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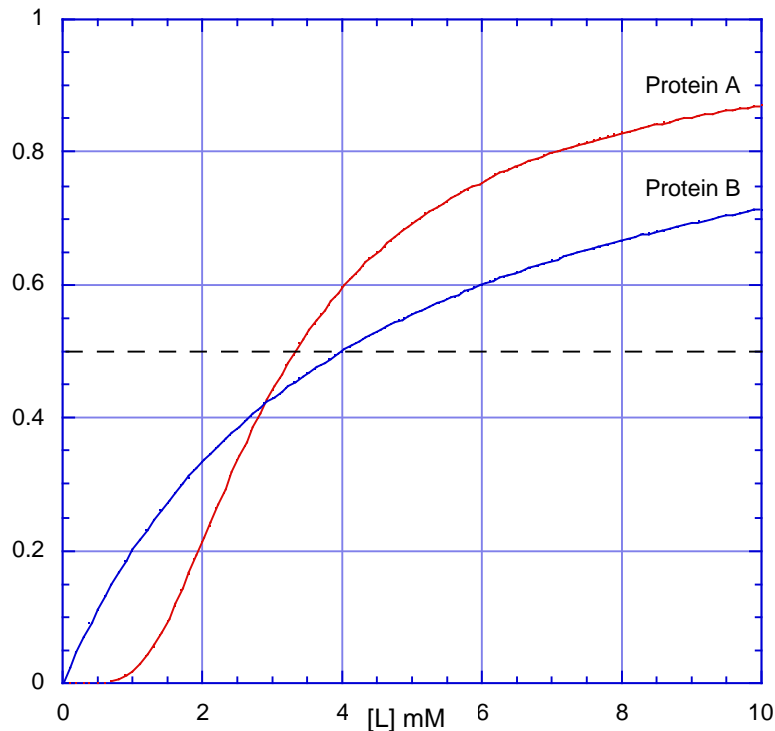
## Exam II - Biochemistry 341

Wednesday, November 17, 1999 - 7:30 AM to 9:00 AM

Show all your work to receive partial credit. Try to use pencil!

Browse over the whole exam and work on the questions that you feel more comfortable with first!

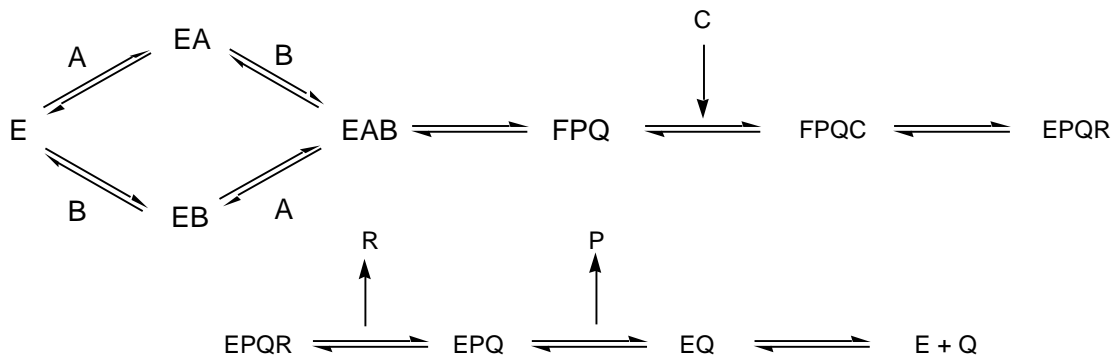
- 1) (20 points) You have recorded binding data for two proteins (**A** and **B**) that bind the same ligand (**L**). The data for both proteins is presented in the following plot.



- Which of the two proteins exhibits cooperative binding?
- Estimate as best as you can from the graph the  $K_{50}$ 's for both proteins.  $K_{50}$  is defined in the same way  $P_{50}$  is defined for hemoglobin.
- At which [L], approximately, does the affinity for **L** of the cooperative protein drop below the affinity of the non-cooperative protein?
- Considering your responses from (ii) and (iii), would you say it is sufficient to compare only  $K_{50}$  values when comparing binding affinities of cooperative and non-cooperative proteins for the same substrate? Explain clearly.

