Name Key

Chem 452 - Fall 2012 - Quiz 3

1. In class, we discussed a number of strategies used by enzymes to speed up the rates of the reactions they catalyze. In one or two sentences, describe a specific example for each of the following strategies, drawing from the four systems that we discussed in class. Include each of the four systems as an example for at least one of these strategies. (The systems discussed in class include, *chymotrypsin*, *carbonic anhydrase*, the *EcoRV* restriction endonuclease, and the *myosin II ATPase*.)

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a. Catalysis by approximation:
Enzymes speed up reactions by placing the various players in a reaction next to one another. The catalytic triad in *chymotrypsin* provides an example. Aspartate 102 forms a hydrogen bond to histidine 57, which plays the role of a general acid base catalyst. The asp 102 orients this histidine so that it can accept a proton from the hydroxyl group of serine 195, turning it into a powerful nucleophile, which can then attack the carbonyl of the peptide bond that is to be hydrolyzed in the reaction. All of the other systems discussed could also be cited as examples.

b. Metal ion catalysis:
The Zn^{2+} ion in *carbonic anhydrase* is a good example. It binds the substrate water, lowers its *pK* so that it more readily gives up a proton to produce the reactive hydroxide ion. A Mg^{2+} ion plays a similar role in the mechanism for the *EcoRV* restriction endonuclease.

c. Covalent catalysis:
The ser 195 in *chymotrypsin* serves as a nucleophile in the proteolysis reactions carried out by serine proteases. It catalyzed the hydrolysis of peptide bonds by attacking the the carbonyl carbon of the peptide bond that is being hydrolyzed. This leads to the formation of an intermediate that is covalently bonded as an ester to the ser 195. The ester is later hydrolyzed to return the enzyme to its initial state.

d. General acid/base catalysis:
The histidine 57 described in a. above, is one example. *Carbonic anhydrase* provides a second example with a histidine that helps to remove a proton from the active site after it has been removed from the substrate water molecule. Another examples is provided by ser 236 in the *myosin II ATPase*, which also accepts a proton (as a base) from the substrate water after if first donates a proton (as an acid) to the γ-phosphate of the substrate ATP.

e. Substrate specificity:
The *EcoRV* endonuclease provides a good example of specificity. It binds non-specifically to a DNA molecule, and then slides along it until finds it recognition. A number of hydrogen bonds then form with the DNA which provides sufficient free energy to kink the DNA and force the phosphate-ribose backbone into the the active site of the enzyme.

f. Transition state stabilization:
The oxyanion hole in the *chymotrypsin* reaction helps stabilize the negatively charge, tetrahedrally-shaped transition state in the chymotrypsin reaction. The *myosin II ATPase* provided a second example by preferentially binding the pentavalent transition state for the γ-phosphate over the tetravalent γ-phosphate of the ATP substrate.

g. A contribution of a buffer component to the rate-limiting step in a reaction:
This was observed with *carbonic anhydrase* for which the presence of the conjugate base component of the buffer has been shown to produce a concentration dependent increase in the rate of the reaction and therefore is playing a role in the mechanism for the reaction.

2. We spent a good bit of time in class discussing the enzyme aspartate transcarbamoylase (ATCase), which is also known as aspartate carbamoyltransferase, as an example of allosteric regulation of enzyme activity.

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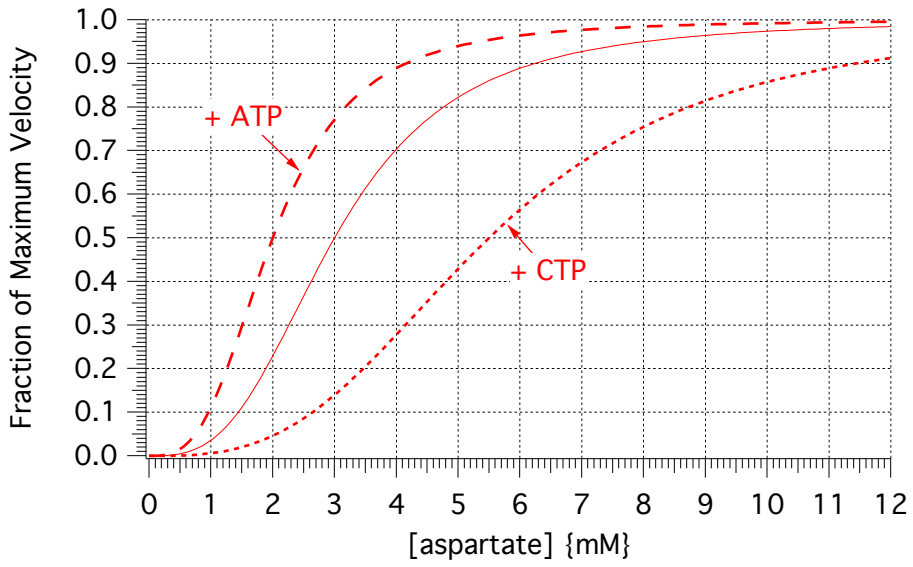
a. What is the function of ATCase?
ATCase catalyzes the first reaction in the metabolic pathway that leads to the synthesis of the pyrimidine nucleotides UTP and CTP. In this reaction, the carbamoyl group is transferred from the carbamoyl phosphate to the α-amino group of aspartate to form N-carbamoylasparate.

b. Which of the six classes of enzymes does ATCase belong to? As its name suggests, it is a transferase.

c. ATCase provides a good example of *feedback inhibition*. Explain what this means.
Feedback inhibition is the phenomenon where the end product of a metabolic pathway serves as an allosteric inhibitor of a reaction occurring near the beginning of the pathway. ATCase, which catalyzes the first reaction in the pyrimidine nucleotide synthesis pathway, is allosterically inhibited the CTP, the end product of the pathway.

d. At *pH* 8.3 and 25°C, the ATCase isolated from *E.coli* displays a K_M value of 3.0 mM when aspartate is varied as the rate limiting substrate. Using the axes shown below, sketch a curve that represent the activity ATCase as a function of the aspartate concentration:



e. Does the curve you drew above represent ATCase as an enzyme displaying Michaelis-Menten kinetics? No Explain:
ATCase displays cooperative binding for its substrate to the 6 active sites. This is why the curves shown above have a sigmoidal (S-shaped) instead of hyperbolic shape that characterizes Michaelis-Menten kinetics.

f. Cytidine triphosphate (CTP) is an *allosteric inhibitor* of ATCase, whereas adenosine triphosphate (ATP) is an *allosteric activator* of ATCase. Sketch curves on the graph above that represents the effect that each has on the kinetics of the ATCase catalyzed reaction. Be sure to label each curve as either "+CTP" or "+ATP".

g. Does ATP stabilize the tense (T) or relaxed (R) state of ATCase? The R state
(The R state is the state that binds the substate with the higher affinity. This therefore lowers the K_M, which shifts the activity curve to the left.)