

PHAR 332 SPRING 2005
FUNDAMENTALS OF DRUG ACTION II
EXAM 1

SEAT NUMBER

YOUR NAME _____.

WRITE YOUR NAME ON THE **SECOND PAGE** IN THE INDICATED PLACE AS WELL.

DIRECTIONS

Sit in the assigned seat. This is a two-hour exam, worth 250 points. The exam is closed-book; no aids are permitted.. The exam must be written **in ink**. If you use a pencil, we will not regrade the exam. Answer all questions completely and accurately. Use the proper number of significant figures, and the correct scientific units, where needed. Indicate final numerical results by circling or double-underlining. In the essay questions, use good English; your score will reflect your use of proper English as well as the scientific content of your answer. The exam has 10 (ten) pages plus a page of formulas; check your exam to make sure it is complete.

Warning! Cheating on an exam may result in failing the exam, dismissal from the course, and/or other penalties!

We reserve the right to photocopy your exam for our files.

YOUR NAME _____

<u>PROBLEM</u>	<u>SCORE/POSSIBLE</u>
I	_____/ 60
II	_____/ 20
III	_____/ 20
IV	_____/ 31
V	_____/ 44
VI	_____/ 45
VII	_____/ 30
TOTAL	_____/ 250
BONUS	_____/02

YOUR NAME _____

I. (60 pts total; 5 pts each) Fill in the blanks with the appropriate word, phrase, or abbreviation.

1. List three basic reasons for studying enzyme kinetics:

a. _____

b. _____

c. _____

2. When there is only the one intermediate species, ES, and when both formation and dissociation of this complex are fast, k_{cat} is simply equal to the rate parameter _____ in the MM model.

3. When enzymatic activity curves, as plots of activity as a function of [S], are **sigmoid**, not hyperbolic, this implies _____ among the sites.

4. Allosteric activators bind preferentially to the _____ form of the enzyme, and increase its concentration at the expense of the _____ form, and so raise activity.

5. Aspartate amino transferase first accepts an amino group from aspartate, converting the aspartate into oxalate; then it binds α -ketoglutarate, and donates the amino group to this to form glutamate. This is an example of a _____ kinetic mechanism.

6. In binding first MgATP, then creatine, the enzyme creatine kinase follows a kinetic mechanism described as _____.

7. Ibuprofen binds non-covalently to the active site to inhibit prostaglandin synthetase, and so acts as an anti-inflammatory. This is an example of _____ inhibition.

8. Enzymes influence chemical reactions in living systems by _____.

9. The "lock and key hypothesis" attempts to explain the basis of _____.

10. At high temperature, the rate of enzyme action decreases because the increased heat can _____.

YOUR NAME _____

II. (20 points total; 5 points each) Define each of the terms correctly.

1. *Allosteric effector*:

2. *Turnover number*:

3. *Induced fit mechanism*:

4. V_{max} :

YOUR NAME _____

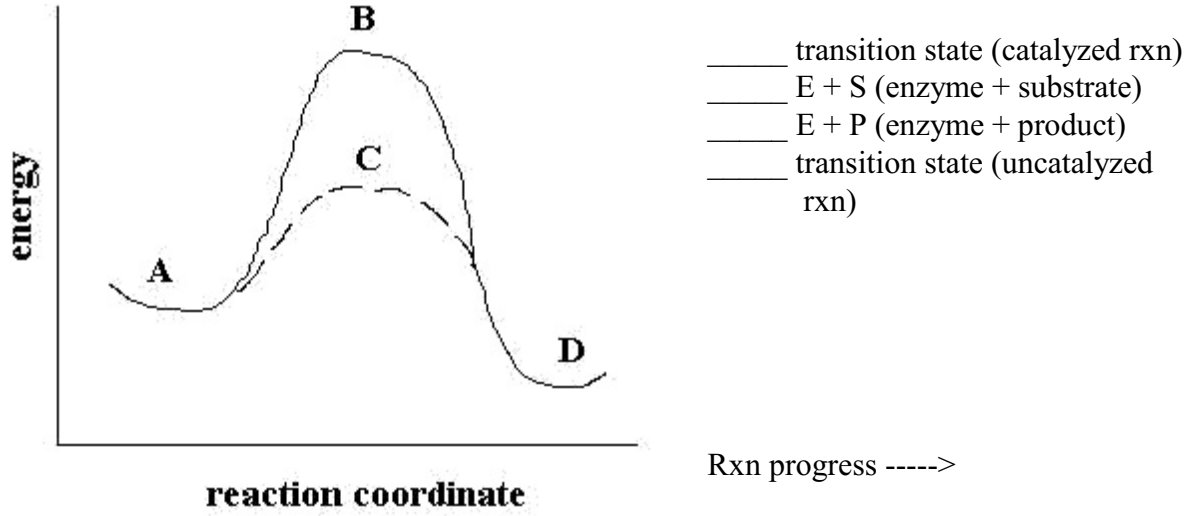
III. (Dr. Woodbury; 20 points)

