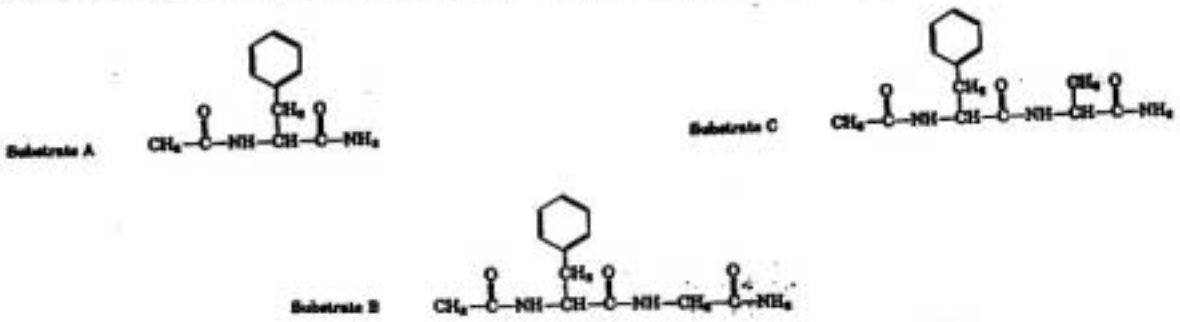
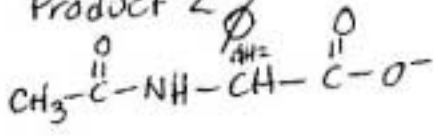
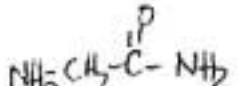
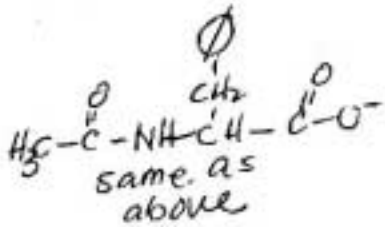
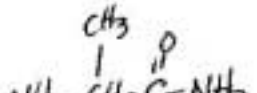
Answers Problem Set II

I. Enzyme Catalysis (30 points)

Shown below are three different substrates for the enzyme chymotrypsin.



a) Write down and label product 1 and product 2 for the amide bond hydrolysis for each substrate. (6 points)

	Product 1	Product 2
Substrate A	NH_3	
substrate B		 same as above
substrate C		same as above

b) The Km's for the three different substrates are 31 mM (Substrate A), 15 mM (Substrate B), and 25 mM (substrate C). If we assume that the ES complex is in equilibrium with the E and S starting material, we can calculate the binding energy directly from the Km. What are the binding energies for each of the ES complexes at 37°C? What interactions might give rise to these differences? (8 points)

$$\Delta G^{o'}_{binding} = -RT \ln K_{binding} = -RT \ln \frac{1}{K_{diss}} = RT \ln K_{diss}$$

	$K_m \approx K_d$	$\Delta G^{o'} = (1.98\ cal\ H^{-1}K^{-1})(310) \ln K_m$
Substrate A	31 mM	− 2.14 kcal/mole
B	15 mM	− 2.59 kcal/mole
C	25 mM	− 2.27 kcal/mole

These differences are very small
binding site pocket prefers backbone upstre of scissile bond, ~~cannot accomodate~~ groups such as -Cl are slightly

c) The k_{cat} for substrates a, b, and c are: 0.06 s^{-1}, 0.14 s^{-1}, and 2.8 s^{-1} respectively. What is the activiation barrier for each reaction at 37°C. Why do you suppose these barriers are so different? (8 points)