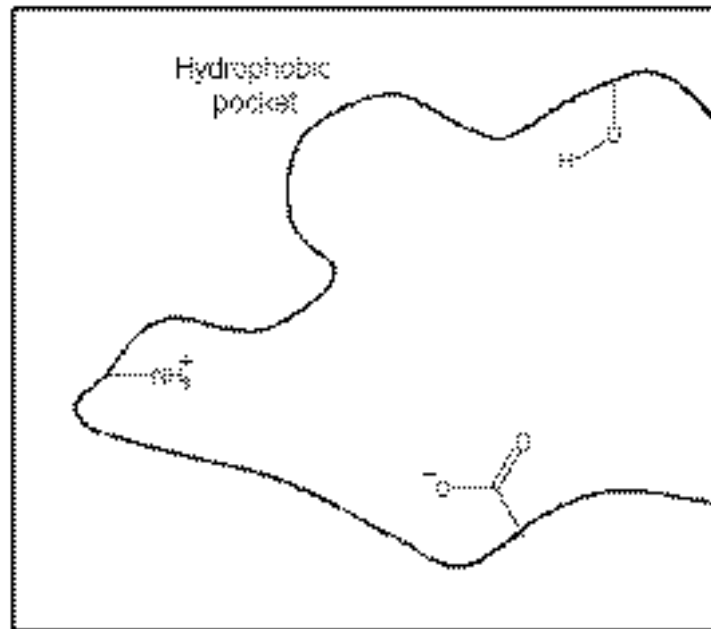
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2) (20 points) You have studied a complex multisubstrate enzyme, and kinetic measurements show that the reaction has the following mechanism:



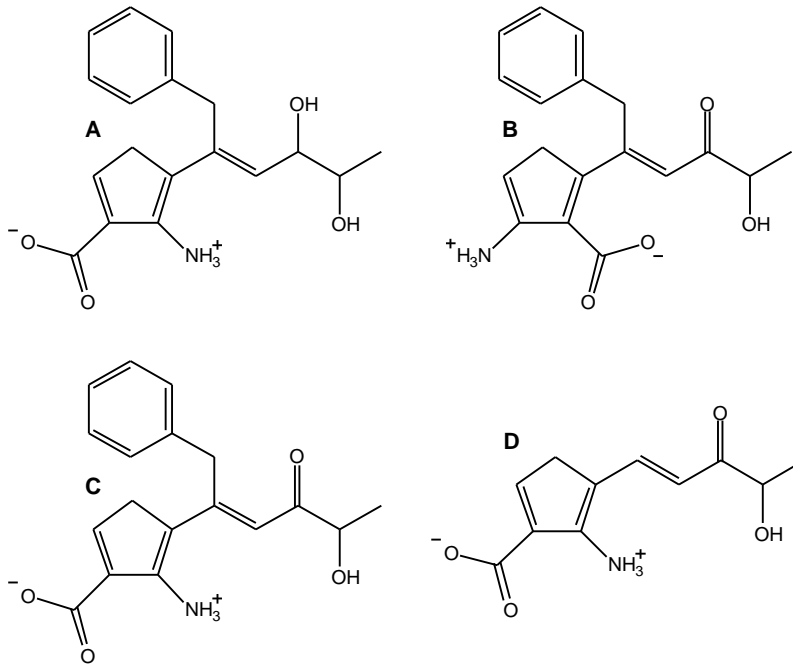
- Draw a detailed Cleeland diagram for this enzymatic reaction, clearly indicating all the species present at each step.
- Indicate clearly substrate binding and product release steps, and steps where chemistry occurs.
- Classify the addition of substrates **A** and **B**, the addition of substrate **C** and release of product **R**, and the release of products **P** and **Q** as sequential ordered, sequential random, or ping-pong mechanisms.
- How many different enzyme species are present through the reaction?

