(1) Draw an approximate denaturation curve for a typical blood protein (*eg* myoglobin) as a function of pH.

(2) Myoglobin is a simple, single subunit binding protein that has an oxygen storage function in diving mammals. In contrast, hemoglobin is a multi-subunit protein that transports nearly all the oxygen in animals.

(a) Draw and label a single graph, indicating the approximate O_2 binding curves for both myoglobin and hemoglobin.

(b) Draw and label a single graph, representing the Hill plot for the binding of O_2 to both proteins.

(3) What is cooperativity and how is it quantified.

(4) Derive the equilibrium expression for the binding of single ligand to a protein in terms of fractional occupation of ligand binding sites.

(5) What is the Bohr effect and how does it affect the biological function of hemoglobin? What binding events or reactions contribute to the Bohr effect?

(6) You have determined the rate of a chemical reaction as a function of substrate concentration. What is the best method for determining the kinetic constants, V_{max} and K_m?

(7) What is an apparant V_{max}?

(8) Consider a modified Michaelis-Menten enzyme mechanism. At $v_o = 0$, how does the equation simplify?

$$v_o = (V_{f,max} [S] K_{s,M}^{-1} - V_{r,max} [P] K_{p,M}^{-1}) / (1 + [S] K_{s,M}^{-1} + [P] K_{p,M}^{-1})$$

(9) Draw and label a double reciprocal plot showing the effect of increasing concentrations of inhibitor. Indicate how you would determine the true K_M?

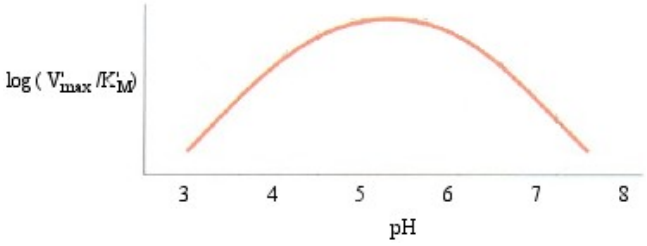
(10) What type of inhibition can be relieved by the addition of excess substrate and why?

(11) Draw a general reaction equation for a mixed inhibitor of a unimolecular reaction.

(12) Draw and label a reasonable pH vs. initial velocity plot for an enzyme with a catalytic His and Cys.

(13) You have been provided with the following plot.

(a) What information can be obtained from this plot?



(b) If the pK_a's of several catalytic residues were perturbed in the enzyme-substrate complex, what type(s) of catalytic mechanism would be enhancing the reactions rates?

(14) Serine proteases (endoproteases) hydrolyze specific peptide bonds using a bi bi ping pong mechanism. Answer the following questions.

(a) Write a reasonable chemical equation for the catalyzed reaction.

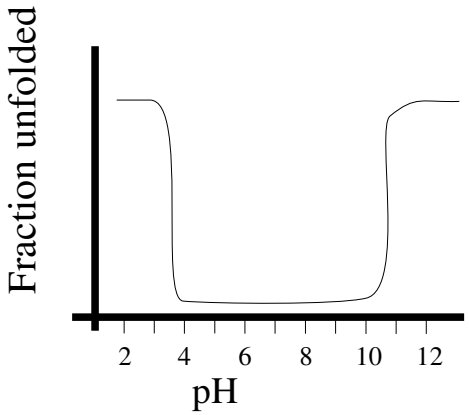
(b) Outline the reaction mechanism using a Clelland schematic.

(c) Draw a reasonable reaction coordinate for the catalyzed and uncatalyzed reaction (on a single diagram).

(d) If an invariant Ser residue is the nucleophile in this reaction mechanism, what types of catalytic mechanisms are being utilized by the enzyme?

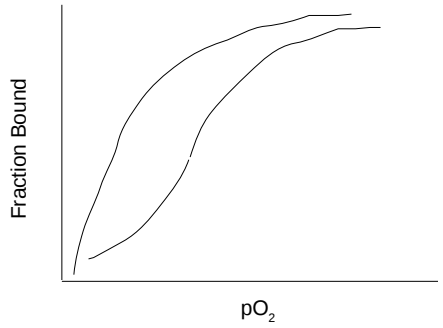
Answers:

1 –

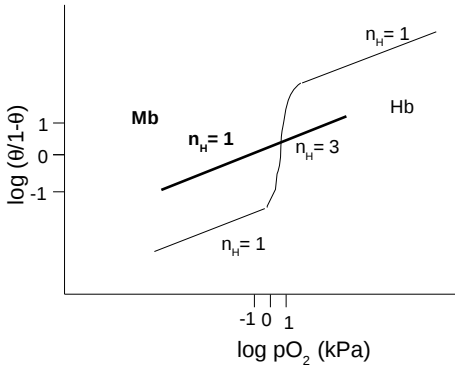


At extremes of pH the protein is unfolded, while in a physiological pH range (5-9) the protein is folded. The folding/unfolding of the protein is cooperative.