$\Delta G^{\ddagger} = 17.6 - 1.36 \log(k)$

Substrate A : $17.6 - 1.36 \log(0.06) = 19.3$ kcal/mol

substrate B : $17.6 - 1.36 \log(.14) = 18.8$ kcal/mol

Substrate C ; $17.6 - 1.36 \log(2.8) = 17.0$ kral/mol

active site chymotrypsin is most complementary to the transition state of "C" with favorable interactions on the order of 1.8 kral with the methylene sidegroup of "C" – probably multiple van der Waals int between enzyme & substrate in tetrahedral transition state involved in formation of acyl enzyme complex ~~or peptide~~ since subsequent acyl enzyme ~~intermediate~~ tetrahedral intermediate is same for as substrate

d) The k_{cat} /Km values help to evaluate which substrate works "best" with an enzyme. Which of the substrates is the "best" substrate for chymotrypsin and why. (8 points)

kcat/Km

A 1.94 worst substrate

B 9.33 pretty good substrate

C* 11.2 best substrate - incorporating good binding (not best, and terrific catalysis
good ground state complementarity.
great transition state complementarity

II. Michaelis Menten Kinetics (25 points)

1. Write down the general form of the Michaelis-Menten equation, identifying every term and keeping careful track of and including all of the subscripts. (5 points)

$$V_0 = \frac{k_{cat}[E_T][S]}{K_m + [S]}$$

V_0 = initial rate

k_{cat} = turnover # (observed rate constant)

E_T = total enzyme concent.

$[S] \cong [S_0]$ = substrate conc. at beginning of reaction

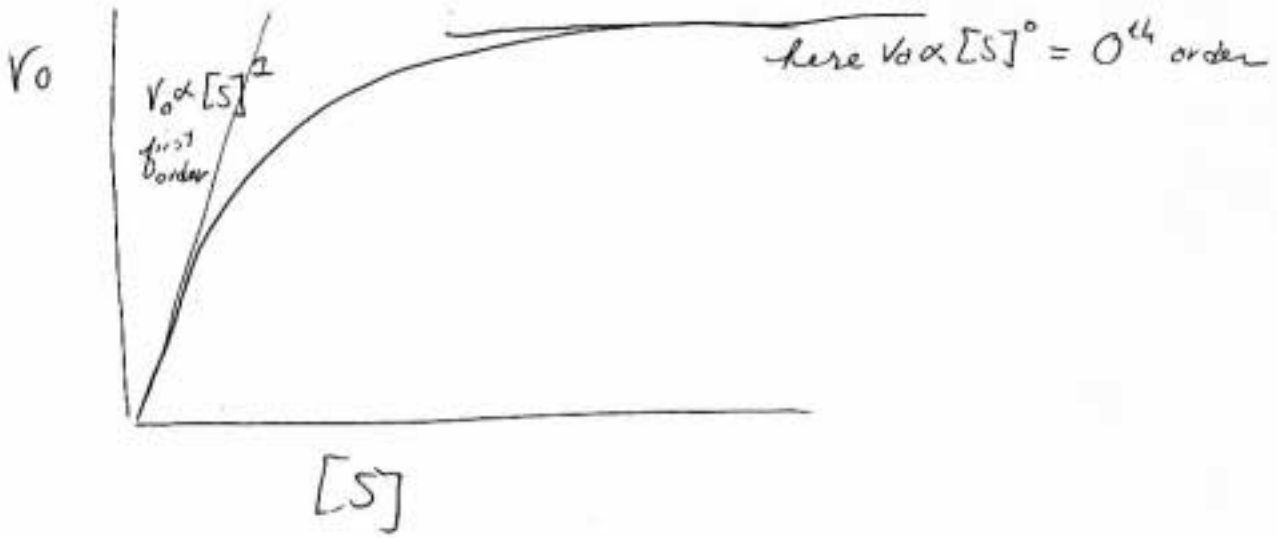
K_m = Michaelis Constant which is a measure of how enzyme dissociates from substrate

2. (8 points)

a) Sketch the form of the Michelis Menten equation on a graph of initial rate vs. substrate concentration for a nonallosteric enzyme.

