

MECHANISM AND PROMISCUITY OF ISOCHORISMATE PYRUVATE LYASE

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Despite their broad utility in the laboratory, pericyclic reactions seem to be rarely exploited in cellular metabolism. The Claisen rearrangement of chorismate to prephenate catalyzed by chorismate mutase is the best known example. Moreover, the catalytic mechanism of some pyruvate lyases initially described as a general base process, have been recently proposed to follow a concerted pericyclic pathway. In order to test this hypothesis, we present a theoretical insight into the reaction catalyzed by isochorismate pyruvate lyase (IPL) from *Pseudomonas aeruginosa* and the uncatalyzed reaction in solution. Two dimensional PMF (2D-PMF) had been carried out in order to unambiguously determine the reaction path. The results show that the enhancement of the rate constant, by comparison to the reaction in solution, is in agreement with the experimental data when a pericyclic mechanism is explored¹. The enzyme possesses also weak chorismate mutase activity. The catalytic promiscuity frequently exhibits both traits plasticity and robustness, which means that the efficiency of secondary reaction may be improved dramatically by means of one or few mutations, while the primary activity is not affected by these mutations. Based in the comparison of the behaviour of *Escherichia coli* chorismate mutase and IPL along the chorismate rearrangement, the mutation of Alanine 38 to Isoleucine has been proposed by us. This mutation increases the catalytic rate constant of the secondary reaction by a factor^{2,3} of 1000 and the rate constant of the primary reaction by a factor¹ of only 6. It must be emphasized that the primary reaction has been improved also by this mutation, showing that IPL enzyme is not a dead end at all.

¹ S. Martí, J. Andrés, V. Moliner, E. Silla, I. Tuñón, J. Bertran, in preparation.

² S. Martí, J. Andrés, V. Moliner, E. Silla, I. Tuñón, J. Bertran, *J. Am. Chem. Soc.* **2008**, *130*, 2894-2895.

³ S. Martí, J. Andrés, V. Moliner, E. Silla, I. Tuñón, J. Bertran, *Chem. Soc. Rev.* **2008**, *37*, 2634-2643.