Draw diagrams to illustrate the difference between the concerted mechanism (MWC model), and the sequential mechanism (KNF model), of enzyme kinetics. Label your diagrams properly, and briefly explain in writing what they signify.

YOUR NAME _____

IV. (Dr. Wang) (31 points total)

1. Shown below is a graph describing energy versus reaction coordinate for a catalyzed and uncatalyzed reaction. Fill in the blanks with the letter that corresponds to each stage of the graph (16 pts).



2. Enzymes carry out different chemical reactions in catalysis. Fill in the blanks with the name of the mechanism that matches with the example described in each line. Possible choices are: covalent catalysis, acid-base catalysis, and metal ion catalysis. These possible choices may be used once or more, or not at all. (15 pts).

_____ catalysis may involve glu, asp, his, lys, or arg residues.

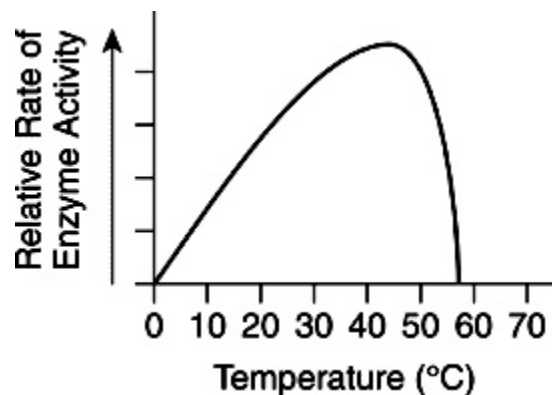
_____ catalysis is performed by Ribonuclease A (RNase A)

_____ catalysis is performed by chymotrypsin.

YOUR NAME _____

V. (Dr. Wang) (44 points)

1. Based on the information in the graph below, briefly explain why a prolonged period of high fever is dangerous to humans (15 pts).



2. Briefly describe the molecular basis of the function of penicillin as an antibiotic. (15 pts)

3. List two very general mechanisms by which enzymes lower the activation barrier and increase reaction rate (NOT “acid-base” or “covalent” catalysis, etc. - be more general!). Briefly explain how these work. (14 pts)