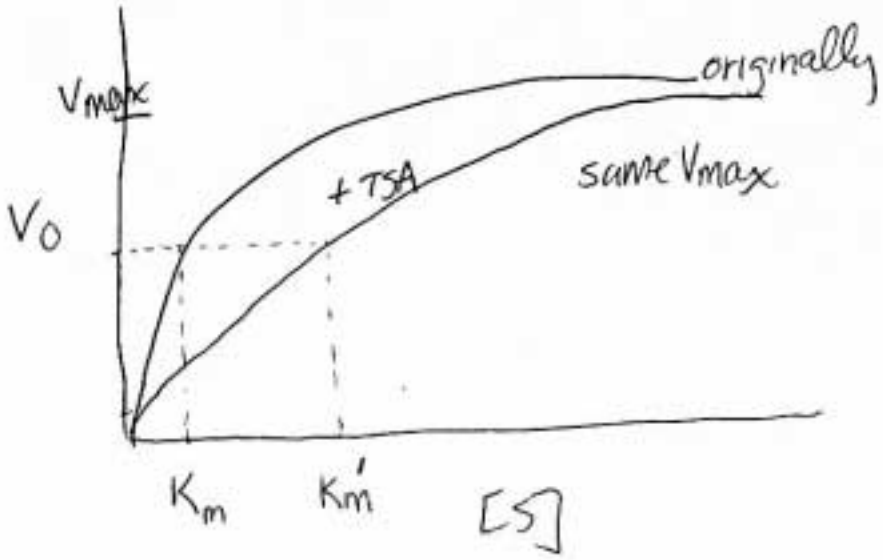
b) How does this graph show that the order of the reaction with respect to substrate varies from first order to zeroth order?

c) What is responsible for this peculiar switching of the order of a reaction?



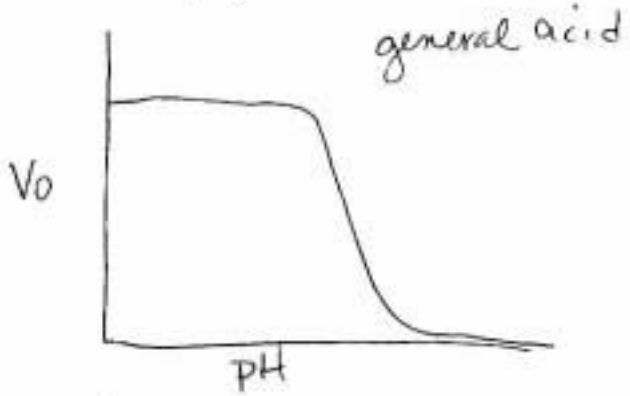
* switch from first to zeroth order happens when the enzymes active sites are all saturated with substrate

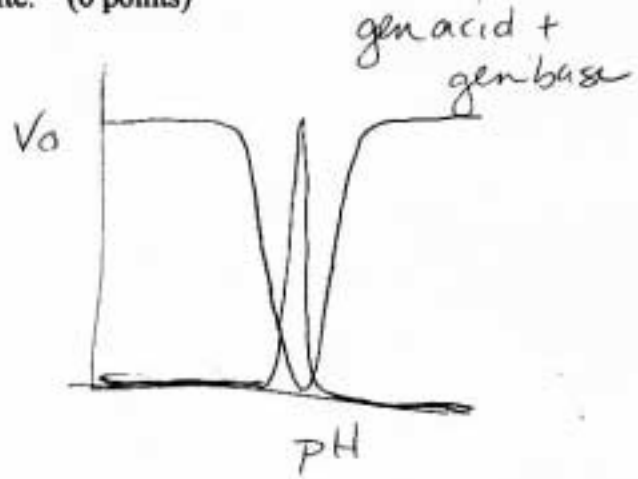
3. How would the graph above change if a transition state analogue was added to the mixture of enzyme and substrate at the beginnning of the reaction. Explain (6 points)

