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Enzymatic Synthesis of Aza-L-tyrosines

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Abstract—Tyrosine phenol-lyase from *Citrobacter freundii* synthesizes 2-aza-L-tyrosine and 3-aza-L-tyrosine from 3-hydroxypyridine and 2-hydroxypyridine, respectively, and ammonium pyruvate.

A convenient method for obtaining optically pure α-amino acids involves using enzymes to establish the desired stereochemistry at the α-carbon. Tyrosine phenol-lyase (deaminating) [EC 4.1.99.2] is a pyridoxal-5′-phosphate (PLP)-dependent enzyme that catalyzes the β-elimination of L-tyrosine to phenol and ammonium pyruvate, as shown in Scheme 1.[1,2] This process is reversible, and hence TPL also catalyzes the reverse reaction which synthesizes L-tyrosine from phenol and ammonium pyruvate.[2,3] In the present study, we were interested in determining if 2-aza-L-tyrosine (**1**) and 3-aza-L-tyrosine (**2**) could be synthesized using tyrosine phenol-lyase. Nagasawa et al. reported the syntheses of numerous optically pure ring-substituted tyrosine derivatives using tyrosine phenol-lyase.[4] However, under the conditions used in their experiments, 2-hydroxypyridine (which exists in solution predominantly as the 2-oxo-1*H* tautomer) and 3-hydroxypyridine failed to give rise to the corresponding amino acids, 3-aza-L-tyrosine and 2-aza-L-tyrosine. One of the purposes for the present study was to determine if changes in the reaction conditions would facilitate the formation of the aforementioned amino acids. We now report that 2-aza-L-tyrosine and 3-aza-L-tyrosine can be synthesized using recombinant tyrosine phenol-lyase from *Citrobacter freundii*,[5] following a series of experiments designed to determine the optimum conditions necessary for production of these amino acids (Scheme 2).

All reactions were performed using 100 mM ammonium chloride, 162 μM pyridoxal-5′-phosphate, 63 mM pyruvic acid, and 20 mM 2- or 3-hydroxypyridine in a total volume of 350 mL. The reactions were performed at pH 8.8–9.0 and 37 °C for 3-azatyrosine and pH 8.0–8.2 and 25 °C for 2-azatyrosine. Synthesis of 3-azatyrosine at 25 °C resulted in a 50% decrease in yield. Tyrosine phenol-lyase (42 mg) was added to the reaction mixture once the pH had been adjusted with concentrated NH_4OH. After 5 h the pH had to be re-adjusted with 2 N NH_4OH. The reactions were then incubated for 5 days at the appropriate temperature. Each day, the pH was checked and adjusted as needed. On the third day, an additional 4.2 mg of tyrosine phenol-lyase was added, after the pH had been adjusted, to compensate for denaturation of the enzyme that may occur during incubation.

2-Aza-L-tyrosine

Following incubation of the reaction mixture with TPL for 5 days, the yellow solution was heated quickly to 100 °C for 1 min to denature the remaining enzyme. After cooling, the denatured enzyme was removed by filtration through Celite. The filtrate was applied to a Dowex 50W X8 (H^+ form) column (2.5×25 cm). The column was washed with 1 L of water to remove unreacted 3-hydroxypyridine and pyruvic acid, and the azatyrosine was eluted with 0.75 M NH_4OH. The solvent was removed in vacuo. The residue was suspended in MeOH and evaporated to dryness. A small amount of MeOH was then added, and the mixture was filtered to give pure 2-aza-L-tyrosine (45.3 mg, 12.5%). ^1H NMR (D_2O) δ 7.69 (bs, 1H), 6.99 (d, J = 8.8 Hz, 1H), 6.89 (dd, J = 2.0, 8.8 Hz, 1H), 3.42 (dd, J = 5.4, 8.6 Hz, 1H), 2.89 (dd, J = 5.4, 13.3 Hz, 1H), 2.63 (dd, 8.6,

