DEAMINATION OF THE MICROBIAL TOXIN TRANS L-2-AMINO-4-METHOXY-3-BUTENOIC ACID AND ITS PARENT VINYLGLYCINE BY SHEEP LIVER SERINE-THREONINE DEHYDRATASE

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Summary

The microbial toxin trans L-2-amino-4-methoxy-3-butenoic acid and its parent vinylglycine are deaminated to alpha keto-acids by sheep liver serine threonine dehydratase. The ability of the dehydratase to deaminate the above compounds is consistent with the proposed mechanism for dehydratases. Since these compounds irreversibly inhibit pig heart aspartate aminotransferase (1), the dehydratase may act as a detoxification pathway.

The microbial toxin trans L-2-amino-4-methoxy-3-butenoic acid (L-AMB) as well as the parent compound, 2-amino-3-butenoic acid (vinylglycine), have been shown to be substrates for sheep liver serine-threonine dehydratase.[1] Both compounds are natural products (1,2). The sheep liver dehydratase has recently been shown to require covalently linked α-ketobutyric acid as a carbonyl cofactor (3). A possible mechanism for the conversion of threonine and vinylglycine to α-ketobutyrate by the dehydratase is:

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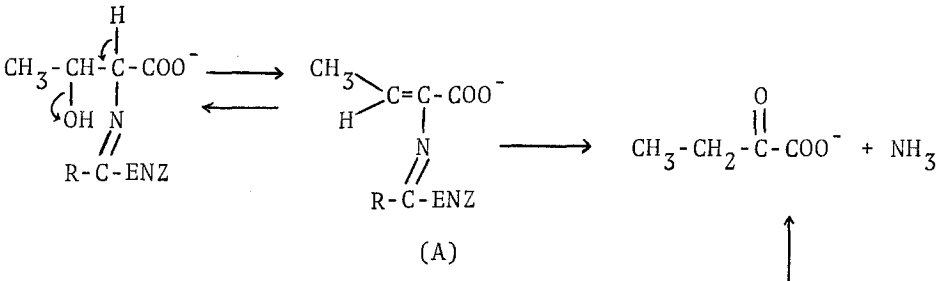
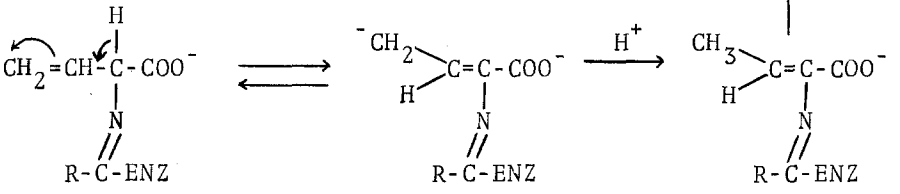
1. Serine-threonine dehydratase of specific activity 500 μmoles of ketoacid per hour per mg protein was used throughout these studies.

THREONINE



(A)

VINYLGLYCINE



(A)

In this conversion scheme, a common intermediate, A, is formed in the dehydration of threonine and the tautomerism of vinylglycine. Trans L-2-amino-4-methoxy-3-butenoic acid presumably is converted to ketoacid by a mechanism similar to the one proposed for vinylglycine.

The α-ketobutyrate formed when serine-threonine dehydratase acts on vinylglycine was identified by comparing its ^{13}C NMR spectrum with that of authentic α-ketobutyrate.

The ketoacid produced from L-AMB has not been unequivocally identified. One reason being that we had a limited amount of material to work with. However, the action of serine threonine dehydratase on L-AMB does produce a product which gives a positive 2,4-dinitrophenylhydrazone test (4), and which when reduced with NaB^3H_4 chromatographed with a R_f different from that of α-hydroxybutyrate in the solvent system n-propyl alcohol-concentrated NH_4OH (3:2).

The ability of the sheep liver dehydratase to convert vinylglycine and L-AMB to α-ketoacids can be related to the enzyme's ability to labialize the alpha proton of these two compounds. Removal of the alpha proton from vinylglycine or L-AMB results in the formation of an allylic carbanion. Reprotonation of the allylic carbanion at the gamma carbon may result in the formation of the enzyme-bound enamine (A). The enzyme-bound enamine upon dissociation from the enzyme, can spontaneously form α-ketoacid and ammonia.

The results in Table I indicate that only one isomer of vinylglycine is a substrate for serine-threonine dehydratase since only 50% of the racemic mixture can be converted to α-ketobutyrate.

The K_m for racemic vinylglycine is 12mM (Fig. 1). The K_m for L-threonine is 13mM (5). However, since only one enantiomer of racemic vinylglycine is degraded (see Table I), the K_m for vinylglycine is actually 6mM.

TABLE I

Stoichiometry of Racemic Vinylglycine Conversion

Time of incubation in hours	μM of vinylglycine in reaction mixture*	μM of α-ketobutyrate formed+
1.0	1.0	0.48 ± 0.05
3.5	1.0	0.61 ± 0.05

* Reaction mixture contained 1μM of vinylglycine (racemic), 120 units of enzyme, 0.1M phosphate buffer pH 7.2, in a total volume of 0.2 ml.

\+ α-Ketobutyrate was determined by the 2,4-dinitrophenylhydrazine method (4).

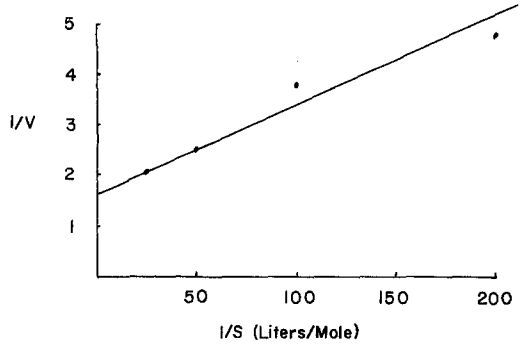


Figure 1. Double reciprocal plot of velocity ($\frac{\mu moles\ ketobutyrate}{min}$) vs. racemic vinylglycine concentration at 37°C and pH 7.4 K_m determined from this plot is 12mM.

TABLE II

Relative Rates of Product Formation

Substrate	$\frac{\mu moles\ ketoacid}{min.\ mg\ enzyme}$	Relative rates based on L-threonine
racemic vinyl-glycine	0.520	12.5
L-AMB	0.056	1.3
L-threonine	4.16	100

α-Ketobutyrate formed from vinylglycine and threonine was determined by the 2,4-dinitrophenylhydrazine method (4). The ketoacid formed from L-AMB was determined by the same method and it was assumed that its phenylhydrazone had an ε identical to that of the phenylhydrazone of α-ketobutyrate.

The relative rates of product formation from L-threonine, racemic vinylglycine and L-AMB are given in Table II. L-Threonine is a better substrate than either vinylglycine or L-AMB. However, vinylglycine has kinetic parameters very similar to those of allo-

threonine which has been shown to be a substrate for the dehydratase (6).

L-AMB and vinylglycine have previously been shown to inhibit aspartate aminotransferase (1), however, we have now demonstrated that these compounds can be degraded by the sheep liver serine-threonine dehydratase. The action of sheep liver serine-threonine dehydratase on these compounds is consistent with the proposed mechanism of the enzyme when acting on serine or threonine.

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