RACEMIZATION OF *D*- OR *L*-ALANINE BY CRYSTALLINE TYROSINE PHENOL-LYASE FROM *ESCHERICHIA INTERMEDIA*

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Summary

Conversion of *D*- or *L*-alanine into the racemate is catalyzed by crystalline tyrosine phenol-lyase prepared from cells of *Escherichia intermedia*. The racemization reaction proceeds optimally at pH 7.2-7.5, as a function of enzyme concentration and incubation time. The *Km* for *L*-alanine and the maximal velocity of racemization are 2.6×10^{-2} M and 0.051 μmole/minute/mg of protein, respectively. Addition of either *D*- or *L*-alanine to holotyrosine phenol-lyase results in the appearance of a new spectral band near 500 mμ which has been ascribed to the intermediates in many pyridoxal dependent reactions.

Tyrosine phenol-lyase (β-tyrosinase) was recently crystallized in our laboratory[1,2] from cells of *Escherichia intermedia* (AKU 0010) and its properties established in some detail[2,3]. We have reported that the crystalline preparation of tyrosine phenol-lyase catalyzes a series of α,β-elimination (Equation 1-4) and β-replacement (Equation 5-8) reactions[2-5].

L-Tyrosine + H_2O ⟶ pyruvate + NH_3 + phenol (1)

S-Methyl-*L*-cysteine + H_2O ⟶ pyruvate + NH_3 + CH_3SH (2)

L-Cysteine + H_2O ⟶ pyruvate + NH_3 + H_2S (3)

L-Serine ⟶ pyruvate + NH_3 (4)

L-Tyrosine + pyrocatechol ⟶ 3,4-dihydroxyphenyl-*L*-alanine + phenol (5)

S-Methyl-*L*-cysteine + pyrocatechol ⟶ 3,4-dihydroxyphenyl-*L*-alanine + CH_3SH (6)

L-Tyrosine + resorcinol ⟶ 2,4-dihydroxyphenyl-*L*-alanine + phenol (7)

S-Methyl-*L*-cysteine + resorcinol ⟶ 2,4-dihydroxyphenyl-*L*-alanine + CH_3SH (8)

α,β-Elimination reaction of *D*-isomer of these amino acids is also catalyzed by the crystalline enzyme[2]. In the course of further investigation on the catalytic properties of tyrosine phenol-lyase, we found that the crystalline enzyme catalyzes the conversion of either *D*- or *L*-alanine to the racemate. This communication describes the racemization reaction of *D*- or *L*-alanine catalyzed by crystalline tyrosine phenol-lyase.

Materials and Methods

Crystalline tyrosine phenol-lyase was prepared from cells of *E. intermedia* grown in a bouillon-peptone medium supplemented with *L*-tyrosine, according to the method of Yamada *et al*[1,2]. *D*-Amino acid oxidase was crystallized from pig kidney by the method of Massay *et al*[6]. Crystalline catalase was obtained from Sigma Chemical Company.

The racemase activity of tyrosine phenol-lyase was followed manometrically by measuring *D*-alanine formed from *L*-alanine with crystalline *D*-amino acid oxidase. The reaction mixture in a test tube contained 200 μmoles of *L*-alanine, 200 μmoles of potassium phosphate buffer, pH 7.3, 0.4 μmole of pyridoxal phosphate and crystalline tyrosine phenol-lyase, in a total volume of 4.0 ml. Incubation was carried out at 30°. The reaction was stopped by immersing the tube in boiling water for 1 minute and the mixture was filtered. The deproteinized filtrate was used for the measurement of *D*-alanine. The reaction mixture in a Warburg flask contained an aliquot of the filtrate, 100 μmoles of sodium pyrophosphate buffer, pH 8.3, 20 mμmoles of FAD, 150 μg of catalase and 75 μg of *D*-amino acid oxidase, in a total volume of 2.8 ml. Two tenth milliliters of 20% KOH was placed in the center well. Incubation was carried out at 30° until *D*-alanine was completely consumed.

Results

When *L*-alanine was incubated with crystalline tyrosine phenol-

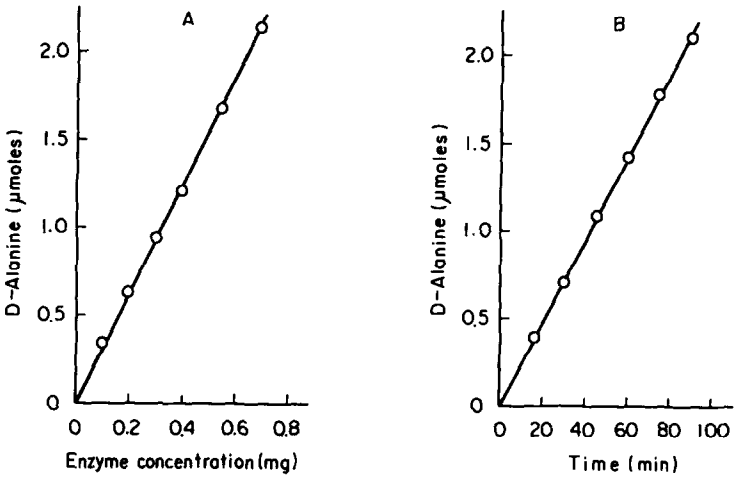

Fig. 1. Racemization of *L*-alanine as a function of enzyme concentration and incubation time. The racemase activity was determined under the standard conditions. In Fig. 1A, incubation was carried out for 60 minutes and in Fig. 1B, 0.45 mg of enzyme protein was used.

Table I. Effect of Pyridoxal Phosphate on Racemization of *L*-Alanine

Native or boiled tyrosine phenol-lyase, 0.52 mg of protein, was used. The racemase activity was determined under the standard conditions, with or without pyridoxal phosphate. Incubation was made at 30° for 60 minutes.