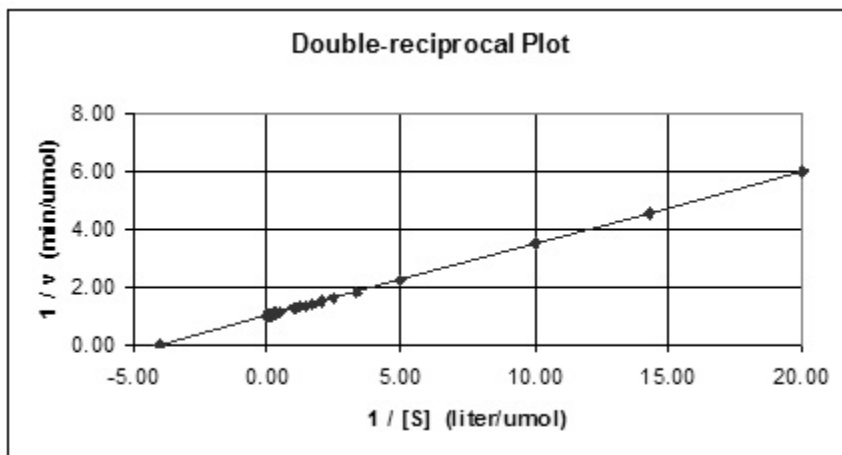
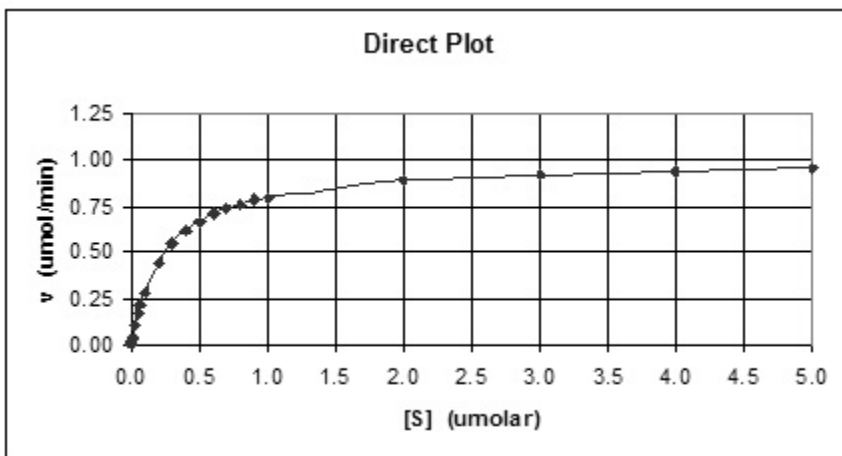
Mechanism A:

Mechanism B:

YOUR NAME _____

VII. (Dr. Woodbury; 30 points total)

Given the plots shown, deduce proper values for K_M and V_{max} . Show your work. Use proper units and the proper number of significant figures. (Note: the symbol “ μmol ” is used to indicate micromoles, and “ μmolar ” indicates micromolar concentration.)



YOUR NAME _____

BONUS POINT QUESTIONS.

Each is worth ONE bonus point. Be sure you have finished with the other parts of the exam before attempting these questions.

A. Only a small section of the enzyme is the active site - so why does the rest of the enzyme exist, making the whole molecule so big and complex?

B. Give an example of active-site directed irreversible inhibition, naming both enzyme and inhibitor, that is relevant to pharmacy.

FORMULAS AND CONSTANTS

Avogadro's number $N_A = 6.0221 \times 10^{23} \text{ mol}^{-1}$

Gas constant $R = 8.3144 \text{ J K}^{-1} \text{ mol}^{-1}$

Faraday constant $\mathcal{F} = 9.6487 \times 10^4 \text{ C mol}^{-1}$

Boltzmann's constant $k_B = 1.38066 \times 10^{-23} \text{ J K}^{-1}$

MM kinetic scheme



MM Rate Law

$$v = \frac{[E_0] [S] k_{+2}}{[S] + \left(\frac{k_{+2} + k_{-1}}{k_{+1}} \right)} = \frac{[E_0] [S] k_{+2}}{[S] + K_M}$$

Double-reciprocal format
of MM Rate Law

$$\frac{1}{v} = \frac{K_M}{V_{\max}} \cdot \frac{1}{[S]} + \frac{1}{V_{\max}}$$

Michaelis constant

$(k_{+2} + k_{-1}) / k_{+1}$ is defined as K_M

ANSWER KEY

I. (50 pts total; 5 pts each) Fill in the blanks with the appropriate word, phrase, or abbreviation.

1. List three basic reasons for studying enzyme kinetics:

a. The study of enzyme kinetics is a powerful way to investigate an enzyme's mechanism.

b. Kinetic assays with enzymes are widely used in drug discovery and development.

c. Tests for the presence or absence of specific enzymes, employing kinetics, are used clinically in diagnosing pathologies.

d. Understanding enzyme kinetics helps in understanding general cell metabolism. It also helps in understanding drug action.

2. When there is only the one intermediate species, ES, and when both formation and dissociation of this complex are fast, k_{cat} is simply equal to the rate parameter k_{+2} in the MM model.

