ENZYMATIC CONVERSION OF O-CARBAMYL-L-SERINE TO PYRUVATE AND AMMONIA*

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SUMMARY

O-Carbamyl-L-serine is converted to pyruvate and ammonia by rat liver homogenates. This enzymatic activity was purified about 90-fold; it is distinct from glutamate-aspartate, glutamate-alanine, and soluble glutamine transaminases, and from O-sulfo-L-serine deaminase. The purified preparation catalyzed the formation of 2 moles of NH_3 per mole of pyruvate formed. The preparation did not act on the L- or DL-forms of threonine, serine, homoserine, cysteine and O-phosphoserine, or L-alanine. Purified rat liver glutamine transaminases L and K and commercially available pig heart glutamate-aspartate and glutamate-alanine transaminases deaminated O-carbamyl L-serine very slowly. β-Chloro-L-alanine irreversibly inactivated glutamine transaminases L and K and the purified O-carbamyl-L-serine deaminase preparation.

During studies on the specificity of highly purified rat liver glutamine transaminase it was found that O-carbamyl-L-serine could effectively replace L-glutamine in the transaminase reaction (1,2). However, when O-carbamyl-L-serine was incubated with partially purified preparations of glutamine transaminase, considerable formation of pyruvate occurred; highly purified preparations of glutamine transaminase formed pyruvate from O-carbamyl-L-serine at a very much lower rate, suggesting that formation of pyruvate from O-carbamyl-L-serine was catalyzed by a different enzyme. An activity capable of forming pyruvate and ammonia from O-carbamyl-L-serine was then partially purified from rat liver. Some properties of the purified deaminase preparation are described here. Since O-carbamyl-L-serine is not known to occur in mammalian tissues, the physiological significance of this enzymatic activity (if any) is not clear. The reaction may possibly be catalyzed by an enzyme whose normal function is not yet evident. A similar problem is posed by the earlier finding (3) in rat liver of an

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activity that deaminates O-sulfo-L-serine, a compound which has apparently not yet been found in nature. The presence in rat liver of an activity that deaminates O-carbamyl-L-serine may be of significance in studies in which this and related compounds are used as glutamine analogs or antagonists.

MATERIALS AND METHODS

O-Carbamyl-L-serine and β-chloro-L-alanine were obtained from Cyclo Chemical Corp. S-Carbamyl-L-cysteine (4) and O-sulfo-L-serine (5) were prepared as described. L-Albizziin was obtained from K and K Laboratories. L-Glutamine, O-phospho-DL-serine, L-cystathionine and DL-homoserine were purchased from Calbiochem. Glutamate-aspartate and glutamate-alanine (pig heart) transaminases were purchased from Boehringer. Glutamine transaminases L and K were isolated as described (1,6).

O-Carbamyl-L-serine deaminase was assayed in mixtures containing 0.1 mM pyridoxal-5'-phosphate and 50 mM O-carbamyl-L-serine in 100 mM sodium borate buffer (pH 8.5) in a final volume of 0.1 ml. Pyruvate was measured as the 2,4-dinitrophenylhydrazone (7). Ammonia was determined with Nessler's reagent or by the indophenol method (8) after diffusion (9). A unit of activity is defined as the amount of enzyme that catalyzes the formation of 1 μmole of pyruvate per hour at 37°. Activity toward β-chloro-L-alanine and O-sulfo-L-serine was determined by substituting these amino acids for O-carbamyl-L-serine. Glutamine transaminase L was assayed by measuring formation of [^{14}C] α-ketoglutaramate in a solution containing 20 mM L-[^{14}C] glutamine and 20 mM glyoxylate (1). Glutamine transaminase K was assayed by measuring the disappearance of phenylpyruvate from a solution containing 20 mM L-glutamine and 0.4 mM phenylpyruvate (10). Glutamate-aspartate (11) and glutamate-alanine (12) transaminases were determined as described.