	D-Alanine formed
	μmoles
Native enzyme	
with pyridoxal phosphate	1.53
without pyridoxal phosphate	0
Boiled enzyme	
with pyridoxal phosphate	0
without pyridoxal phosphate	0

lyase in the presence of added pyridoxal phosphate, the formation of *D*-alanine proceeded as a function of enzyme concentration and incubation time (Fig. 1A and 1B). No pyruvate was detected in the reaction mixture before incubation with *D*-amino acid oxidase, indicating that

neither α,β-elimination nor transamination occurred. Without pyridoxal phosphate, or with boiled enzyme, the racemization of *L*-alanine was not observed (Table I).

Fig. 2 shows that tyrosine phenol-lyase converts either *D*- or *L*-alanine into the racemic mixture and that the ratio of conversion of either isomer never exceeds 50%.

Tyrosine phenol-lyase, in the presence of potassium phosphate buffer, has an optimal reactivity in the pH range of 7.2-7.5 for the racemization

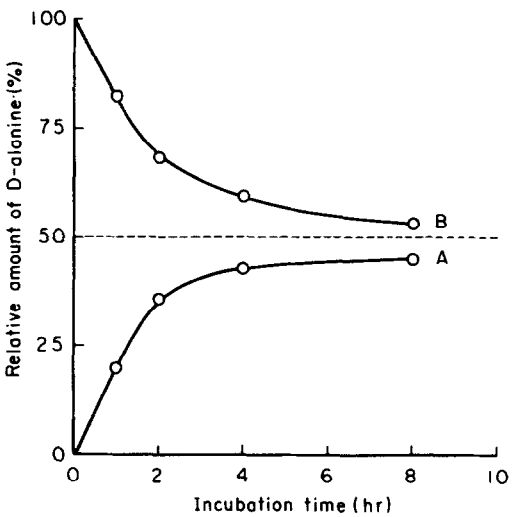

Fig. 2. Racemization of *D*- or *L*-alanine by tyrosine phenol-lyase. The racemase activity was determined with 13.1 mg of enzyme protein under the standard conditions. *L*- (curve A) or *D*-alanine (curve B) was used as the substrate.

of *L*-alanine. The *Km* value for *L*-alanine and the maximum velocity of racemization were determined from the Lineweaver and Burk plots[7], to be 2.6×10^{-2} M and 0.051 μmole/minute/mg of protein, respectively.

L-Alanine is not a substrate of α,β-elimination or β-replacement reactions catalyzed by tyrosine phenol-lyase, but it does act with the enzyme as a competitive inhibitor[2]. Addition of *L*-alanine to holo-tyrosine phenol-lyase resulted in the appearance of a new spectral band

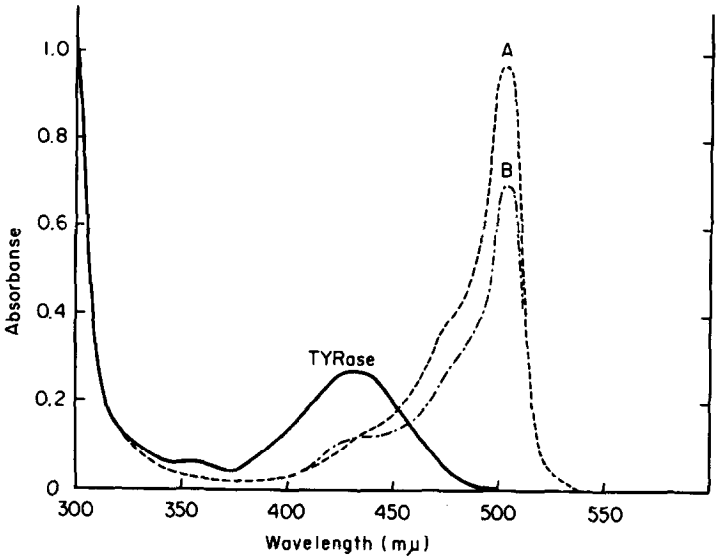

Fig. 3. Absorption spectra of holotyrosine phenol-lyase in the presence of *D*- or *L*-alanine. The solutions contained 3.9 mg of the holoenzyme in 1 ml of 0.01 M potassium phosphate buffer, pH 8.0. The spectra were taken at 25° 10 minutes after 67 μmoles of *L*- (curve A) or *D*-alanine (curve B) were added to the solutions. The spectrum of enzyme alone was indicated as TYRase.

near 500 mμ with a shoulder at 470 mμ (Fig. 3, curve A). A similar spectral change was also found on the addition of *D*-alanine to the holoenzyme (Fig. 3, curve B).

Discussion

The appearance of a new absorption peak around 500 mμ on addition of substrate or inhibitor was observed in many pyridoxal phosphate dependent enzymes. According to the general mechanism proposed by Metzler, Ikawa and Snell[8], many nonenzymatic reactions of amino acids with pyridoxal proceed by several intermediates. Schiff bases of aldimine and ketimine are proposed as the intermediates of such reactions. From spectral studies on crystalline tryptophanase of *E. coli*, Morino and Snell[9] have concluded that the absorption at 500 mμ can be ascribed to the deprotonated aldimine Schiff base or to a species in equilibrium with one of the aldimine and ketimine Schiff bases.

The racemization of *D*- or *L*-alanine by tyrosine phenol-lyase can be explained by adopting the general mechanism for pyridoxal dependent reactions. The racemization proceeds by the temporary abolition of the center of asymmetry at the α-carbon atom of alanine, attending reversible rearrangement of the aldimine Schiff base to the ketimine Schiff base.

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