Poster Presentation

MS29.P15

Substrate distortion in the catalysis of orotidine monophosphate decarboxylase

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One way for enzymes to affect reactions they catalyze is through transition state stabilization. Another factor to be considered is the contribution of substrate distortion, although it has been thoroughly described for only a few enzymes. We have a longstanding interest in the reaction mechanism of orotidine monophosphate decarboxylase (ODCase) and determined various crystal structures bound with distorted substrates at around 1.5 Å resolution. The enzyme is known as one of the most proficient enzymes, which accelerates the decarboxylation of orotidine 5'-monophosphate (OMP) to form uridine 5'-monophosphate (UMP) by 17 orders of magnitude. One argument against the contribution of substrate distortion to the ODCase reaction is the weak affinity of UMP. The distortions observed so far all appear at the C6-substituent of the pyrimidine ring, which corresponds to the carboxylate of OMP. Since the carboxylate is removed by the reaction, the product UMP should bind more tightly to ODCase than OMP, if the distortion of C6-substituent contributes to the catalysis. In order to investigate this inconsistency, we determined the crystal structure of ODCase with UMP at atomic resolution (1.03 Å). The structure showed an unfavorable interaction between UMP and the catalytic residue K72, an interaction considered to be absent in the OMP complex. Surface plasmon resonance analysis indicated that UMP binds stronger to the K72A mutant than to the wild-type enzyme by 5 orders of magnitude. These analyses invalidate the argument against a contribution of substrate distortion to ODCase catalysis. Finally, we estimated how much the distortion contributes to the catalysis using computational simulation methods. The results indicated that 10-15% decrease of the ΔΔG‡ value is contributed by substrate distortion.

[1] *M. Fujihashi, K. Mito, EF Pai, et al. J. Biol. Chem., 2013, 288, 9011-6,* ***[2]*** *M. Fujihashi, T. Ishida, S. Kuroda, et al., J. Am. Chem. Soc., 2013, 135, 17432-43*

Keywords: Enzyme mechanism, Substrate distortion