Stereochemistry of the Conversion of Serine and Tyrosine into Pyruvate by Tyrosine Phenol-lyase

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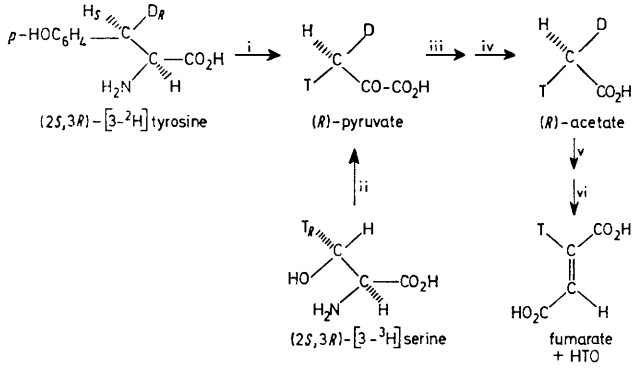
Summary (2*S*,3*R*)- and (2*S*,3*S*)-[3-^{3}H]serine in D_2O and (2*S*,3*R*)-[3-^{2}H]tyrosine in HTO were incubated with tyrosine phenol-lyase to give pyruvate which was trapped as lactate; chirality analysis of the methyl group in the products showed that the replacement of the β-substituent in these amino acids by hydrogen had occurred stereospecifically with retention of configuration.

TYROSINE PHENOL-LYASE [L-tyrosine phenol-lyase (deaminating) EC 4.1.99.2 formerly known as β-tyrosinase] is a pyridoxal phosphate-dependent microbial enzyme, which catalyses β-replacement and reversible αβ-elimination reactions of L-tyrosine and some other β-substituted α-amino acids.[1] Recent work from two laboratories has established the steric course of the β-replacement reactions of L-serine to give L-tyrosine[2] and tyrosine to give L-2,4-dihydroxyphenylalanine.[3] In both cases tyrosine phenol-lyase catalysed the replacement reaction with retention of configuration at the β-carbon atom, in conformity with the stereochemistry observed for related pyridoxal phosphate enzymes, *i.e.*, tryptophan synthetase,[4,5] tryptophanase,[6]



SCHEME. Steric course of the tyrosine phenol-lyase-catalysed deamination of serine and tyrosine. i; H^3HO, tyrosine phenol-lyase; ii; D_2O, tyrosine phenol-lyase; iii; lactate dehydrogenase–NADH; iv; dichromate–2N H_2SO_4; v; acetate kinase–ATP, phosphotransacetylase–CoASH, malate synthetase–glyoxylic acid vi; fumarase.

and *O*-acetylserine sulph-hydrase.[7] We now report on the steric course of the αβ-elimination process catalysed by tyrosine phenol-lyase, *i.e.*, reactions (1) and (2).

L-Serine ⟶ pyruvic acid + NH_3 (1)

L-Tyrosine + H_2O → phenol + pyruvic acid + NH_3 (2)

(2*S*,3*R*)- and (2*S*, 3*S*)-[*U*-^{14}C,3-^{3}H]serine[7] (< 0·1 μmol) were incubated at 30 °C for 3h in D_2O (0·25 ml) with potassium phosphate (25 μmol; pH 8·3), tyrosine phenol-lyase (0·18 IU), pyridoxal phosphate (0·5 μmol), NADH (1 μmol), and an excess of lactate dehydrogenase (5 IU).† After the reaction, lithium lactate (20 μmol) was added as a carrier, and the lactate was isolated by paper chromatography (EtOH–conc.NH_4OH–H_2O, 80:4:16, R_f 0·55), and oxidized with dichromate to give acetate.[8] Conversion of serine into lactate in these first incubations was only about 15%; the remaining serine was therefore reisolated and incubated again in essentially the same manner. The four acetate samples were analysed separately for the chirality of the methyl group by the method of Cornforth *et al.*[9] and Arigoni *et al.*,[10] following Eggerer's procedure.[9] In this analytical procedure, which involves conversion into malate with malate synthetase followed by reaction with fumarase, retention of more than half of the tritium in the malate–fumarate mixture during equilibration with fumarase indicates the (*R*) configuration of the acetate analysed, whereas retention of less than half of the tritium indicates the (*S*) configuration.