3. When enzymatic activity curves, as plots of activity as a function of [S], are **sigmoid**, not hyperbolic, this implies **_(positive) cooperativity_** among the sites.

4. Allosteric activators bind preferentially to the **_R (relaxed)_** form of the enzyme, and increase its concentration at the expense of the **_T (taut)_ form**, and so raise activity.

5. Aspartate amino transferase first accepts an amino group from aspartate, converting the aspartate into oxalate; then it binds α -ketoglutarate, and donates the amino group to this to form glutamate. This is an example of a **_ping-pong_** kinetic mechanism.

6. In binding first MgATP, then creatine, the enzyme creatine kinase follows a kinetic mechanism described as **_ordered sequential_**.

7. Ibuprofen binds non-covalently to the active site to inhibit prostaglandin synthetase, and so acts as an anti-inflammatory. This is an example of (competitive / uncompetitive / noncompetitive / mixed - chose one) **_competitive_** inhibition.

8. Enzymes influence chemical reactions in living systems by **_affecting the rate at which reaction occurs. ___**

9. The "lock and key hypothesis" attempts to explain the basis of **_enzyme specificity_**.

10. At high temperature, the rate of enzyme action decreases because **__the increased heat can _alter the active site of the enzyme_ .**

II. (20 points total; 5 points each) Define each of the terms correctly.

1. *Allosteric effector*:

The (usually small organic) molecules that cause the conformational changes in a protein, and so affect its activity, are called allosteric effectors. This is because

(1) the small molecule causing these effects is different in shape from substrate; (2) it binds at a separate site away from the active site; and (3) the protein changes conformation or shape.

2. *Turnover number*:

The turnover number, k_{cat} , is the maximum number of substrate molecules converted, or turned over, to product per unit time per active site.

3. *Induced fit*:

Binding of the substrate causes or “induces” the enzyme to change its shape/conformation, which results in catalytic activity.

4. V_{max} :

Maximal (maximum) velocity of reaction is achieved when all of the active sites are filled so that the only limiting factor is the step in which product P is formed from the complex. This rate of reaction is symbolized by V_{max} , and V_{max} is given by

$$V_{max} = k_{cat} [E_0]$$

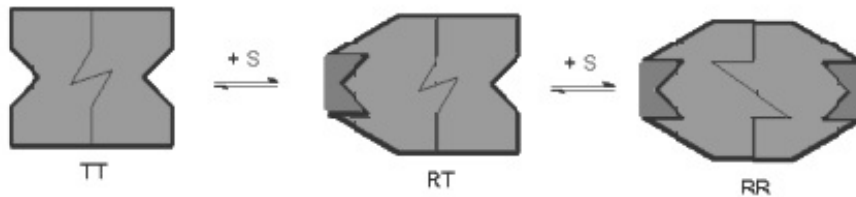
III. (Dr. Woodbury; 20 points)

Draw diagrams to illustrate the difference between the concerted mechanism (MWC model), and the sequential mechanism (KNF model), of enzyme kinetics. Label your diagrams properly, and briefly explain in writing what they signify.

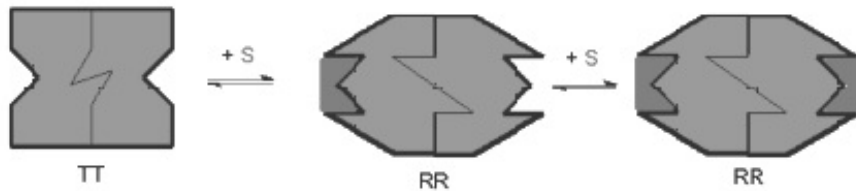
From the notes:

COMPARISON OF ALLOSTERIC MODELS

A) SEQUENTIAL MODEL



B) CONCERTED MODEL



Subunits can switch between 2 (or more) conformations, eg., R (relaxed, usually more catalytically active if we are dealing with an enzyme) and T (taut, usually less catalytically active, but the form that is more stable thermodynamically).

Activators bind preferentially to the R form, increase its concentration at the expense of the T form, and so raise activity.