3) (20 points) The active site of the enzyme **examtwoase**, an isomerase, has the following chemical and structural characteristics:



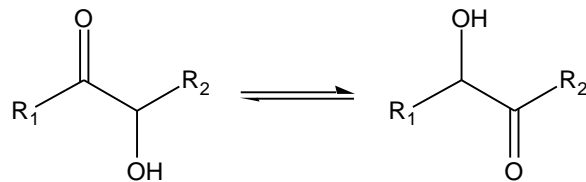
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A chemist has designed four substrates (**A - D**) for the enzyme, shown below.

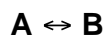


- Which of the substrates will bind better to the enzyme? Which one will bind worst? Justify your answer by dissecting the contributions to the binding energy.
- Draw the best substrate on the binding site diagram provided, and indicate all the interactions that make it the better substrate
- Order the expected reaction rates for the different substrates taking into account your answer of part (i). If you think one of the molecules won't undergo any reaction with this enzyme, mention it.

Hint: Remember that an isomerase catalyzes the following reaction:



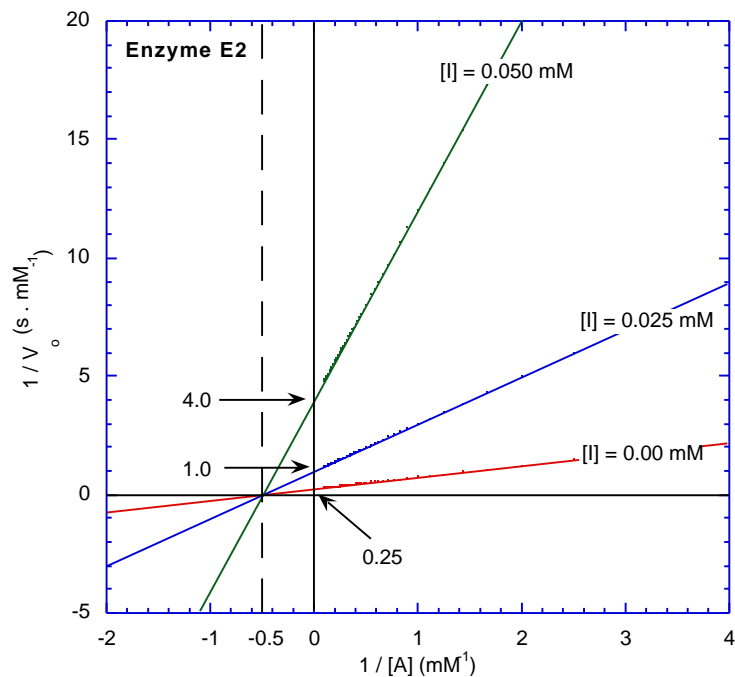
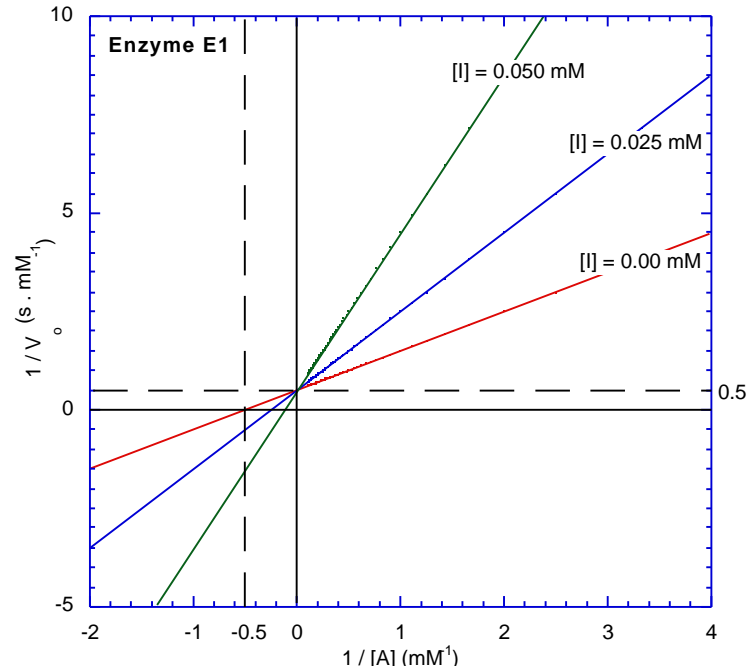
- 4) (20 points) You need to use an enzymatic reaction as part of a large chemical process. The reaction is the typical



