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Stereochemistry of ammonia elimination from L-tyrosine with L-phenylalanine ammonia-lyase

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SUMMARY

The *trans* stereochemistry was established for the enzymatic deamination by L-phenylalanine ammonia-lyase from *Rhodotorula texensis*, based on the spectroscopic analysis of the deamination products from stereospecifically labeled L-(3*R*)-[3-$^2H_1$]- and L-(3*S*)-[2,3-$^2H_1$]tyrosine used as substrate. The exchange of hydrogen on C-2 was not observed during the ammonia-elimination process.

L-Phenylalanine ammonia-lyase has been found in higher plants[1–4], *Basidiomycetes* fungi[7] and yeast[8]. In both higher plants and fungi, it is frequently found in conjunction with tyrosine ammonia-lyase. On the other hand, L-phenylalanine ammonia-lyase[9,10] from *Rhodotorula texensis* is known to catalyze the elimination of ammonia not only from L-phenylalanine but also from L-tyrosine to give *trans*-cinnamic acid and *p*-coumaric acid, respectively[11]. The enzymes from potato tubers[4] and *Ustilago bordi*[6] have been highly purified, but they both are inactive toward L-tyrosine.

The stereochemistry of deamination from L-phenylalanine was defined by Hanson *et al.*[12] as anti-periplanar elimination with abstraction of the *pro-S* hydrogen from C-3. In order to establish the steric course of deamination of L-tyrosine (Fig. 1), stereospecifically deuterated L-tyrosine was treated with phenylalanine ammonia-lyase from *R. texensis*.

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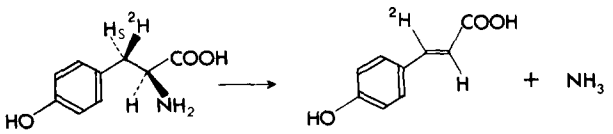

Fig. 1. Ammonia elimination of L-(3*R*)-[3-$^2H_1$]tyrosine by L-phenylalanine ammonia-lyase of *Rhodotorula texensis*.

L-Phenylalanine ammonia-lyase was prepared from cells of *R. texensis* [IFO 0932] grown in a tyrosine-containing medium, according to Ogata *et al.*[9] with the following modification. *R. texensis* [IFO 0932] was cultivated in a half-synthetic medium containing 0.1% L-tyrosine at 28 °C for 48 h. Harvested cells were rinsed with water and disrupted with a sonic oscillator. Crude extract was treated with $MnSO_4$ to remove coloring matter and fractionated by 0.4–0.8 ammonium sulfate saturation. The dialyzed ammonium sulfate fraction was treated with protamine sulfate and subjected to DEAE-cellulose column chromatography followed by Sephadex G-200 gel filtration.

L-(3*R*)-[3-$^2H_1$]Tyrosine was synthesized by the method of Kirby and Michael[13] and by subsequent resolution of racemic tyrosine ethyl ester with chymotrypsin; (T-1, m.p. 294–295 °C decomposition, deuteration 95 ± 5%, $[\alpha]_D^{30}$ −10.0 °C in 5 M HCl c = 1.14) L-(3*S*)-[2,3-$^2H_2$]Tyrosine was obtained by nearly the same procedure as described above, except the reduction with deuterium over Pd on carbon in a mixture of ^2H_2O and dioxane; (T-2, m.p. 288–290 °C decomposition, deuteration 75 ± 2% on C-2 and C-3, respectively, $[\alpha]_D^{30}$ −3.2 in 1 M HCl, c = 1.35).

A reaction mixture containing 200 mg of substrate, 16 mmoles of Tris–HCl buffer (pH 8.5) and 60 mg of L-phenylalanine ammonia-lyase (641 units per mg protein), in total volume 160 ml, was incubated at 30 °C for 12 h. After termination of reaction by heating the mixture at 80 °C for half an hour, the denatured protein was removed by filtration and the filtrate was extracted with ether. The extract was dried over anhydrous sodium sulfate. After removal of solvent, the residue was submitted to sublimation under reduced pressure to yield *p*-coumaric acid (m.p. 201–204 °C, 20 mg from T-1 and 5 mg from T-2, respectively).

Deuterium retention in these acids was assessed by both NMR (60 Mc Varian) and mass spectroscopies. The mass spectrum of *p*-coumaric acid from T-1 showed a molecular ion peak at m/e = 165 and revealed the presence of one ^2H atom, per molecule. The NMR spectrum of this acid (5% in [^2H]acetone, internal tetramethyl silane) was analyzed as follows; δ = 6.75–7.70 AA′BB′ 4H in *p*-substituted aromatic ring, δ = 6.35 quasi triplet 1H vinyl proton on C-2, which coupled with deuterium on C-3, $J_{HD,trans}$ = 2.2 Hz, and δ = 6.00–6.50 broad singlet 2H of hydroxyl groups (Fig. 2). These results are reasonably responsible to the location of deuterium on C-3. This spectrum also indicated the contamination of unlabeled *p*-coumaric acid (6 ± 5%), in which olefinic protons on C-2 and C-3 were detected by chemical shifts at δ = 6.35 and 7.65, in doublet, respectively, with J_{trans} = 15 Hz. This acid must have originated in unlabeled L-tyrosine contained in the substrate used, and the amount of which compares well that in the starting material

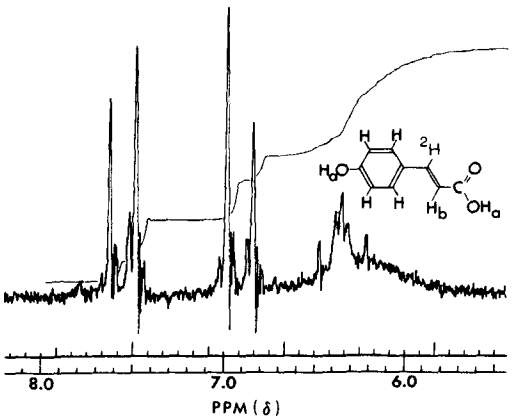

Fig. 2. NMR spectrum of enzymatically synthesized *p*-[3-^{2}H] coumaric acid. The spectrum was taken in [U-^{2}H]ethyl ether with internal tetramethyl silane by Varian A-60 spectrometer.

(5 ± 5%). Thus, the result indicates that the *pro-S* hydrogen on C-3 is eliminated together with amino group on C-2 in a *trans* fashion to give *p*-coumaric acid, and the possibility of *cis*-elimination may well be excluded.

L-(3*S*)-[2,3-$^2H_2$]Tyrosine (T-2) was used to check the probability of proton exchange on C-2 with water during the enzymatic reaction course. The NMR spectrum showed the product *p*-coumaric acid to contain deuterium to the extent of 25% on C-2 and no deuterium on C-3. The absence of labeling on C-3 in the product rejects again the possibility of *cis*-elimination, and the residual deuterium on C-2, though on a very low level, reveals that there is not significant loss or exchange of hydrogen at C-2 in the enzymatic process. The low reaction yield and decreased content of isotope in the acid obtained may be due to a remarkable primary isotope effect which operates in the elimination of *pro-S* hydrogen (deuterium in this case) on C-3. So the unlabeled L-tyrosine involved may probably be responsible for the non-deuterated product acid.

The stereochemistry of the deamination of L-tyrosine catalyzed by L-phenylalanine ammonia-lyase from *R. texensis* is defined as *trans*, without exchange of hydrogen at C-2, which parallels those of L-phenylalanine[12] and L-histidine[14].

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