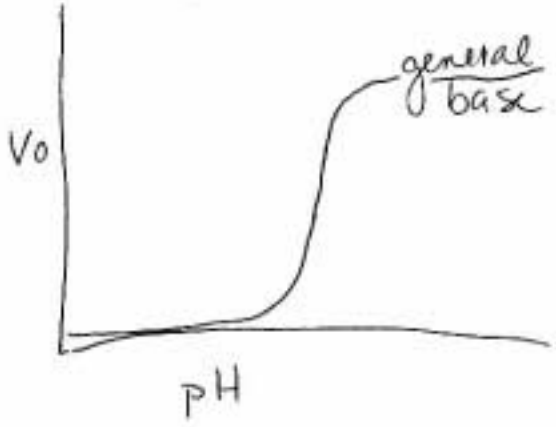


t.s. analogue is a competitive inhibitor

4. Many, many enzymes utilize general acid (proton donor) and/or general base (proton acceptor) catalysis in their active sites. On a graph below, sketch how the rate of a reaction would vary as a function of pH for a general acid catalyst, a general base catalyst, and an enzyme which employed both acid and base functions in its active site. (6 points)







III Glycolysis (30 points)

Step	Reaction	Enzyme	Type*	ΔG°'	ΔG
1	Glucose + ATP ⟶ glucose 6-phosphate + ADP + H^+	Hexokinase	a	−4.0	−8.0
2	Glucose 6-phosphate ⟷ fructose 6-phosphate	Phosphoglucose isomerase	c	+0.4	−0.6
3	Fructose 6-phosphate + ATP ⟶ fructose 1,6-bisphosphate + ADP + H^+	Phosphofructokinase	a	−3.4	−5.3
4	Fructose 1,6-bisphosphate ⟷ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate	Aldolase	e	+5.7	−0.3
5	Dihydroxyacetone phosphate ⟷ glyceraldehyde 3-phosphate	Triose phosphate isomerase	c	+1.8	+0.6
6	Glyceraldehyde 3-phosphate + P_i + NAD^+ ⟷ 1,3-bisphosphoglycerate + NADH + H^+	Glyceraldehyde 3-phosphate dehydrogenase	f	+1.5	−0.4
7	1,3-Bisphosphoglycerate + ADP ⟷ 3-phosphoglycerate + ATP	Phosphoglycerate kinase	a	−4.5	+0.3
8	3-Phosphoglycerate ⟷ 2-phosphoglycerate	Phosphoglyceromutase	b	+1.1	+0.2
9	2-Phosphoglycerate ⟷ phosphoenolpyruvate + H_2O	Enolase	d	+0.4	−0.8
10	Phosphoenolpyruvate + ADP + H^+ ⟶ pyruvate + ATP	Pyruvate kinase	a	−7.5	−4.0

Note: $\Delta G^{\circ\prime}$ and ΔG are expressed in kcal/mol. ΔG, the actual free-energy change, has been calculated from $\Delta G^{\circ\prime}$ and known concentrations of reactants under typical physiological conditions.

1. Next to each of the reactions above, write one of the following letters which best describes the type of chemical reaction catalyzed: (10 points)

a Phosphoryl transfer
b Phosphoryl shift
c Isomerization
d Dehydration
e Aldol Cleavage
f Phosphorylation coupled to oxidation

2. In each of the reactions cited above, the free energy for the reactions written under actual cellular conditions is much more negative than the free energy under biological standard state conditions (pH 7, 37 C). Why is that? (4 points)

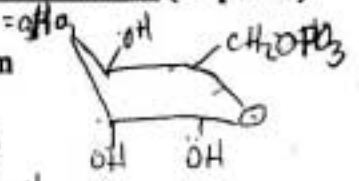
Actually in 7, 10 this isn't case.

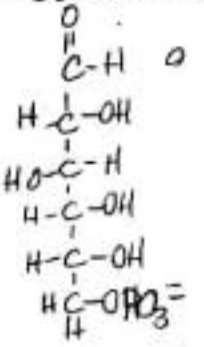
For 1-6, 8,9 Reactions are much further away from standard state concentrations of 1M reactants & products, and system is NOT at equilibrium. A negative ΔG means energy is released, a larger neg ΔG means more energy is released

$\Delta G = \Delta G^\circ + RT\ln K$

3. Give an example of an enzyme we have studied which exhibits the following phenomena and draw a chemical structure of its normal biological substrate (16 points)