where **B** is the product you want. You found two enzymes, **E1** and **E2**, that will catalyze your reaction. They have different  $K_M$  and  $V_{max}$  parameters, and they both show inhibition by **I**, which is a compound that is present as part of

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other reactions in your process. The double-reciprocal plots of  $1 / V_o$  vs  $1 / [A]$  at different concentrations of **I** for both enzymes are shown below:

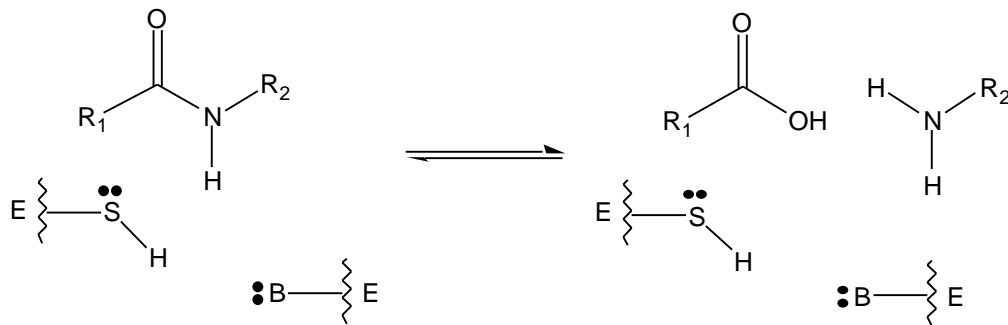


- What type of inhibition does each enzyme exhibits vs **I**?
- Calculate the *real*  $V_{max}$  and  $K_M$  parameters from the graphs for both enzymes. Which enzyme is faster (i.e., has a higher  $V_{max}$ ) at  $[I] = 0$ ?

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iii) You know that under the conditions of your experiment,  $[I]$  will be 0.05 mM. Considering that you can use a large excess of substrate **A** in your system, which enzyme, **E1** or **E2**, would you buy to accomplish your process faster?

5) (20 points) The mechanism of the cysteine proteases is very similar to that of the serine proteases. The main differences are that a cysteine residue is used instead of a serine residue in the initial attack to the peptide group, and that no evidence for a catalytic triad exists. There is, however, evidence that the cysteine residue is activated by general acid-base catalysis as with serine proteases. Consider the following peptide bond hydrolysis:



- Write a detailed mechanism for the enzymatic hydrolysis of the peptide bond presented above, accounting for all nucleophilic attacks and hydrogen abstractions ('push electrons wisely...')
- In the reaction mechanism, indicate which steps can be considered as covalent catalysis and which steps can be considered as general acid-base catalysis.
- Indicate all the transition states that form during the reaction. Hint: There should be two of them...
- You investigated the pH dependence of this cysteine protease, and found that it is active in a narrow pH range, between 5.5 and 6.5. Considering this information, what could be the identity of the residue acting as a base in the reaction?

Additional data:      Glu -  $pK_R = 4.25$       Lys -  $pK_R = 10.53$   
                         Asp -  $pK_R = 3.65$       Arg -  $pK_R = 12.48$   
                         His -  $pK_R = 6.00$

6) **Bonus Question** (20 points) You came across a cysteine protease while investigating a really nasty virus. This protease, which is crucial for the virus to self-replicate, cleaves peptide bonds flanked by small aliphatic residues (like A) to the left, and bulky aromatic residues (like F) to the right. Based on the mechanism of cysteine proteases you outlined in question 5, design a transition state analog that will inhibit this enzyme. Justify your design.