-free solution was passed through a column (volume, 2 ml) of Dowex 50 (H+), the effluent was then treated with ferric chloride. Ultraviolet spectral studies of reaction mixtures indicated the compounds formed from the glutamine analogs by transamination do not exhibit characteristic ultraviolet absorbance; on acidification of such reaction mixtures ultraviolet absorbance similar to that shown in Fig. 2 was observed only in the experiments with S-carbamyl-L-cysteine and L-albizzin.

![Fig. 2. Ultraviolet spectra of the products obtained by the action of L-amino acid oxidase on L-glutamine and glutamine analogs.](http://www.jbc.org/)

### Table V

| Compound                          | Solvents | Method of detection |
|-----------------------------------|----------|---------------------|
| Fyruvic acid                      |          | a                   |
| Glyoxylic acid                    |          | b, c                |
| 2-Pyrrolidone-5-hydroxy-5-carboxylic acid |          |                     |
| 2-Thiazolidone-4-hydroxy-4-carboxylic acid |          |                     |
| 2-Imidazolidone-4-hydroxy-4-carboxylic acid |          |                     |
| 2-Oxazolidone-4-hydroxy-4-carboxylic acid |          |                     |



\[ \text{C}_5\text{H}_6\text{O}_2\text{NS} \]

Calculated: C 33.1 H 2.07 N 9.66

Found: C 33.5 H 2.40 N 9.60
2-Imidazolinone-4-carboxylic Acid—L-Albizziin (200 mg; 1.36 mmoles) was oxidized with L-amino acid oxidase and the protein-free solution was processed as described above. The crystalline product melted at 250° with decomposition; yield 92 mg (56%).

C₇H₈O₄N₂
Calculated: C 37.5 H 3.13 N 21.9
Found: C 37.4 H 3.29 N 21.9

Hilbert (19) reported a melting point of 261° for this compound; however, a product synthesized by the published procedure by Dr. Ralph Stephani of this laboratory exhibited a melting point of 251°, and a mixed melting point between this material and the product obtained enzymatically was 250°. The ultraviolet spectrum (Fig. 2) of the enzymatically prepared product was identical to that reported by Ditmer et al. (20). The infrared spectrum of the enzymatically prepared material and of the chemically synthesized product prepared in our laboratory were identical (Fig. 3).

Barium 2-Oxazolidone-4-hydroxy-4-carboxylate—O-Carbamyl-L-serine (300 mg; 2.0 mmoles) was dissolved in 10 ml of water containing 200 mg of Crotalus adamanteus venom and 1000 units of beef liver catalase; the procedure described above was followed. The free acid obtained after passing the solution of the product through Dowex 50 (H+) was found to be extremely deliquescent, and this product was therefore dissolved in 0.5 ml of water and treated with 0.1 M barium hydroxide until the pH of the solution reached 5.0. After removal of insoluble material by filtration, the barium salt was precipitated by addition of 5 volumes of ethanol at 0°. The product was reprecipitated twice from water, washed with ethanol, and dried in vacuo over phosphorus pentoxide at 25°. Yield, 137 mg (30%).

C₁₆H₁₂O₁₂N₂Ba₂
Calculated: C 22.4 H 1.87 N 6.52
Found: C 21.9 H 2.12 N 6.22

The infrared spectrum of 2-imidazolinone-4-carboxylic acid (Fig. 3) exhibits a peak at 1600 cm⁻¹ which may be ascribed to the C=O moiety; this band is also present in the spectrum of 2-thiazolinone-4-carboxylic acid, but is not found in the infrared spectra of 2-pyrrolidone-5-hydroxy-5-carboxylic acid or 2-oxazolidone-4-hydroxy-4-carboxylic acid. The infrared spectra of the last two mentioned compounds are similar between 1800 and 1100 cm⁻¹; these findings offer additional evidence that the product obtained by oxidation of O-carbamyl-L-serine remains on acidification in the lactam form.

Studies in which the initial products of oxidation by L-amino acid oxidase of S-carbamyl-L-cysteine and L-albizziin were incubated with ω-amidase did not lead to the formation of ammonia; similar results were obtained when the ω-amidase was incubated with 2-thiazolinone-4-carboxylate, 2-imidazolinone-4-carboxylate, and 2-oxazolidone-4-hydroxy-4-carboxylate. None of these compounds was found to be a substrate in enzymatic transamination with amino acids under conditions in which ω-ketoglutaramate was shown to be active (1).