2a -



2b -



3 – Cooperativity is a special case of allostery. Ligand binding to a site on the protein results in an altered affinity for the same ligand at identical sites on other subunits within the protein. Cooperativity is quantified using the Hill Plot (log (q/1-θ) vs log (L)). In particular, the Hill coefficient, n_H, is a measure of cooperativity. The Hill coefficient takes values between 0 and the maximum number of ligand molecules that can bind to the protein.
A Hill coefficient of 1.0 indicates the absence of cooperativity.
A Hill coefficient below 1.0 indicates negative cooperativity.
A Hill coefficient above 1.0 indicates positive cooperativity.

4 -

$$\theta = \frac{(binding\ sites\ occupied)}{(total\ binding\ sites)} = \frac{[PL]}{([PL]+[P])}$$

Since [PL] = K_a [L] [P], we can

$$\theta = \frac{(K_a[L][P])}{(K_a[L][P]+[P])} = \frac{(K_a[L])}{(K_a[L]+1)} = \frac{[L]}{([L]+\frac{1}{K_a})}$$

5 – Bohr effect refers to changes in hemoglobin function in the presence of $[H^+]$ (and $[CO_2]$). Biologically, the presence of slightly acidic conditions favors the T state of Hb and the actual binding of H^+ to Hb at His146 (and other sites) also further favors the T state of Hb. H^+ are produced near tissues that have high levels of CO_2, as CO_2 is converted to bicarbonate. In a second mechanism, the reaction of CO_2 which the amino terminus also produces H^+.

6 – To estimate the helical content we must estimate the helical content of the random coil (0% helix) and all helix (100% helix) polypeptides on the standard curve. Assuming a molar ellipticity of -12000 (0% helix) and -38000 (100% helix) respectively, the helical content is
(-12000 - -26000) / (-12000 - -38000) = 14000/26000 = 0.54 or 54%

7 – An apparant V_{max} is an experimentally determined V_{max} for an enzyme that does not obey the fundamental assumptions of Michaelis-Menten enzymes. This includes enzymes in the presence of inhibitors or far from their optimal pH.

8 – v_0 is only zero at equilibrium (ie. Haldane conditions)

$0 = (V_{f,max} [S] K_{s,M}^{-1} - V_{r,max} [P] K_{p,M}^{-1}) / (1 + [S] K_{s,M}^{-1} + [P] K_{p,M}^{-1})$

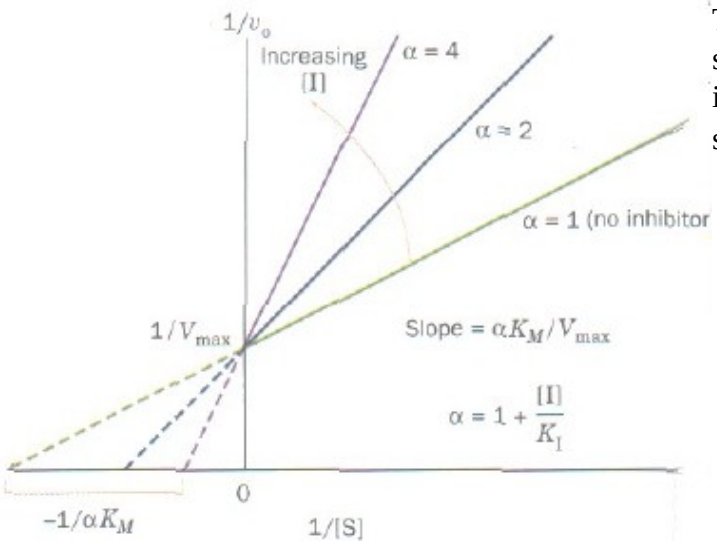
$0 = (V_{f,max} [S] K_{s,M}^{-1} - V_{r,max} [P] K_{p,M}^{-1})$

$V_{r,max} [P] K_{p,M}^{-1} = V_{f,max} [S] K_{s,M}^{-1}$

$[P]/[S] = V_{f,max} K_{s,M}^{-1} / V_{r,max} K_{p,M}^{-1}$

This is the Haldane equation which shows the relationship between K_{eq} and the kinetic parameters.

