CHEM/BIOC/MMG 205

October 23, 2006

BIOCHEMISTRY I

EXAM II

Read the questions carefully.

Extra pages are available. Put your name on every page.

This exam is a closed-book, closed-notes exam. No outside material may be used and you may not discuss the exam with anyone else.

Due to conflicts with the exam schedule, not all students are taking the exam at the same time. Do not discuss the exams until they are handed back.

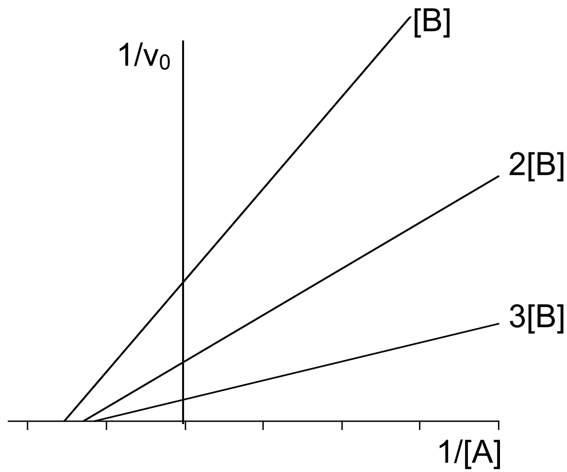
The exam has 6 questions for a total of 60 points. The exam duration is 2 hours.

Good Luck!

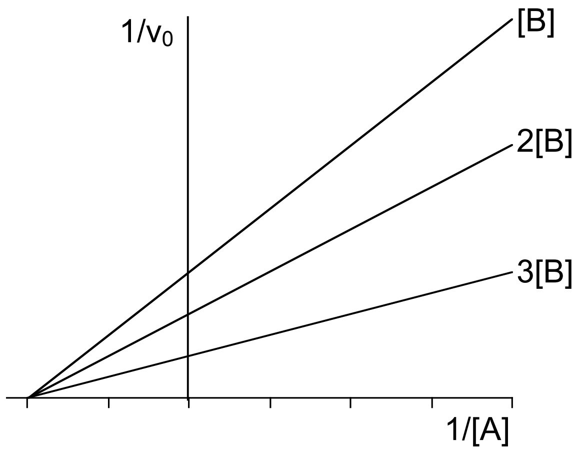
1. (12 points) What classes of enzymes perform the following transformations

(a) oxidation-reduction reactions	oxidoreductases
(b) transfer of functional groups	transferases
(c) hydrolysis reactions	hydrolases
(d) addition to double bonds	lyases
(e) isomerization reactions	isomerases
(f) formation of bonds with ATP cleavage	ligases

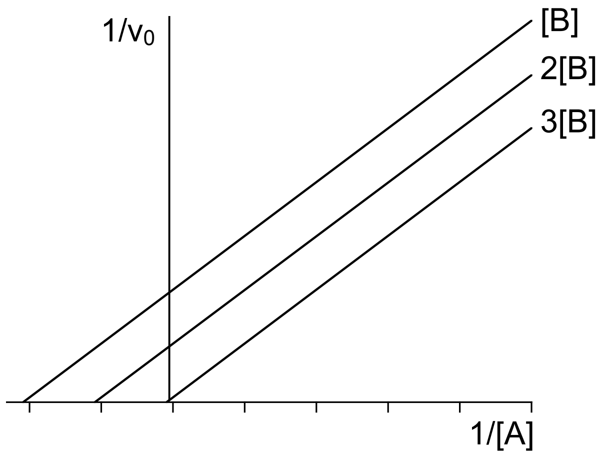
2. (9 points) Double reciprocal plots of bisubstrate enzyme mechanisms are shown below. Indicate which mechanism gives rise to which plot.



Ordered single displacement or random single displacement where A influences B binding.



Random single displacement. A has no effect on binding B and vice versa.



Double displacement (ping-pong)

3. (10 points) Mutation of asp102 in the catalytic triad of trypsin to asn (D102N) leads to a 100-fold reduction in the V_{max} of the enzyme, although the mutant enzyme continues to display burst-phase kinetics.

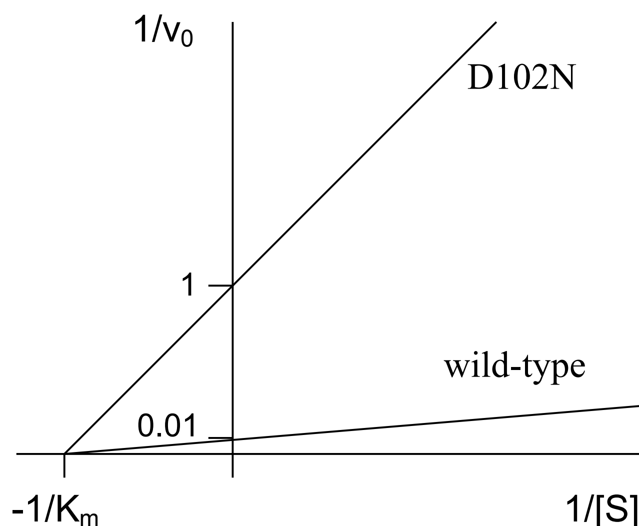
(a) Explain the observed reduction in V_{max}

Trypsin operates by a ping-pong mechanism in which the second step is rate-determining. A reduction in V_{max}, means the second step has been slowed. The second step is hydrolysis of the covalent enzyme-product acyl intermediate. Asp102 orients his57 by H-bonding to one of the imidazole ring nitrogens. The other nitrogen of his57 is a general base that deprotonates water as it attacks the acyl intermediate.

