Towards the elucidation of structural events during the catalysis by tyrosine phenol-lyase: crystal structures of different enzyme forms and their complexes with substrate analogues

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Tyrosine phenol-lyase (TPL; EC 4.1.99.2) is a homotetrameric pyridoxal-5'-phosphate (PLP)-dependent enzyme that catalyses the β-elimination of L-tyrosine (the reversible hydrolytic cleavage of L-tyrosine to phenol and ammonium pyruvate [1]). Except for the physiological reaction, TPL also catalyzes the β-elimination reactions of some other amino acids and their derivatives, β-replacement reactions, racemization of alanine and the synthesis of some L-tyrosine derivatives (*e.g.* 3,4-dihydroxy-L-phenylalanine (L-DOPA) – the precursor in the synthesis of dopamine [2]). The β-elimination proceeds via several intermediate steps, including the cleavage of the C_β-C_γ bond. In order to reveal details in the enzymatic reaction and understand structural events during the catalysis, we determined the X-ray structures of several different forms of TPL from *Citrobacter freundii*, including the structures of noncovalent complexes which resemble the key reaction intermediates. The previously known crystal structures of the apoenzyme [3] and the holoenzyme complexed with a substrate analogue [4] were solved at a lower resolution and using the data obtained from the crystals grown at pH 6.0. In contrast, we studied the crystals grown at pH 8.0, which is pH closer to the physiological value, and observed some interesting differences between the structures at the two pH values. There are two subunits in the asymmetric unit constituting the "catalytic dimer" with two active sites located at the interface between the two noncrystallographically related subunits. One active site is in the "closed", while the other is in the "open" conformation. It is assumed that the transition from the "open" to the "closed" form of the active site during the enzymatic reaction is important for the enzyme catalytic efficiency.

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