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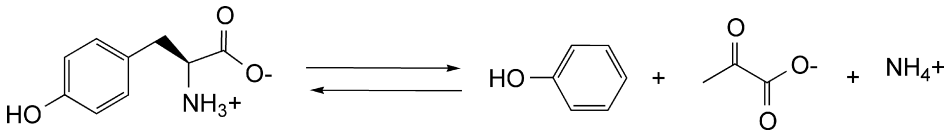
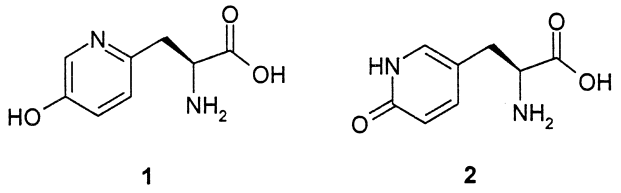


Scheme 1. Reaction catalyzed by tyrosine phenol-lyase.



Scheme 2. Structures of 2-aza-L-tyrosine (**1**) and 3-aza-L-tyrosine (**2**).

13.3 Hz, 1H); MS (ESI) m/z 183 (MH^+); UV (0.1 M HCl), $\lambda_{max} = 290$ nm (log $\varepsilon = 3.83$); $[\alpha]_D^{20}$ +51.6 (c 0.91, 0.1 M HCl), lit., +55 (c 1.1, 1.0 M HCl).[6] The NMR, MS and UV properties of 2-aza-L-tyrosine were identical with those of synthetic racemic 2-azatyrosine.

3-Aza-L-tyrosine

Following incubation of the reaction mixture with TPL for 5 days, the yellow solution was heated to 100 °C quickly for 1 min to denature the remaining enzyme. After cooling, the denatured enzyme was removed by filtration through Celite. The filtrate was applied to a Dowex 50W X8 (H^+ form) column (2.5×25 cm). The column was washed with 1 L of water to remove unreacted hydroxypyridine and pyruvic acid. The azatyrosine was eluted with 0.75 M NH_4OH. The solvent was removed in vacuo, and the residue was dissolved in a minimum amount of water. Acetone was added until the solution just remained cloudy. After sitting at room temperature overnight, 3-aza-L-tyrosine was collected by filtration (36.5 mg, 10%). ^{1}H NMR (D_2O/NaOD) δ 7.65 (dd, $J = 2.4$, 9.0 Hz, 1H), 7.42 (d, $J = 2.4$ Hz, 1H), 6.63 (d, $J = 9.0$ Hz, 1H), 3.89 (t, $J = 6.5$ Hz, 1H), 3.01 (t, $J = 6.5$ Hz, 2H); MS (ESI) m/z 183 (MH^+); UV (H_2O), $\lambda_{max} = 230$ nm (log $\varepsilon = 3.99$), $\lambda_{max} = 300$ nm (log $\varepsilon = 3.75$); $[\alpha]_D^{20}$ +17.9 (c 0.10, H_2O). The NMR, MS, and UV properties of 3-aza-L-tyrosine were identical with those of synthetic racemic 3-azatyrosine.

Aza analogues of L-tyrosine are of interest as antimetabolites of tyrosine and inhibitors of enzymes which react with free L-tyrosine in solution or tyrosyl residues in proteins. Thus, azatyrosines are potentially useful as antibiotics or drugs. The antitumor activity of 2-aza-L-tyrosine,[7] a natural product originally isolated from a *Streptomyces* sp., has stimulated interest in the stereoselective synthesis of this compound. 3-Aza-L-tyrosine is also a natural product isolated from a toxic mushroom, *Clitocybe acromelalga*.[8] A number of syntheses of 2-aza-L-tyrosine and 3-aza-L-tyrosine have been reported.[8,9] Although the yields are low, the new procedure reported herein provides 2-aza-L-tyrosine and 3-aza-L-tyrosine in a single step from readily available achiral starting materials.

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