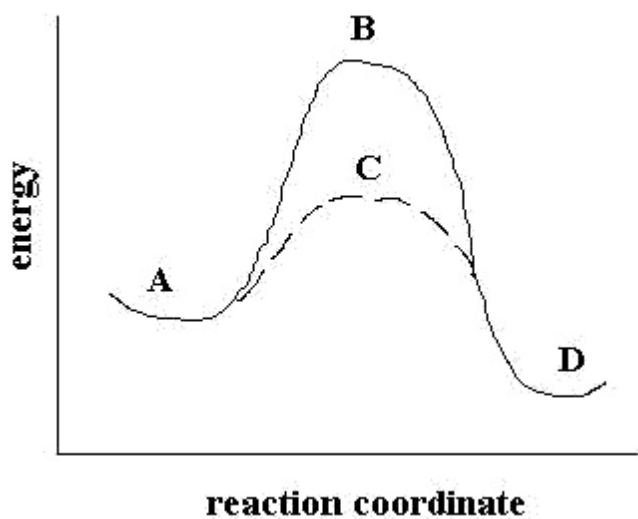
Inhibitors bind to the T form, causing transition to R state to be more difficult, and shifting activity curve to the right (higher [S] required for same activity).

For MWC, conformational changes in subunits occur in **concerted** manner, e.g., all-R or all-T but not mixed R and T in the same enzyme molecule ("concerted," "two-state," "All-or-none" Model).

For KNF model, intermediate or "mixed" states are permitted, e.g., RT now allowed.

IV. (Dr. Wang) (31 points total)

1. Shown below is a graph describing energy versus reaction coordinate for a catalyzed and uncatalyzed reaction. Fill in the blanks with the letter that corresponds to each stage of the graph (16 pts).



___C___ transition state (catalyzed rxn)

___A___ E + S (enzyme + substrate)

___D___ E + P (enzyme + product)

___B___ transition state (uncatalyzed rxn)

Rxn progress ----->

2. Enzymes carry out different chemical reactions in catalysis. Fill in the blanks with the name of the mechanism that matches with the example described in each line. Possible choices are: covalent catalysis, acid-base catalysis, and metal ion catalysis. These possible choices may be used once or more, or not at all. (15 pts).

___Acid-base catalysis___ catalysis may involve glu, asp, his, lys, or arg residues.

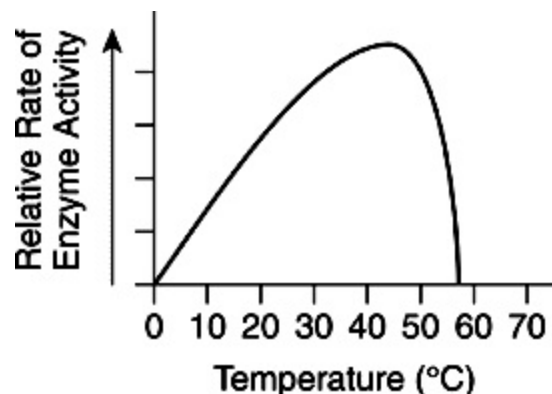
___Acid-base catalysis___ catalysis is performed by Ribonuclease A (RNase A)

___Covalent___ catalysis is performed by chymotrypsin.

V. (Dr. Wang) (44 points)

1. Based on the information in the graph below, briefly explain why a prolonged period of high fever is dangerous to humans (15 pts).

Temperature can influence the action of an enzyme. A high fever will denature the enzymes in living cells. The cells will not be able to carry on their metabolic reactions and the human will ultimately die.



2. Briefly describe the molecular basis of the function of penicillin as an antibiotic. (15 pts)

Penicillin forms a covalent complex with the enzyme, with irreversible inhibition to block the synthesis of cell walls in bacteria.

