

Enzymes: Mechanisms, Kinetics & Inhibition

- 1) Before the crystal structure of orotidine 5'-monophosphate decarboxylase (ODCase) was solved in 2000, three alternative mechanisms for the decarboxylation of OMP were considered likely. While the atomic-level analysis of this enzyme has helped to disprove some of these mechanisms, the molecular mechanism of catalysis by this highly proficient enzyme remains largely unsolved.
 - a) Draw (with appropriate chemical structures) the three alternative mechanisms that were considered prior to availability of the crystal structure. If you cannot find out from the literature what these three mechanisms were, you can come up with your own hypotheses.
 - b) Using molecular visualization software¹ (Maestro, SYBYL, PyMOL, or any other program of your choice), prepare an image of the ODCase active site showing the bound nucleobase and the residues that are in direct contact with the nucleobase. You do not need to show the sugar nor 5'-phosphate moieties. Please do not use images that somebody else has made.
 - c) Discuss how the crystal structures of ODCase complexed with inhibitors disprove some of the mechanisms that you outlined earlier.

2. In order for cells replicate, sufficient quantities of nucleotides, such as deoxythymidylate (dTTP), are needed. dTTP is made from deoxyuridylate (dUTP) by thymidylate synthase. Enzymes responsible for synthesis of dUTP from uridine are ribose-1-phosphate uridine phosphorylase, uridine phosphorylase, UMP kinase, ribonucleotide reductase, orotate phosphoribosyltransferase, thymidine kinase, and dUTP diphosphohydrolase. Significant decrease in thymidylate synthase activity is seen after administration of drug 5-fluorouracil. However, this compound does not significantly reduce the activity of the enzymes that are involved in conversion of uracil to dUTP.
 - a) Explain why 5-fluorouracil does not inhibit enzymes that convert uracil to dUTP.
 - b) Explain why administration of 5-fluorouracil leads to reduced thymidylate synthase activity.

a) ¹ If you are not familiar with the use of molecular visualization software, please see a tutorial at <http://www.chem.ucsb.edu/~kalju/chem110L/public/tutorial/start.html> and examples at <http://www.chem.ucsb.edu/~molvisual/>