PURIFICATION OF O-CARBAMYL-L-SERINE DEAMINASE ACTIVITY. All steps were carried out at 4° unless otherwise stated; centrifugations were performed at 45,000 g

for 10 minutes. Step 1: Male Sprague Dawley rats were decapitated and exsanguinated. The livers (40 g) were removed and homogenized in a Waring Blender for 60 seconds in 3 volumes of 0.1 M KCl. The homogenate was centrifuged and the sediment was discarded. Step 2: The supernatant solution was heated at 50° for 20 minutes with constant stirring, and then chilled in ice and centrifuged to remove precipitated protein. Step 3: Solid ammonium sulfate (25 g/100 ml) was slowly added with constant stirring to the supernatant solution. After stirring for 20 minutes the precipitate was removed by centrifugation, and the supernatant was treated with solid ammonium sulfate (18g/100 ml); after stirring for 20 minutes, the precipitate was recovered by centrifugation and dissolved in 20 ml of 5 mM potassium phosphate buffer (pH 7.2). This solution was dialyzed against 10 liters of 5 mM potassium phosphate buffer (pH 7.2) containing 1 mM 2-mercaptoethanol. Step 4: The dialyzed solution was applied to the top of a column (2 x 10 cm) of hydroxylapatite previously equilibrated with the same buffer used for dialysis. The column was successively eluted with 5 mM, 10 mM, and 20 mM potassium phosphate buffer (pH 7.2). Elution was continued with a linear gradient established between 300 ml of 20 mM potassium phosphate (pH 7.2) and 300 ml of 100 mM potassium phosphate buffer (pH 7.2); both buffers contained 1 mM 2-mercaptoethanol. The enzyme was collected from tubes 40-48 (Figure 1) and concentrated to 5 ml with a Diaflow XM 50 membrane. A summary of the purification is given in Table I. The purified enzyme was stored in 20% glycerol containing 0.1 mM pyridoxal 5'-phosphate, 1 mM 2-mercaptoethanol and 10 mM potassium phosphate buffer (pH 7.2); no loss of activity was observed after 2 weeks of storage at -20°.

PROPERTIES OF THE ENZYME. The enzymatic decomposition of O-carbamyl-L-serine leads to formation of 2 moles of ammonia per mole of pyruvate formed (Table II). The eliminated group is probably carbamic acid, which under these conditions is unstable and breaks down to NH_3 and CO_2. With large amounts of enzyme the reaction proceeds

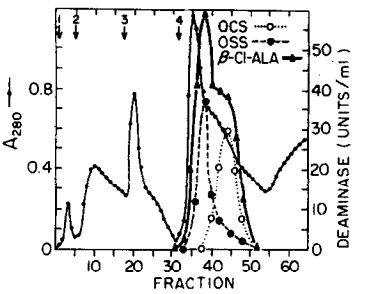

Figure 1. Hydroxylapatite chromatography of various activities. The column was eluted successively with 5 mM (1), 10 mM (2), and 20 mM (3) potassium phosphate buffers (pH 7.2); a potassium phosphate gradient (4) was then begun (see text). OCS = O-carbamyl-L-serine deaminase, OSS = O-sulfo-L-serine deaminase, β-Cl-ALA = β-chloro-L-alanine deaminase. Fractions of 10 ml were collected.

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to at least 80% of completion. Pyruvate, rather than β-hydroxypyruvate, was shown to be a product by paper chromatography; pyruvate and β-hydroxypyruvate are well separated in the following solvent: tert-butyl alcohol, methylethylketone, formic acid, water (40:30:15:15 v/v). The α-keto acids were detected by spraying the paper with o-phenylenediamine in 20% trichloroacetic acid (13). The apparent Km value for O-carbamyl-L-serine is 17 mM and the pH optimum for the deamination reaction (determined in 0.1 M sodium borate buffers) is in the range 7.8-8.8. Omission of pyridoxal 5'-phosphate in the assay led to a 60% decrease of deaminase activity.

O-Carbamyl-L-serine deaminase activity co-purifies with glutamine transaminase L in the first 3 steps of the purification procedure, but these activities are separated by chromatography on hydroxylapatite (Table I). Thus, purified O-carbamyl-L-serine deaminase (fractions 40-48; Fig. 1) exhibits very little glutamine transaminase activity. Glutamine transaminase L, which eluted in fractions 52-60 (not shown in Fig. 1), exhibited only very slight O-carbamyl-L-serine deaminase activity (about 1% of the transaminase activity). At first we suspected that O-carbamyl-L-serine (OCS) deaminase might be a property of O-sulfo-L-serine (OSS) deaminase, but as shown in Fig. 1 the peaks observed for these activities are significantly different. The major

TABLE I

Purification of O-Carbamyl-L-Serine Deaminase Activity from Rat Liver

Step	Vol ml	Protein mg	O-Carbamyl-L-Serine Deaminase Activity Total units	Specific units/mg	Yield %	Purif. Factor	Glutamine Transaminase units
1. Crude extract from 40 g of liver	172	10,400	3,300	0.317	100	1	3,440
2. After heat treatment	152	7,200	3,100	0.430	94	1.4	3,700
3. Ammonium sulfate fractionation	20	1,860	2,640	1.42	80	4.5	2,600
4. Hydroxylapatite chromatography	150	75	2,210	29.5	67	93	20