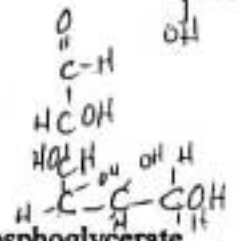
a) binds a six carbon sugar keto in its open form





(2) phosphoglucoisomerase
gly4- aldolase

b) binds to many different hexose sugars



(1) hexokinase

c) is inhibited by high levels of 2,3 Diphosphoglycerate

(3) phosphofructokinase

gly 8? phosphoglycero mutase

d) needs to bind a second molecule of oxidized substrate before it can release product

(6) glyceraldehyde 3phosphate dehydrogenase

e) incorporates radioactive oxygen from solvent into one of its products

(5) ~~triosephosphate isomerase~~ (4) aldolase, chymotrypsin

f) would have its activity destroyed by an inhibitor that reacted with thiols

(6) glyceraldehyde 3P dehydrogenase

g) Has as its main job recycling of its second product which would otherwise go to waste

(5) lactate dehydrogenase
triosephosphate isomerase

e) Dehydrates its substrate

(9) enolase

IV. Regulation/Control/Kcat/S (15 points)

Substrate	Concentrations		Change as a result of ischemia (%)
	Initial	Ischemia	
Glucose	2560	1930	−25
Glucose-6-P	224	91	−59
Fructose-6-P	50	27	−46
Fructose-1,6-bis-P	27	153	+467
Dihydroxyacetone-P	13	39	+200
Glyceraldehyde-P	0.9	3.3*	+267
1,3-bis-P-glycerate	<1	<1	0
3-P-glycerate	25	85	+240
2,3-bis-P-glycerate	29	29	0
2-P-glycerate	2.8	8.8	+214
P-pyruvate	3.5	8.5	+151
Pyruvate	39	72	+85

Shown above are data for concentrations of glycolytic intermediates in a resting animal (intial) and in one deprived of oxygen for 25 seconds (ischemia).

I) Given the concentrations of the glycolytic intermediates, and the Kms noted above, identify the degree of saturation of each of the enzymes of glycolysis. → last page, handout 30.006

a) in the resting animal
b) in the ischemic animal

enzyme		Km (mM)	(a) Km/S	(b) Km/S	Δ	
hexokinase	gly1	0.1	.04	.05	(+)	here, as [S] decreases, Km/S increases, enzymes become more unsaturated since gly3 cranl
		0.7	3.13	7.7	(+)	
phosphoglucoisomerase	gly2				-	can highly regulated step
phosphofructokinase	gly3	-	-	-		
aldolase	gly4	0.1	3.7	0.7	(-)	after gly3 substrate levels have all increased to effectively lower Km/S, enzymes are more saturated, glycolysis "revved" up.
TIM	gly5	0.87	66.9	22.3	(-)	
GAPdehyd.	gly6	0.07	78	21	(-)	
PG kinase	gly7	1.2	large	large	same	
PG mutase	gly8	5.0	200	60	(-)	
PG enolase	gly9	.07	25	8	(-)	
pyr kinase	gly10	.6	15.4	8	(-)	

II) How do allosteric effectors play a role in regulating the flow through glycolysis in an animal suffering from ischemia?

gly 3, Phosphofructokinase is the key allosterically regulated enzyme in glycolysis. glycolytic intermediates upstream are depleted by stepping up its activity & intermediates downstream build up to push flow through glycolysis when cells don't have access to TCA cycle & electron transport

Exams prior to Spring 2000

I. Enzymes of Glycolysis and Citric Acid Cycle

1a). The reactions catalyzed by the ten enzymes of glycolysis can be chemically classified into the five following groups. What is the general name for an enzyme which catalyzes this kind of chemical reaction and which enzymes of glycolysis fall into these categories. Answers for the first group have been provided as a guide.