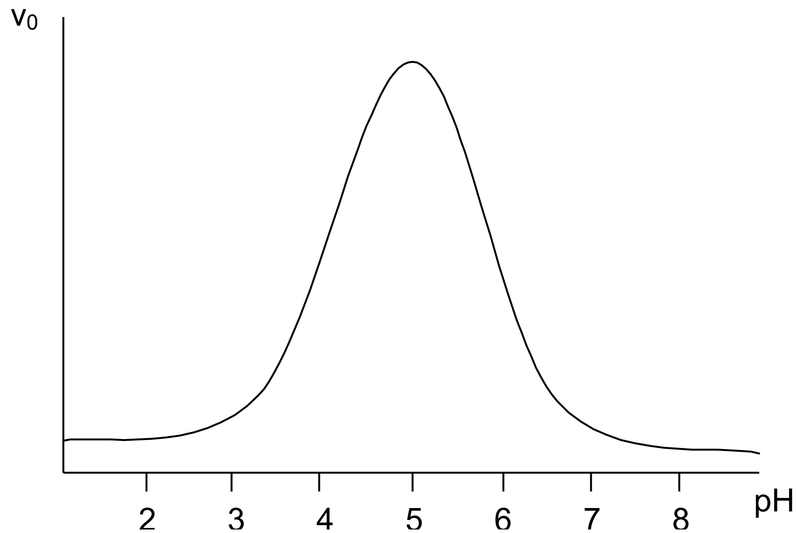
The D102N mutation does not allow the formation of the H-bond to his57, so the rate determining step is slowed, and the measured V_{max} is smaller.

(b) Analysis of the X-ray crystal structure of the D102N mutant indicates that the specificity pocket, oxyanion hole and orientation of the nucleophilic ser195 are identical to the wild type protease. On the same axes, sketch the double-reciprocal plots you would expect for the wild-type enzyme and the D102N mutant.

All the factors that influence substrate binding and product dissociation are unchanged, so K_m remains the same. V_{max} is reduced 100-fold.

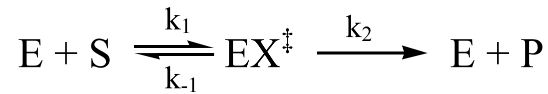


4. (8 points) The initial rates of an enzyme-catalyzed reaction change with pH according to the following profile. Given that the mechanism involves a general acid and a general base, what two amino acids might perform these functions? Briefly explain your reasoning.



Activity is low at pH less than 4, and increases at pH greater than 4. This suggests that a carboxylate group COO^- is necessary for activity (no activity: COOH below pKa). COO^- is a general base, so the partner must be a general acid (RH^+). The activity falls off above pH 6, so we're looking for an amino acid with a side chain pKa of about 6. Histidine would be a good choice. The general base could be aspartate or glutamate.

5. (11 points) The model for Michaelis-Menten kinetics is as follows:



A simple model of positive homotropic allostery is that substrate binding at a site remote from the active site increases substrate binding at the active site, increasing k_1 according to:

$$k_1^{app} = c[S]k_1$$

where c is a constant

Show that this model leads to non-hyperbolic Michaelis-Menten kinetics.

Assuming steady state in $[EZ^{\ddagger}]$ and measurements of initia rates at [P] = 0, we can write the Michaelis-Menten equation as:

$$v_0 = V_{max}[S]/([S] + (k_{--1} + k_2)/k_1)$$

Substituting for k_1^{app}

$$v_0 = V_{max}[S]/([S] + (k_{--1} + k_2)/c[S]k_1)$$

Rearranging

$$\mathbf{v_0 = V_{max}[S]^2/(K + [S]^2)}$$

where K = $(k_{--1} + k_2)/ck_1$

A plot of v_0 vs [S] is a sigmoid, not a rectangular hyperbola.

6. (10 points) What are the advantages and disadvantages of covalent regulation of enzyme activity versus allosteric regulation?

Allosteric Regulation

Advantages: Allosteric effectors are metabolites that reflect the prevailing cellular conditions. Effector binding is reversible, and adjusts enzyme activity to the momentary needs of the cell. Allosteric enzymes are rapidly controllable according to effector concentration.

Disadvantages: Allosteric control is determined by the amount of effector at any given moment, so the enzyme can't stay "on" if the effector concentration is momentarily reduced.

Covalent Regulation

Advantages: Covalent regulation (zymogens, phosphorylation/dephosphorylation) is rapid and reversible in the case of regulation by phosphorylation. The process is efficient (ie many enzymes can be regulated simultaneously) because the regulating enzymes (kinases and phosphatases) are catalytic. Enzyme function can be decoupled from cellular conditions.

Disadvantages: The effector enzymes are metabolically costly, and often form part of elaborate enzyme cascades (blood clotting factors are a good example). Covalent regulation may be irreversible, as in the case of zymogens.