TABLE II

Formation of Pyruvate and Ammonia from O-Carbamyl-L-Serine by the Purified Liver Enzyme Preparation*

Time (mins.)	Pyruvate (μmoles)	Ammonia (μmoles)
10	0.16	0.28
20	0.35	0.76
40	0.68	1.44
60	1.01	2.20

*The reaction mixture (vol., 1ml) contained purified enzyme (1 unit), 0.05 M O-carbamyl-L-serine, 0.1 mM pyridoxal 5'-phosphate, 1 mM 2-mercaptoethanol, and 0.1 M sodium borate buffer (pH 8.4); 37°. Similar results were obtained with Tris buffer.

O-carbamyl-L-serine deaminase activity of rat liver is clearly not a catalytic property of glutamate-aspartate transaminase, which eluted in fractions 34-38. Glutamate-aspartate transaminase is known to deaminate β-chloro-L-alanine (14,15); as indicated in Fig. 1 the peak of this deaminase activity corresponds to fractions 34-38. β-Chloro-L-alanine deaminase activity was also associated with the fractions that deaminate O-carbamyl-L-serine and O-sulfo-L-serine. Glutamine transaminase L and K also exhibit slight β-chloro-L-deaminase activity (about 0.3% of the transaminase activities). When purified glutamine transaminases (L and K) and the purified O-carbamyl-L-serine deaminase preparation were incubated with β-chloro-L-alanine, there was irreversible inactivation. Thus, after these enzymes (10 μg) were incubated at 37° in reaction mixtures (final volume, 0.02 ml) containing 50 mM β-chloro-L-alanine and 0.1 M sodium borate buffer (pH 8.5) for 1 hour, only 0-20% of the initial activity was detected. Similar results were obtained with preparations of pig heart glutamate-aspartate (in confirmation of earlier work (14,15)) and glutamate-alanine transaminases; these enzymes deaminated O-carbamyl-L-serine at less than 0.1% of the rates of transamination. None of these enzymes was inactivated by O-carbamyl-L-serine.

Incubation of the purified O-carbamyl-L-serine deaminase with 50 mM L- or DL-serine, threonine, allothreonine, homoserine, serine, and O-phosphoserine or 1 mM L-cystathionine did not lead to α-keto formation*, Incubation with S-carbamyl-L-cysteine led to pyruvate formation at a rate that was about 10% of that observed with O-carbamyl-L-serine. [Since S-carbamyl-L-cysteine breaks down spontaneously at pH 8.5 to cysteine and cyanate (16), these assays were carried out for 5 minutes]. L-Glutamine and L-albizziin (20 mM) inhibited O-carbamyl-L-serine deaminase 60% and 20%, respectively. Albizziin does not appear to be a substrate; thus, no pyruvate or ammonia could be detected

*Purified serine transhydroxymethylase (22), kindly supplied by Dr. A.N. Palekar of this laboratory, did not act on O-carbamyl-L-serine.

on incubating 50 mM L-albizziin with 1 unit of enzyme for 3 hours at 37° under the conditions of assay.

DISCUSSION

The enzymatic α,β-elimination described here is analogous to the nonenzymatic conversion of O-carbamylserine to ammonia and pyruvate in the presence of pyridoxal and metal ions reported by Longenecker and Snell (17). Although several transaminases can catalyze this reaction at low rates, most of the O-carbamyl-L-serine deaminase activity of rat liver is evidently not associated with any of these enzymes. It is quite possible that this reaction may be catalyzed by a number of vitamin B_6-enzymes. Previous work has shown that glutamate-aspartate transaminase can catalyze several α,β-elimination reactions (see, for example, 14, 15, 18-20); deamination of O-carbamyl-L-serine may now be added to this list. The β-elimination of β-chloro-L-alanine is catalyzed by aspartate β-decarboxylase (21), and by glutamate-aspartate transaminase (14, 15) and these enzymes are irreversibly inactivated and alkylated by incubation with this substrate analog. The present studies show that β-chloro-L-alanine acts in a similar manner toward the glutamine transaminases and the deaminase activity studied here; β-chloro-L-alanine might therefore be useful as an active site labeling reagent for these enzymes.

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