3. List two very general mechanisms by which enzymes increase reaction rate (NOT “acid-base” or “covalent” catalysis, etc. - be more general!). (14 pts)

Mechanism A: **Proximity effect**

Mechanism B: **Transition state stabilization**

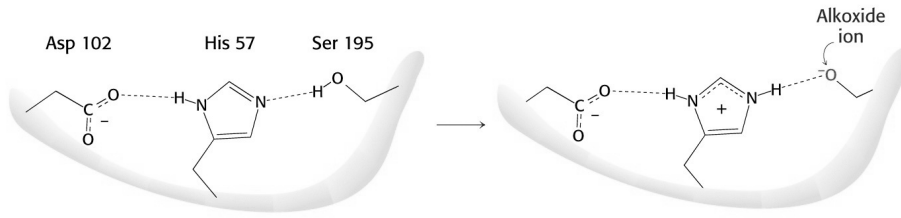
VI. (Dr. Wang) (45 points)

Examination of homologous serine proteases such as chymotrypsin shows the following:

- 1) Crystal structures show that Ser 195, His 57, Asp 102 are close in space.
- 2) Ser 195, His 57, and Asp 102 are conserved (found in all serine proteases) and are called the catalytic triad.

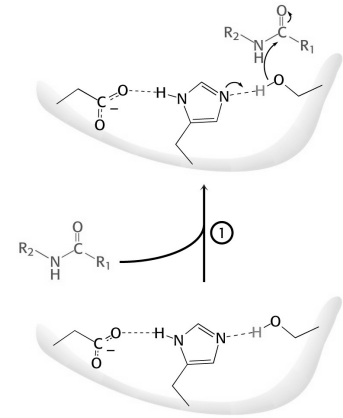
Answer the following questions:

1. Draw the proper arrangement of the three amino acids for the catalytic function (15 pts).

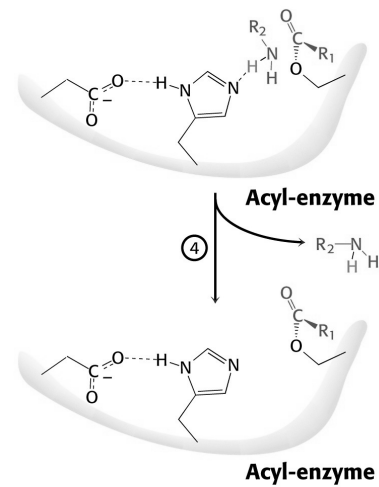


2. For cleavage of peptide bonds, what is the function of the Serine residue in the catalytic triad? (15 pts).

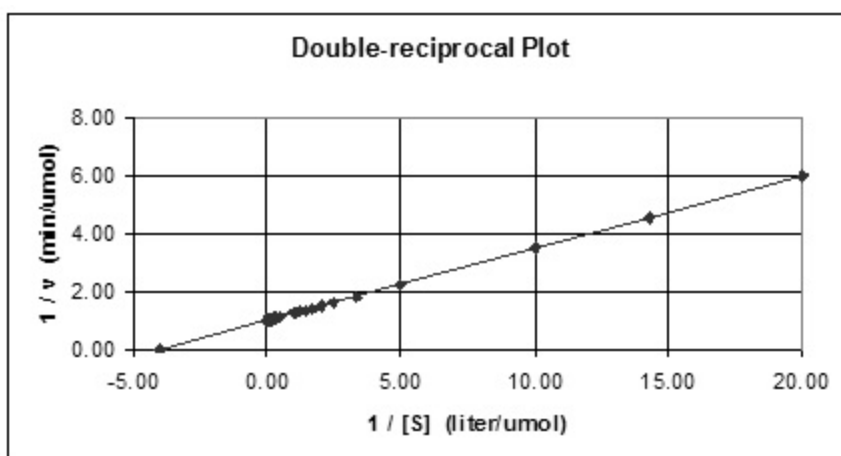
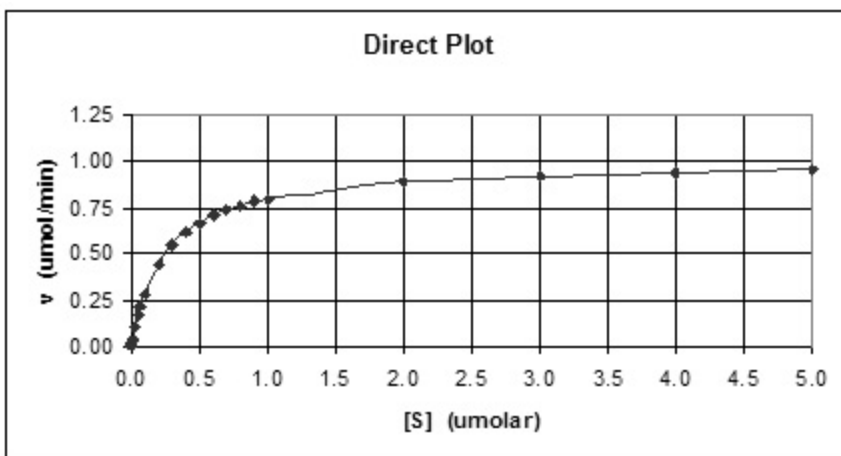
Ser 195: Nucleophile