The results (Table) indicate that the two acetate samples from the first incubation were chiral; therefore the protonation at the β carbon atom occurs stereospecifically. The configurations of the acetate samples, (3*R*)-serine giving (*R*)-acetate and (3*S*)-serine giving (*S*)-acetate, indicate that the replacement of the β-substituent by a proton proceeds with retention of configuration. The chirality analyses of the acetate samples from the second incubation, in which the serines reisolated from the first incubation were converted into lactate, show that serine apparently undergoes extensive racemization at the β carbon during incubation with tyrosine phenol-lyase.

The same stereochemical course, replacement of the β-substituent by hydrogen with retention of configuration, was also observed in the conversion of (2*S*,3*R*)-[3-^{2}H]-tyrosine[11] into lactate in tritiated water. The methyl group formed had the (*R*) configuration (Table), although for reasons which are not apparent the stereochemical purity of the sample was low. As is also shown in the Table, (2*S*,3*S*)(2*R*,3*R*)-[2,3-$^2H_2$]tyrosine in HTO gave lactate in which the methyl group was achiral, in accordance with the known ability of tyrosine phenol-lyase to deaminate both D- and L-tyrosine.[12]

The results reported here for tyrosine phenol-lyase parallel those obtained recently for tryptophanase[6] and D-serine dehydratase,[13,14] two other pyridoxal phosphate-

† 1 IU (International Unit) of the enzyme converts 1 μmol of the substrate per minute under standard conditions.

TABLE. Chirality analysis of acetate samples obtained from degradation of lactate produced by deamination of serine or tyrosine with tyrosine phenol-lyase and reduction with lactate dehydrogenase.

Substrate for deamination T/^{14}C of	(2*S*,3*R*)-[*U*-^{14}C, 3-^{3}H]serine in D_2O (expt. 1)	(2*S*,3*S*)-[*U*-^{14}C, 3-^{3}H]serine in D_2O (expt. 2)	(2*S*,3*R*)-[*U*-^{14}C, 3-^{3}H]serine (reisolated from expt. 1) in D_2O	(2*S*,3*S*)-[*U*-^{14}C, 3-^{3}H]serine (reisolated from expt. 2) in D_2O	(2*S*,3*R*)-[3-^{2}H] tyrosine in HTO	(2*S*,3*S*)(2*R*,3*R*) [2,3-$^{2}H_2$]tyrosine in HTO
Serine	3·11	2·38	n.d.[a]	n.d.	—	—
Lactate	2·82	2·41	n.d.	n.d.	—	—
Acetate	4·10	3·91	3·80	2·72	8·57[b]	8·43[b]
Malate	3·01	2·84	2·54	1·79	5·39	4·61
Malate : fumarate (after equilibration with fumarase)	2·18	1·10	1·44	0·89	3·32	2·24
% T-retention in fumarase reaction[c]	72·4	38·7	56·7	49·7	61·6	48·6

[a] n.d. = not determined. [b] ^{14}C-Acetate added to tritiated samples to obtain this ratio. [c] Values for authentic reference samples of chiral acetate were (*R*)-acetate 72·2%; (*S*)-acetate 33·7%.

containing enzymes functioning primarily in $\alpha\beta$-elimination reactions. These enzymes, also, catalyse stereospecific protonation at the β-carbon on the same side as the leaving β-substituent, *i.e.*, with retention of configuration. In contrast, the protonation at C_β in the deamination of serine catalysed by tryptophan synthetase β_2 protein is nonstereospecific.[6] This latter enzyme normally functions in a β-replacement rather than an $\alpha\beta$-elimination reaction.

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