DISCUSSION

The findings indicate that L-albizziin, S-carbamyl-L-cysteine, and O-carbamyl-L-serine are substrates of glutamine transaminase (Fig. 1, Reaction I), and are converted initially to an open chain α-keto acid (II), which is in each case, like α-keto glutamate, a substrate for ω-amidase. Thus, in experiments in which the glutamine analogs were incubated with both the transaminase and the ω-amidase, we obtained evidence for the formation of ammonia (Table I), and the corresponding β-substituted pyruvic acid derivative (Fig. 1, Reactions 5 and 6). The data also indicate that the open chain α-keto acids (II) are rapidly converted to the lactam form (III); conversion of these cyclic forms (except for X—CH₂) to the open chain form (Reaction 2a) was not demonstrated in the present studies. The mechanism by which compounds of Structure III are formed from the corresponding amino acids by L-amino acid oxidase differs from that involved in the transaminase pathway. Thus, the initial enzymatic product of the L-amino acid oxidase reaction has been shown by Hafner and Wellner (18) to be the corresponding imino acid, this compound, which has been estimated to have a half-life of several seconds at pH 8.1 is hydrolyzed (in the case of most amino acids) to yield the corresponding α-keto acid and ammonia. The present findings (Table III) suggest that the imino acid formed in the oxidation of albizzinin must cyclize very rapidly to Structure III (X=NH), presumably by hydrolysis of the intermediate 2-imidazolinone-4-amino-4-carboxylic acid. Such cyclization is also very rapid when O-carbamyl-L-serine is the substrate, although some imino acid formation was detected with this substrate. In contrast, the results with glutamine indicate a pathway typical of many other amino acids in which imino acid formation occurs; cyclization of the imino acid derived from glutamine would yield Structure III (X=CH₂O) without intermediate formation of the corresponding open chain form (Structure II, X=CH₂). It seems probable that in the transamination reactions, the ketimine intermediate formed with enzyme-bound pyridoxal 5'-phosphate undergoes hydrolysis to give initially the corresponding open chain α-keto acids. This interpretation is supported by the studies in which ω-amidase was added to the reaction mixtures; thus, Reactions 5 and 6 could not be demonstrated in studies in which the glutamine analogs were incubated with L-amino acid oxidase and ω-amidase. Reaction 5 can be demonstrated with L-glutamine in the presence
It is of interest that although albizziin can replace glutamine in the reaction catalyzed by liver glutamine transaminase, it is apparently not a substrate for certain other enzymes that utilize glutamine, notably the glutamine amidotransferases. However, albizziin inhibits formylglycinamidine ribonucleotide synthetase competitively, and after prolonged incubation inactivates the enzyme by binding to it covalently (22). Albizziin also inhibits glutamine-dependent asparagine synthetase (23), and protects glutamine-dependent carbamyl phosphate synthetase against inhibition by L-2-amino-4-oxo-5-chloropentanoic acid (24). Recent studies in this laboratory have shown that an analog of albizziin, \( \beta \)-ureidopropionic acid, is hydrolyzed by \( \omega \)-amidase at about 0.5% of the rate observed with \( \alpha \)-ketoglutaramate. \( \beta \)-Ureidopropionate (10 mm) inhibits the hydroxylamination of glutamate (25) by about 50%. It thus appears that \( \beta \)-ureidopropionate can bind to \( \omega \)-amidase; the evidence for intermediate acyl enzyme formation in the reaction catalyzed by \( \omega \)-amidase (26) suggests that \( \beta \)-ureidopropionate may form an acyl enzyme that is stabilized by the \( \beta \)-nitrogen atom, perhaps in a manner analogous to that proposed for the inhibition of formylglycinamidine ribonucleotide synthetase by albizziin (22). Formation of a similar type of stabilized acyl enzyme may account in part for the low rate of hydrolysis of the \( \alpha \)-keto analog of albizziin by \( \omega \)-amidase observed in the present studies.

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Action of Liver Glutamine Transaminase and L-Amino Acid Oxidase on Several Glutamine Analogs: PREPARATION AND PROPERTIES OF THE 4-S, O, AND NH ANALOGS OF α-KETOGLUTARAMIC ACID

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