Crystallographic Analysis Identifies a Novel Enzymatic Reaction

Masahiro Fujihashi[ab], Angelica M. Bello[cd], Lakshmi P. Kotra[cd], Emil F. Pai[ae], [a]*Ontario Cancer Inst.*, [b]*Grad. School of Science, Kyoto Univ.* [c]*MDIT center*, [d]*Faculty of Pharmacy*, [e]*Dept. of Biochem., Univ. of Toronto.* E-mail: bxk04063@nifty.com

A novel enzymatic reaction has been identified with the help of crystallographic analysis. *In vivo*, orotidine 5'-monophosphate decarboxylase (ODCase) catalyzes the conversion of orotidine 5'-monophosphate (OMP) to uridine 5'-monophosphate (UMP), most probably through a carbanion intermediate and lasting about 50 milliseconds. During crystallographic studies of ligand binding, we discovered the slow (several days to completion) hydrolysis of 6-CN-UMP to 6-OH-UMP (BMP), the most potent inhibitor of ODCase known. Interestingly, the only obvious mechanism for this reaction seems to be a nucleophilic substitution.

Three independent approaches have confirmed this novel reaction: First, an electron density map (1.45 Å) from a crystal of ODCase incubated with 6-CN-UMP at room temperature for 2 months clearly identifies the compound at the active site as BMP. Second, MS analysis of ODCase freshly mixed with 6-CN-UMP shows a peak consistent with the presence of an ODCase-6-CN-UMP complex; after 7 days incubation, the spectrum displays a peak corresponding to an ODCase-BMP complex. Third, kinetic studies, taking advantage of the very low dissociation constant of BMP, confirmed the transformation. As 6-CN-UMP is converted to BMP, the product binds (almost) irreversibly to the enzyme's active site rendering it inactive. We conclude ODCase is catalyzing the conversion of 6-CN-UMP into BMP. Experiments to trap the 6-CN-complex of ODCase and to elucidate its crystal structure are in progress.

Keywords: enzyme catalysis, biomacromolecules, enzyme structure function