9 –

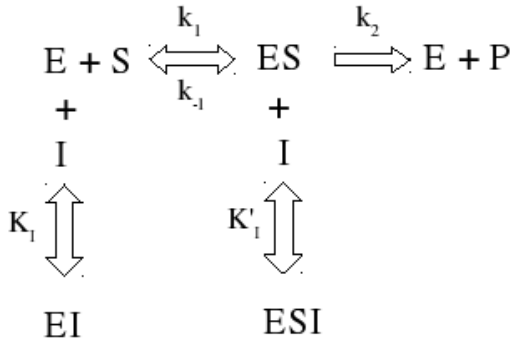


The true K_M can be determined from the slope (or x-intercept) in the absence of inhibitor. Otherwise, you need to know something about the inhibitor (eg. K_I or α)

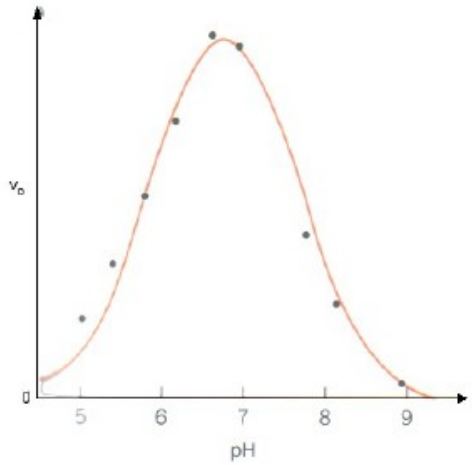
10 – Only competitive inhibition can be overcome by the addition of excess substrate. This is because

competitive inhibitors affect the concentration of $[E]_{free}$ while uncompetitive and mixed inhibition both affect the concentration of [ES].

11 –



12 –



The midpoints of the sigmoidal transition would be expected to be near the pK_a of the free amino acids (ie. 6.0 and 8.5). Of course, the transitions are unlikely to fall at exactly the pK_a of the free amino acids due to local environmental effects.

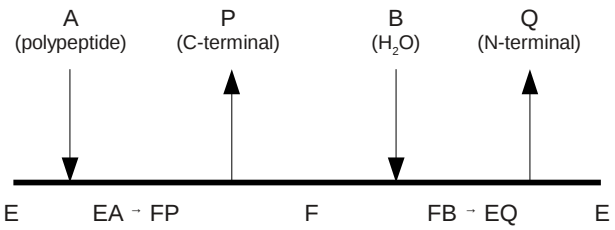
13a – This plot allows us to calculate the pk_as of functional groups in the free enzyme that are required for catalysis. From the plot, the enzyme has pk_as near 4.5 and 6.0. These likely correspond to a carboxylate containing residue (Glu most likely) and His.
Note: the pk_as can be determined by extrapolating the slope of each side of the bell curve and the plateau region. The intersection between the extrapolated slope and plateau lines gives the pk_as.

13b – If the catalytic residues had pK_a's that were perturbed, at the very least we would have evidence of “electrostatic catalysis”. It is likely, but not certain that acid-base catalysis is occuring. More general catalytic mechanisms such as preferrential binding of the transition state are also likely but not certain to be occuring.

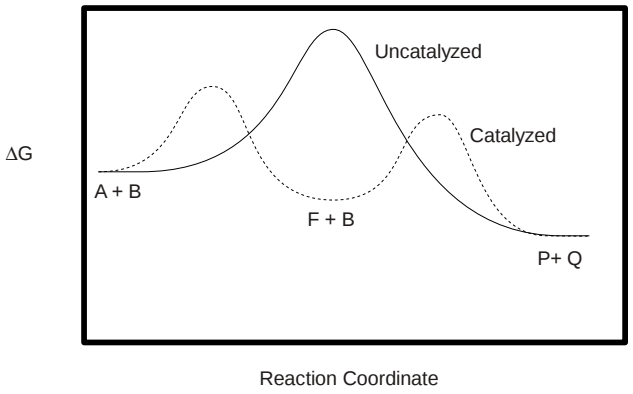
14a – Since the reaction mechanism is bi bi ping pong there must be two substrates and two products. Given the protease hydrolyzes the peptide bond, the second substrate is H_20.

Polypeptide + H_20 ----> $Polypeptide_{(N\text{-terminal fragment})}$ + $Polypeptide_{(C\text{-terminal fragment})}$

14b -



14c -



The uncatalyzed reaction is represented as a single reaction step with a large E_a while the ping-pong mechanism has two elementary steps each with a smaller E_a than the uncatalyzed reaction.

14d – All ping pong mechanism exploit covalent catalysis. If Ser is the nucleophile, both a general acid-general base and electrostatic catalysis are also being utilized, as the Ser must be deprotonated in order to function as a nucleophile.