	CLASS	ENZYMES
oxidation:	dehydrogenases	Gly-6 or GAP dehydrogenase
phosphoryllation:	kinases	Gly-1 or hexokinase
		Gly 3 or phosphofructokinase
		Gly-7 or 3PG kinase
		Gly-10 or pyruvate kinase
isomerization:	isomerase, mutases	Gly-2 or phosphoglucoisomerase
		Gly-5 or triosephosphate isomearase
		Gly-8 or phosphoglycerate mutase
dehydration:	enolase	Gly-9 or 2-phosphoglycerate enolase
aldol condensation:	aldolase	Gly-4 or aldolase

1b) In as concise and compact prose as possible, compare the different mechanisms used by the enzymes which catalyze isomerizations. Identify catalytically active side groups and predict how the activity of the enzyme might vary with pH.

Gly 5, TIM, catalyzes the conversion of DHAP to GAP, a ketone to an aldehyde, via a "cis-ene-diol" intermediate. Glu 165 acts as a base to abstract a dissociable proton from the carbon adjacent to the carbonyl, and then acts as an acid to deliver it back to the carbonyl carbon, forcing protonation of the oxyanion via His 95. The pH profile for the conversion of DHAP to GAP is shown below, high rate requires a deprotonated glu and a protonated His. (Gly-2 similar mechanism)

Gly 8, phosphoglyerate mutase, catalyzes the conversion of 3PG to 2PG, via a HIS-HIS duo at the active site. Phosphoryllation of the active site His is a prerequisite for the reaction, and the isomerization proceeds by HisA abstracting a phosphate group from 3PG, at the same time that the oxygen at postion 2 of 3PG is abstracting the phosphate from HisB. Product dissociation is followed by an internal rearrangement where phosphate group is handed from HisA to HisB. A lysine at the active site pocket is necessary to ion pair with the carboxlyate of the substrate. High catalytic rates are mainained by a deprotonated His and a protonated Lys.

2. Schiff base formation is a common catalytic strategy used by proteins when interacting with a carbonyl-containing substrate. Which of the following statements is not true regarding Schiff base formation in aldolase. Explain.

a) The Shiff base serves to anchor the substrate in the binding pocket by forming a covalent enzyme-substrate adduct.
b) The Schiff base activates FBP for bond cleavage by providing electrons to stabilize the electrophillic intermediate.
c) The nitrogen used in Schiff Base formation is derived from a Lys side group.
d) Proteins employing Schiff base mechanisms should show greater activity at higher pH.

3. Identify by name an enzyme we have studied which: * = not yet studied in 19
(sometimes more than one correct answer exists, just list one)

* a) uses a cofactor to bind to the substrate and assist in the decarboxylation of its substrate by acting as an electron sink
pyruvate dehydrogenase; α-ketoglutarate dehydrogenase
b) uses a lysine side group to form a Schiff Base intermediate which binds and activates its substrate
aldolase, phosphoglucomutase
c) uses a metal ion to activate a water molecule and form an acylenzyme intermediate
carboxypeptidase, thenmolysin, endopeptidase
d) uses a histidine side group in catalysis and forms a phosphorylated enzyme intermediate
phosphoglycerate mutase, succinate thiokinase
e) uses a cysteine side group as a nucleophile and forms a thioester intermediate
GAP- dehydrogenase
* d) turns an achiral substrate into a chiral product
aconitase
* e) scrambles the C^{13} label on acetyl-CoA: H_3C^*-C-CoA
fumarase
* f) is a transmembrane protein in the mitochondrial membrane which binds ATP but is not a kinase
ATP synthetase
g) under normal cellular conditions operates below its V_{max}
glucokinase, GAP dehydrogenase
h) under normal cellular conditions operates at or near its V_{max}
hexokinase, TIM
i) exists in at least two different isozymes
LDH,
* j) is not really an enzyme but a protein which acts as a mobile carrier of electrons in the electron transport chain
cytochrome c
* k) needs to reduce its substrate via a four electron transfer
Cyt. c. oxidase
l) incorporates a radiolabeled oxygen from O^{18} water into its substrate in glycolysis
anything that forms Schiff base (see b)
* m) incorporates a radiolabeled oxygen from O^{18} water into its substrate in the TCA cycle
aconitase, fumarase, citrate synthase

4. In trying to elucidate the mechanism of TIM, experiments were conducted with glyceraldehyde 3-phosphate (GAP) tritiated at C2. The dihydroxyacetone phospate (DHAP) product had lost the label at the middle carbon and showed less than 5% labelling at the C1 carbon. Which of the following is true. Explain.

a) This experiment suggests that a single catalytic group on the enzyme could not be responsible for deprotonation at C2 and subsequent reprotonation at C1.

