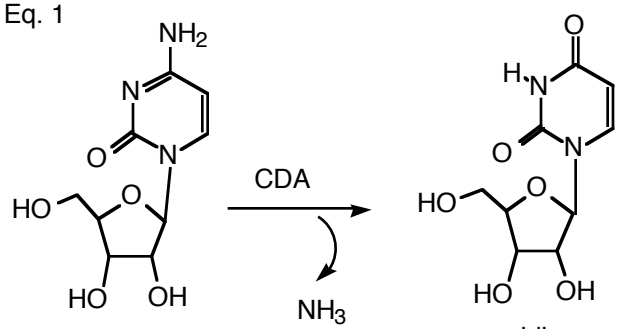
Problem Set 2 Chemistry 5.50 Sept. 16, due Sept 25

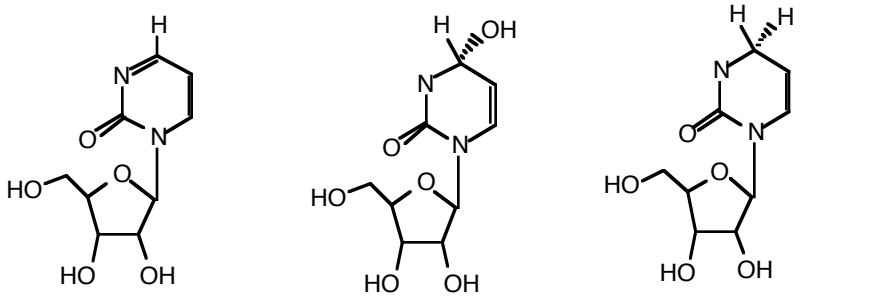
Cytidine deaminase (CDA) catalyzes the conversion of cytidine to uridine with

Eq. 1



cytidine uridine

loss of ammonia (Eq. 1). CDA increases the rate of the hydrolytic deamination of cytidine relative to the non-enzymatic rate, by a factor of 10^{11}.



zebularine ZEB

zebularine 3-4 hydrate, ZEB-H_2O

3-4 Dihydrozebularine DHZ

The Kd (dissociation constants) for cytidine and uridine for CDA have been determined to be 2.1 x 10^{-4} M and 2.5 x 10^{-3} M, respectively. Recently a number of pyrimidine analogs (zebularine (ZEB), zebularine 3-4 hydrate (ZEB-H_2O), and 3-4 dihydrozebularine (DHZ) have been synthesized and tested as inhibitors of CDA. The Ki (thermodynamic dissociation constants) values for ZEB-H_2O and DHZ are 1.2 x 10^{-12} M and 3 x 10^{-5} M, respectively. The difference of a factor of 10^7 created by the replacement of a H of DHZ with a OH group in ZEB-H_2O is remarkable. The structure of CDA has been determined in the presence of uridine (see 1AF2) and ZEB-H_2O (see 1CTU).

An additional piece of information has been obtained that is mechanistically informative. Investigators showed that incubation of zebularine (ZEB) with CDA resulted in a change in its λmax from 303 nm for the free species to 239 nm for the species bound to CDA.

Given this information and using your knowledge of mechanisms for proteases (see class notes and handouts) as potential models for thinking about the CDA reaction, answer the following questions.

1. Without looking at the structures given in the ps 2 folder, propose two different mechanisms by which cytidine might be converted to uridine given the available information above. Your proposal should be based on the information given in class about the mechanisms established for peptide bond hydrolysis by different proteases. One mechanism could involve direct nucleophilic attack by an amino acid residue within the active site (see chymotrypsin) and a second could involve direct nucleophilic attack by a water mechanism similar to aspartate or metallo-proteases. Use generic acid (A) and base (BH) catalysts in your mechanisms.

2. Now pull up the structures of uridine bound to CDA (1AF2) and ZEB-H_2O (1CTU) bound to CDA. You will immediately notice when you look at the PDB header or display all of the heteroatoms associated with your structure is that in addition to the nucleoside bound there is also a zinc. The presence of zinc was missed by previous biochemical studies. Draw a picture of uridine and zinc and its ligands and all of the catalytically interesting residues and their residue numbers. Indicate the distance of the appropriate atoms of these residues to the appropriate atoms of the nucleoside and zinc. Repeat this process for ZEB-H_2O and zinc. [Note: Use the OMP decarboxylase handout as a model for describing what you see in the active sites. Do not print out a diagram from a web site.] I really want you to learn to think about which distances and interactions are important. Looking at a structure takes alot of practive. This exercise may be easier if you use Rasmol (or InsightII) to look within 10 Å of the atom where the chemistry occurs or the zinc. You need to think about which distances are important for bond forming reactions and H-bonding interactions. **Once again your figure should clearly show the interesting amino acid side chains and <u>their residue number</u> and the distance that the reactive atom of these side chains is to the atom of interest in the nucleoside or the metal.**

3 Given the information you have obtained by looking at the structure, you are now in a position to insert details into one of your mechanisms used to answer question 1. Define clearly the role for the appropriate residues that you think are involved in catalysis. Replace A and BH with actual amino acid residues. If neither of your generic mechanisms proposed in question 1 is consistent with your observations in question 2, you are now in a position to propose a detailed third mechanism.

4. Most amazing about the data with the inhibitors described above, is that ZEB-H_2O has a Ki approximately 10^7 lower than that for DHZ and 10^8 lower than the Kd for cytidine.

a. Given your mechanism in question 3, why might ZEB-H_2O have a binding constant so much greater than the normal substrate?

b. Why might ZEB-H_2O have an inhibition constant so much greater than DHZ?

c. Calculate the difference in binding energy in kcal between uridine and ZEB-H_2O?

5. Can you use the two structures to give you insight into the basis of **rate acceleration**

(binding energy, acid/base, etc) over the nonenzymatic reaction by a factor of 10^{11}? Be specific about which aspects of the structure provided the basis for each part of your answer.

6. You are now, hopefully, in a position to provide an explanation for the observed shift in the UV spectrum of ZEB-H_2O on binding to CDA. What is your explanation?'

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