

Communications to the Editor

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The Formation of O-Ureidoserine by an Enzyme in Leguminous Seedlings

A cell free system from leguminous plants has been shown to catalyze the formation of O-ureidoserine(4) from O-acetyl-L-serine(2) and N-hydroxyurea(3). Other possible products, β -(1-hydroxyureido)alanine(5) and β -(3-hydroxyureido)alanine(6), were not formed under the same conditions.

Some other properties of the enzyme are also described.

Keywords—biosynthesis; enzyme; amino acid; O-acetyl-L-serine; O-ureidoserine; β -(1-hydroxyureido)alanine; β -(3-hydroxyureido)alanine; N-hydroxyurea; cycloserine; *Streptomyces hygroscopicus*

O-Ureidoserine(4)¹⁾ may be regarded as a carbamoyl-derivative of cycloserine (8). D-Cycloserine,²⁻⁴⁾ produced by *Streptomyces* species,⁵⁾ is a broad-spectrum antibiotic which is known to inhibit cell wall synthesis in certain bacteria.⁶⁾

During our continuing study of the enzymic synthesis of heterocyclic β -substituted alanines in higher plants,⁷⁻¹²⁾ we have examined the possibility that certain other types of the β -substituted alanines may also be synthesized from O-acetyl-L-serine(2) as a donor of the alanyl-moiety by reactions analogous to those involved in the syntheses of mimosine⁷⁾ and lupinic acid.¹²⁾

This communication presents such an example describing an enzyme in the seedlings of higher plants that catalyzes the formation of O-ureidoserine (4) from O-acetyl-L-serine (2)¹³⁾ and N-hydroxyurea(3), as shown in Figure 1.

The enzyme preparations were obtained from the hypocotyls of seedlings of *Albizzia julibrissin*, *Leucaena leucocephala*, *Lupinus luteus*, *Pisum sativum* and *Citrullus vulgaris*, grown in the dark for 5—6 days at 28°, and *Fagus crenata* for 13—15 days. Unless otherwise stated, enzyme fractions were prepared from the *Albizzia* seedlings essentially as described in previous papers⁷⁻¹²⁾: the enzyme preparations partially purified by ammonium sulfate precipitation, heat treatment and desalting on Sephadex G-25(fine) column were used directly as the source of enzyme activity.

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- 13) O-Acetylserine has been found in the greenish-white epicarp and the reddish-mesocarp of the intact fruits of watermelon (*Citrullus vulgaris*) in about $1.05 \times 10^{-6}\%$ and $0.98 \times 10^{-7}\%$ yields, respectively (I. Murakoshi, F. Ikegami, T. Ariki and J. Haginiwa, presented at the Meeting of Kanto Branch, Pharmaceutical Society of Japan, Tokyo, October, 1977).

with aspartic acid, whilst other possible products, β -(1-hydroxyureido)alanine (5)¹⁶⁾ and β -(3-hydroxyureido)alanine(6),¹⁷⁾ appeared together at a position (225 min (119 ml)) shortly after O-ureidoserine.

Further proof that the reaction product was O-ureidoserine was obtained with the aid of chemical degradations: hydrogenation of the reaction product (PtO₂, water) yielded urea and serine (a very small amount of alanine and glycine was also recognized as by-products arising secondarily from serine), whereas 5 and 6 resulted in the formation of 2-amino-3-ureidopropionic acid (albizziine). When the reaction product was treated with 20% HCl-hydrolysis for 2 hr, it was degraded readily to serine and urea as the major products, whereas 5 and 6 were converted to 2, 3-diamino-propionic acid. These observations agree with the results of Stammer, *et al.*,^{1,15)} Takemoto, *et al.*,¹⁶⁾ and Inouye, *et al.*¹⁷⁾ for the authentic O-ureidoserine (4), β -(1-hydroxyureido)alanine (5) and β -(3-hydroxyureido)alanine (6), respectively.