(b) This experiment suggests that a single catalytic group on the protein is responsible for deprotonation and subsequent reprotonation and has the opportunity to exchange trittium with the solvent.

c) This experiment suggests that two basic groups in close proximity must shuttle the trittium from one carbon to another.

5. In converting glucose to pyruvate in glycolysis, the substrate is oxidized from an aldo sugar to a α-keto acid. Which of the statements below best describes the oxidative processes in glycolysis. Explain.

a) oxidation occurs in gly-1, gly-3, gly-6.
b) oxidation occurs in gly-1 and gly-3.
(c) oxidation occurs in gly-6.
d) none of the above.

6. In testing the catalytic mechanism of gly-8, phosphoglycerate mutase, mutant enzymes were prepared with each of the possible mutations below:

a) His_A at the binding/active site was changed to Arg.
b) His_B at the binding/active site was changed to Tyr.
(c) Lys at the binding/active site was changed to Gly.

Which sample is most likely to have the same k_{cat} and very different K_m for isomerization of 3-phosphoglycerate? Explain.

7. Km/S values are used to evaluate the "degree of saturation" of an enzyme with a particular substrate under particular cellular conditions. Underline the correct word in parentheses in each of the sentences below and choose one enzyme we have studied for which the statement is true.

a) Km/S values (greater or (less) than one mean that the enzyme is working at full speed and that the rate of the reaction will not change rapidly with changes in the substrate concentration.
enzyme: hexokinase
b) Km/S values (greater) or less) than one mean that the enzyme is working far below its Vmax and that the rate of the reaction will change drastically with small changes in the enzyme concentration.
enzyme: GAP dehydrogenase

Answers

8. Enzyme Catalysis

The enzyme HIV-1 protease has been the the most successful target for treatment of HIV-1 infection. When combined with a reverse transcriptase inhibitor, this potent "cocktail" has brought patients back from death's door and restored them to full "health", though the capacity of the virus to "hide out" in the CNS for many years has meant that no one can yet claim that they are cured.

The protease is an endoprotease that cleaves the peptide bond on the amino terminal side of the amino acid proline. In a series of experiments on various peptide substrates, the following catalytic constants were measured. In the table below, a dash represents the scissile bond cleaved by the protease.

Substrate $P_3P_2P_1$-$P_1'P_2'P_3'$	Km (mM)	kcat(s^{-1})	kcat/Km ($mM^{-1}s^{-1}$)
(A) GNY-PVQ	0.60	2.4	4.0
(B) RNF-PVA	1.25	0.8	0.6
(C) LAA-PQF	0.13	1.9	14.6
(D) LNL-PVA	0.02	2.2	110.0

I don't have a detailed answer sheet for this. These noted are key points/me

Assume a simple mechanism, where $k_{cat} = k_2$, and $K_d = K_m$

$$E + S \Longleftrightarrow ES \longrightarrow E + P$$

1. Sketch a reaction coordinate diagram indicating the heights of the activation barriers for the four substrates from the data above. $\Delta G\ [ES] = -RT \ln (1/Km)$

$$\Delta G^{\ddagger} = 17.6 - 1.36 \log k_{cat}$$

2. What information about the enzyme binding site can you get from the various Km values?

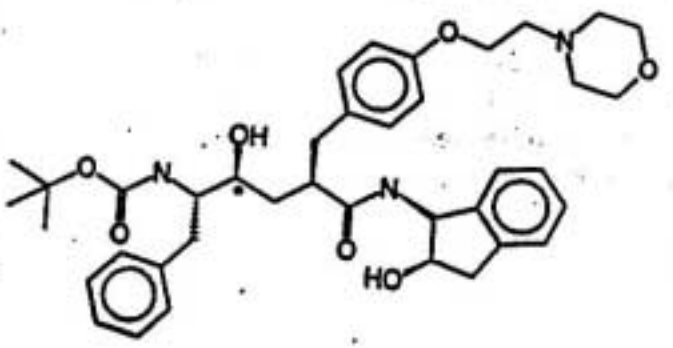
"D" binds most tightly; B least tightly; yet they differ functionally in the P_3 position. This suggests a positively charged group may exist near the P_3 position which destabilizes the E-S interaction for B. Sensitivity is low for $P_2'P_3'$ positions

