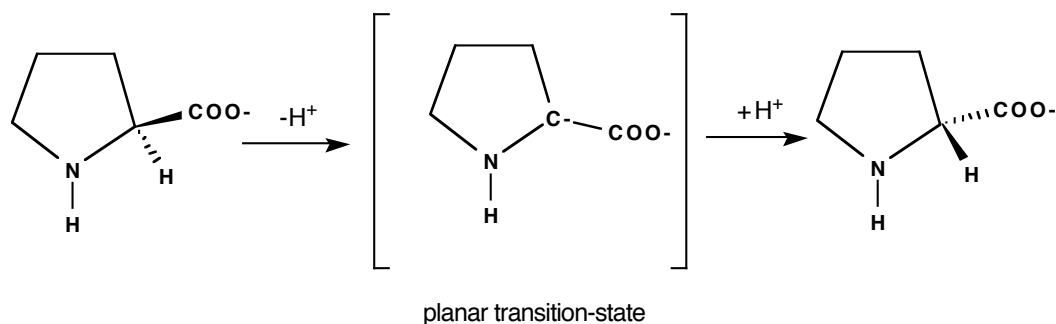


BMB 100B Problem Set 3
Due on Tuesday Feb. 4th, 2003.

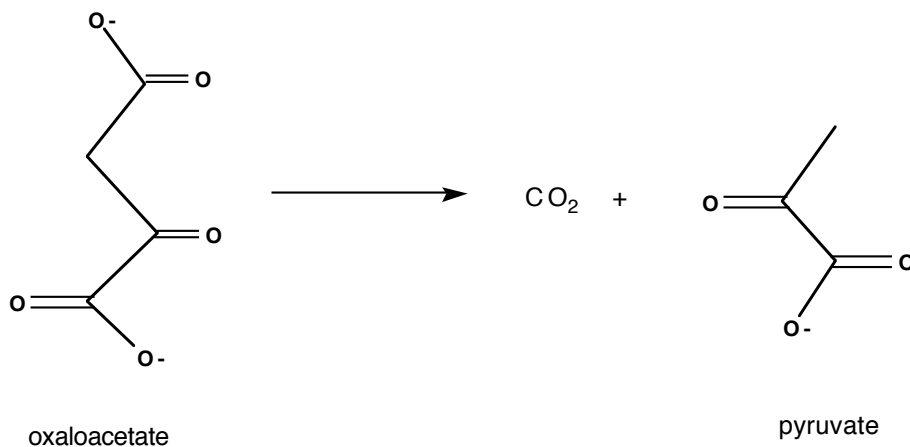
- 1.a). Describe the difference between a transition state and an intermediate in a reaction pathway.
- b). Define 'transition state analogue' and describe the usefulness of designing a transition state analogue.
- c). Proline racemase carries out the conversion of L-proline to D-proline. The reaction is thought to proceed *via* a planar transition state:



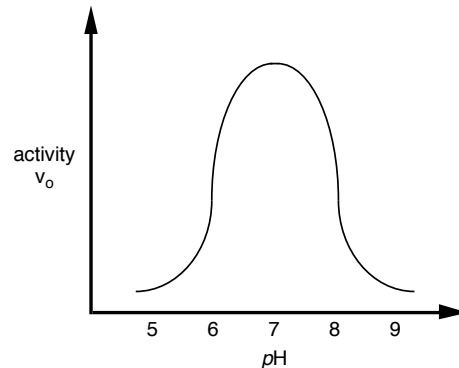
Suggest a transition state analogue for this enzyme and explain what features of the molecule make it a good transition state analogue.

- d). How would one test whether a molecule actually *is* a transition state analogue?

2. Oxaloacetate decarboxylase converts oxaloacetate to pyruvate. Explain why oxalate ($^{\ominus}\text{OOC}\text{COO}^{\ominus}$) is an inhibitor of the enzyme.



- a). The enzyme is inactivated by alkylation with stoichiometric amounts of iodoacetate. What does this suggest in terms of catalytic residues? Draw how this inactivation might occur.
- b). The enzyme quantitatively transfers ^3H from C1 of GAP to NAD^+ to form NADH. In addition, the enzyme catalyses the exchange of ^{32}P between $[\text{P}]\text{P}_i$ and acetyl phosphate. Draw the mechanism for the enzyme.
5. An enzyme has the pH-activity profile shown below.
- a). How might you explain this behavior?
- b). How would you test your hypothesis
- c). Suppose you were told that an Asp residue is responsible for a portion of this pH curve. Explain how this might be so.



6. For the example of a dimeric macromolecule having two identical but non-interacting subunits, as given in class, show that $K_2 / K_1 = 4$, where K_2 and K_1 are macroscopic equilibrium dissociation constants.
7. Prove that

$$K_{\text{eq}}(i) = \left(\frac{\square_{n, i-1}}{\square_{n, i}} \right) k_{\text{eq}}$$

where $K_{\text{eq}}(i)$ is the *macroscopic* equilibrium constant corresponding to having i ligands bound, and k_{eq} is the *microscopic* equilibrium constant for identical and independent ligand binding sites, *i.e.*, $k_{\text{eq}} = k_1 = k_2 = \dots = k_n$. $\square_{n, i}$ is another way of abbreviating the binomial coefficient.

8. A molecule has six sites ($n=6$) for a certain ligand. A prominent scientist takes a rare break

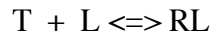
from writing research grant proposals and measures in his laboratory three of the macroscopic equilibria and finds $K_5 = 15K_1$ and $K_4 = 8K_1$. This scientist then claims that he has demonstrated negative cooperativity. His new graduate student, having recently completed BMB 100B, disagrees. She says not only is there no cooperativity, but she also ventures predictions of the relative values for the still unmeasured equilibrium constants K_2 , K_3 and K_6 (relative to K_1). (a) Who is correct? (b) Is it possible for the graduate student to make these predictions? If so, what are they? If not, why not?

9. Derive the following result for the fractional binding function for the Koshland-Nemethy-Filmer tetrahedral model for a tetrameric ($n=4$) protein exhibiting allosteric cooperativity in binding a ligand L:

$$\langle Y_F \rangle = \frac{k_{TR}^3 \square + 3k_{TR}^4 k_{RR} \square^2 + 3k_{RR}^3 k_{TR} \square^3 + k_{RR}^6 \square^4}{1 + 4k_{TR}^3 \square + 6k_{TR}^4 k_{RR} \square^2 + 4k_{RR}^3 k_{TR} \square^3 + k_{RR}^6 \square^4}$$

where $\square = K_s K_t [L]$ and $k_{TT} = 1$ and we make the following assumptions:

- (i). The sequential model holds, and as the protein, predominantly in the T state, binds ligand L, it is induced to the R state, *i.e.*,



and

$$K_t = \frac{[R]}{[T]} \ll 1$$

- (ii). $R + L \rightleftharpoons RL$ $K_s = [RL] / [R] [L]$

- (iii). Between any two protomers in our tetrameric protein, there are three possible strengths of interaction characterized by these three microscopic (intrinsic) binding (or interaction) constants:



10. What is the analogous expression for a square-planar tetrameric protein? (You should be able to do this by inspection of the answer given in number 9.)