Some properties of the enzyme-dependent formation of O-ureidoserine were studied: O-ureidoserine was estimated quantitatively by the ninhydrin-method of Atfield, *et al.*¹⁸⁾ as described in previous papers.⁷⁻¹²⁾ The O-ureidoserine synthetase clearly appears to be specific for the O-acetyl-L-serine: O-acetyl-D-serine or other esters of L-serine, such as the phosphate and sulfate,¹⁹⁾ or L-serine(1) itself could not serve as a donor of the alanyl-moiety. The optimum pH for the enzyme-dependent formation of O-ureidoserine was 7.5 using 0.1 M K-phosphate buffers. Activity was half maximal at pH 7.0 and 7.9 although there is a rapid O- to N-acetyl shift in the substrate above about pH 8.0. The addition of exogenous pyridoxal phosphate as a possible coenzyme to the reaction mixtures at 10, 20 and 82.5 μ g/ml caused 25%, 34% and 50% inhibition, respectively, of the synthetase activity: maximum reaction rates were observed in a mixture containing no added pyridoxal phosphate. When the reaction mixtures were incubated at 28° for varying periods of time up to 4 hr, the rate of O-ureidoserine formation was related linearly with time for at least 40 min but the rates then gradually decreased. The enzyme was reasonably stable: when stored at 0° for 25 hr, the residual enzyme activity for O-ureidoserine formation was about 65–68% of the activity initially assayed. The synthetase activity for O-ureidoserine was very dependent upon the concentration of N-hydroxyurea used: a relatively high final concentration of N-hydroxyurea at 500 mM was required to give maximum rate of O-ureidoserine formation and even M concentrations did not lead to any marked substrate inhibition. From the Lineweaver-Burk Plot of these results a K_m value for N-hydroxyurea of 1.25×10^{-4} M was obtained for O-ureidoserine. Complete inactivation of the enzyme was achieved with KCN at concentration of 11 mM but the addition of hydroxylamine at 1.3 mM, 6.5 mM and 13 mM resulted only in 7%, 21% and 31% inhibition respectively of the enzyme activity.

Enzyme preparations from other plant species were also examined for their ability to catalyze the formation of O-ureidoserine: the specific activity of enzyme preparations from *Albizia* seedlings was approximately 1.5- and 2-fold greater than those from *Leucaena* and *Lupinus*, and *Pisum* seedlings, respectively. Enzyme preparations from *Citrullus* and *Fagus* seedlings did not catalyze the formation of O-ureidoserine under the same conditions although analogous reactions for the formation of β -pyrazol-1-ylalanine⁷⁾ and

15) C.H. Stammer, *J. Org. Chem.*, **27**, 2957 (1962).

16) β -(1-Hydroxyureido)alanine(5) is a product generated from the ring cleavage of natural quisqualic acid by an alkali-treatment under the release of CO₂ (T. Takemoto, T. Nakajima, S. Arihara, and K. Koike, *Yakugaku Zasshi (J. Pharm. Soc. Japan)*, **95**, 326 (1975)).

17) β -(3-Hydroxyureido)alanine(6) is a metabolite in the cultured broth of *Streptomyces hygroscopicus* SF-1293 (S. Inouye, T. Shomura, T. Tsuruoka, Y. Ogawa, H. Watanabe, J. Yoshida, and T. Niida, *Chem. Pharm. Bull. (Tokyo)*, **23**, 2669 (1975)).

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19) L-Serine O-sulfate lyase, an enzyme capable of degrading L-serine O-sulfate to pyruvate, ammonia and sulfuric acid, was newly found in higher plants (I. Murakoshi, A. Sanda and J. Haginiwa, *Chem. Pharm. Bull. (Tokyo)*, **25**, 1829 (1977)).

willardiine,²⁰⁾ etc.^{9,11,12)} have been described in *Citrullus* and *Fagus* seedlings. These results suggest that enzymes catalyzing the formation of β -substituted alanines from O-acetyl-L-serine have different substrate specificities when prepared from different species.

O-Ureidoserine(4) conceivably might be an intermediate in the biosynthesis of cycloserine(8) formed from β -aminooxy-alanine(7) by decarbamoylation.

A more detailed investigation of the enzymes from higher plants responsible for the formations of O-ureidoserine and other types of β -substituted alanines²¹⁾ is in progress in our laboratory.

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- 21) Neither urea nor hydroxylamine could serve as an acceptor of the alanyl-moiety for the formation of the corresponding β -substituted alanines under the same conditions, using the enzyme preparations from *Albizia* seedlings.

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Determination of Uric Acid using 4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole and Urate Oxidase-Catalase System

A new method of colorimetric determination of uric acid, using uricase and catalase is reported. This method is based on the combination of enzyme reactions and colorimetric procedure with using 4-amino-3-hydrazino-1,2,4-triazole, a highly sensitive reagent for formaldehyde. The color developed in a straight line passing through the original point at least within the absorbance unit of 1.5 at 550 nm, corresponding to 16 mg/dl of the uric acid concentration of the sample.

Keywords—determination; uric acid; urate oxidase; catalase; 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole

Determination of uric acid in biological fluids is essential to diagnose the case as gout. Until now, for the assay of uric acid the phosphotungstic acid method¹⁾ and the ultraviolet method have been most commonly employed.²⁾

Recently, urate oxidase-catalase enzyme system has been introduced into the assay of uric acid by Kageyama.³⁾ In this assay system final product, lutidine derivative, which is

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