3. For cleavage of peptide bonds, draw the structure of the key intermediate linked to the enzyme that is isolable (15 pts).



VII. (Dr. Woodbury; 30 points total) Given the plots shown, deduce proper values for K_M and V_{max} . Show your work. Use proper units and the proper number of significant figures. (Note: the symbol “ μmol ” is used to indicate micromoles.)



From the direct plot, 50% activity is achieved at $[S]=0.25$ micromolar, so $K_M = 0.25$ micromolar. Also, it appears that maximum activity is reached at about 1.0 micromole/min, so this must be V_{max} .

From the double-reciprocal (Lineweaver-Burk) plot we see an “x-axis” intercept at -4.0 L/micromol; this equals $-1/K_M$, so $K_M = 0.25$ micromolar. The “y-axis” intercept is at 1.0 min/micromole, which equals $1/V_{max}$. Thus V_{max} must equal 1.0 micromole/min.

BONUS POINT QUESTIONS.

Each is worth ONE bonus point. Be sure you have finished with the other parts of the exam before attempting these questions.

A. Only a small section of the enzyme is the active site - so why does the rest of the enzyme exist, making the whole molecule so big and complex?

1. **Conformation** While active sites can be fairly small, they almost always require an exact, three-dimensional shape to function properly. The problem comes in getting the active site to both acquire and keep its shape in the context of the cell. For many active sites - like the heme-binding site in hemoglobin - the amino acids that compose the site are so widely spaced that bringing them close together requires a lot of very specific folding and turning driven by the characteristics of the many amino acids not involved in the active site. In other words, for the active site to have the correct spatial orientation, the entire enzyme must assume and keep a tightly controlled shape.
2. **Regulation** For many enzymes, it is important for the cell to control the rate of reaction and the amount of active enzyme. Many enzymes contain regulatory sites that specifically bind inhibitors allowing the cell to quickly affect activity. Beyond inhibition, many enzymes require binding by other proteins to become active, and so protein-binding domains often account for a large portion of the enzyme's structure. In addition, most of these modifications do not occur in the active site, and either activate or inactivate the enzyme in other ways. So for the cell to control an enzyme's activity, the enzyme has to have built-in regulatory regions as well as an active site.
3. **Targetting** With all of the different compartments within the cell, it is essential that each enzyme be transported and kept in its relevant place. Almost all of this trafficking and targetting is accomplished through protein recognition tags composed of stretches of amino acids that are contained within the sequence of the enzyme.

B. Give an example of active-site directed irreversible inhibition, naming both enzyme and inhibitor, that is relevant to pharmacy.

From the notes:

cyclo-oxygenase and aspirin

bacterial transpeptidase and penicillin

Some other examples of active-site directed irreversible inhibitors

<u>Drug</u>	<u>Enzyme Inhibited</u>	<u>Clinical Use</u>
Neostigmine	Acetylcholinesterase	Glaucoma, myasthenia gravis
Organo-arsenicals	Pyruvate dehydrogenase	Antiprotozoal agents
D-cycloserine	Alanine racemase	Antibiotic
Azaserine	Formylglycinamide ribonucleotide aminotransferase	Anticancer
4-hydroxy- androstenedione	Aromatase	Estrogen-mediated breast cancer
Chloramphenicol	Peptidyl transferase	Antibiotic
5-fluorouracil	Thymidylate synthase	Anticancer
Disulfiram	Aldehyde dehydrogenase	Alcoholism