3. How would you interpret the kcat/Km values in the last column?

Enzyme would select "D" over other substrates, worst is "B"

4. One inhibitor, L698-502, which is in phase III clinical trials is shown below. This drug is thought to be a competitive inhibitor. How, if at all, would the presence of a competitive inhibitor change the experimentally derived constants in the table above?

Competitive inhibitor effects Km, not kcat



L-689,502
K_i = 0.051 nM
MW = 672.84 Da

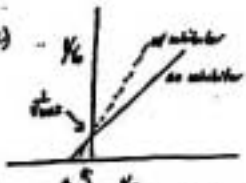
"Km" increased in presence of inhibitor

Problem III

4. Best method of calculating k_{cat} is to extract it from a Lineweaver Burke plot of $1/v_o$ vs $1/s$ where the Y intercept is $1/V_{max}$ and $V_{max} = [E_T]k_{cat}$. Substituting:

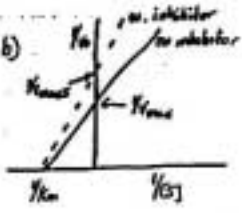
$$k_{cat} = (1/Y\ intercept) / E_T$$

5) a)



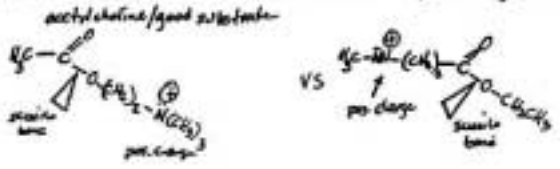
competitive inhibition with no good substrate - V_{max} - same. K_M ↑ since $1/K_m$ decreases

b)



non-competitive inhibitor with poor substrate. V_{max} ↓ since $1/V_{max}$ ↑. K_m remains same.

7. Compare molecular structure of substrates particularly with to the relative orientations of scissile bond and pos. charged amine group:



III. 7 cont'd.

In the two substrates, the relative positions of the amino term - or pos. charge and the ester bond is reversed. Given typical specificity of binding and active sites on enzymes, this juxtaposition leaves an empty "part" of the binding site in the poor substrate with.

open for binding tertiary amine even when substrate bound, ∴ non-competitive

No open holes in binding or active site

Problem III cont'd (5 pts each)

1. From the information on the previous page, what is the k_{cat} for hydrolysis of acetylcholine by acetylcholine-esterase at 25°C.

$k_{cat} = 25,000\ s^{-1}$

2. How long does each acetylcholine substrate molecule stay attached to the enzyme?

$[k_{cat}]^{-1} = 40\ \mu sec$

3. If the mechanism is a simple one such as discussed in class (equilibrium of E and S, or steady state of E and S) what would be the activation barrier for the rate determining step?

$\Delta G^{\ddagger} = 17.6 - 1.36 \log k_{cat} = 11.6$ kcal/mole @25°C

4. Once the initial rate of the hydrolysis has been measured at several different concentrations of substrate, how is k_{cat} calculated? see attached

5. What would the Lineweaver-Burke plots look like for the case of:

a) the inhibition of hydrolysis of acetylcholine by a protonated tertiary amine? Label all axes and lines.

b) the inhibition of hydrolysis of substrate [2] by a protonated tertiary amine? Label all axes and lines.

see attached

6. What happens to V_{max} and K_m in each of these circumstances.

see attached

*7. Does the inhibitor bind at the active site or doesn't it? Can you offer a possible explanation of the contradictory data? see attached

The end.