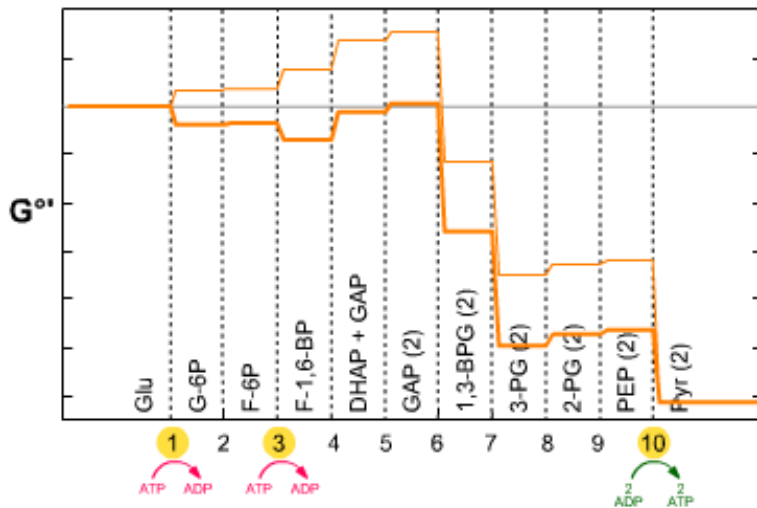


Problem Set II
 Enzyme Catalysis, Glycolysis, TCA, Electron Transport, ATP synthesis

From Spring '02



I. Energy Coupling: You have heard a lot about how glycolysis “conserves” energy released in the process of burning glucose that would otherwise have been lost to heat and stored in the phosphoranhydride bond of ATP or in other high energy molecules. Shown at left is an image from your text’s website used to illustrate energy coupling. Each of the ten steps of glycolysis are listed, and the overall standard state free energies graphed in bold. The lighter graph represents the standard state free energy change for just the sugar, and

is therefore in some cases is only a partial story.

1. Identify two steps in which energy coupling **directly** drives an otherwise energetically unfavorable chemical reaction (i.e. by its effects on **that** reaction alone and not because a product is being drained off by the next reaction). In each case, draw chemical structures for the glucose based substrate and product and name the enzyme that catalyzes this step. Finally, predict what happens to the overall free energy when you take into account physiological concentrations of reactant and product.
2. Identify steps in which energy coupling reduces the standard state free energy released in conversion of glucose based substrate to product (i.e. identify steps in which energy is stored). Draw the chemical structures for the glucose based substrate and product and name the enzyme formally. Finally, predict what happens to the overall free energy when you take into account physiological concentrations of reactant and product?

II. Hexokinase (2.7.1.1) is the first enzyme of glycolysis, and exhibits multisubstrate binding.

1. Shown below are K_m values for several different substrates of hexokinase from rabbit erythrocytes. A check of the structures for the first three sugars on page 297 should convince you that they vary primarily in the stereochemistry and geometry of the 2 position. D-fructose, shown on page 298, is a keto rather than an aldo sugar. Convert these “equilibrium constants” into binding energies and comment on the importance of the 2- position in binding.

<u>K_m (mM)</u>	<u>substrate</u>
0.06	D-glucose
0.71	D-mannose
1.33	2-deoxy D-glucose
17.8	D-fructose

2. What variation might you expect to see (if any) for the k_{cat} for this reaction?
3. The specific activity (μ moles of substrate converted to product per min per mg enzyme) of hexokinase with D-glucose and $MgATP^{-2}$ is 165, and the molecular weight is 110,000 g/mole. What is the value for the k_{cat} in s^{-1} ?
4. Mg-ATP binds with a $K_m = 0.6$ mM in the presence of glucose, and almost not at all in the absence of glucose. What does this suggest about the structure of the protein with and without glucose.

III. Glycolysis, Pyruvate Dehydrogenase, Citric Acid Cycle

1. Assuming the protein is still folded, would the aldolase enzyme be more active at pH 6 or 8? Explain.
2. What would be the effect of mutating residue 149 in GAP dehydrogenase from a cysteine (a thiol) to a serine (an alcohol)?
3. In pyruvate dehydrogenase complex, identify the substrate atom (or atoms) that is (are) oxidized and trace the path of the electrons to their final destination in products of this step.
4. Citrate synthase takes oxaloacetate and acetyl CoA and generates citrate and free CoA-SH by a two step reaction. Which of these steps might you think is rate limiting and why?
5. Aconitase contains an Fe-S cluster in its active site. What is the role of that cluster?

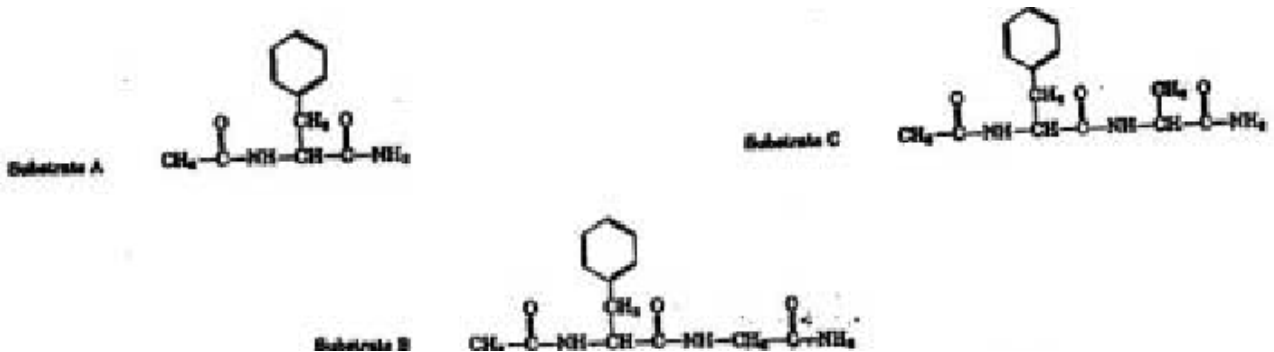
IV. Electron Transport and ATP synthase

1. a) Calculate standard state free energy for the reaction catalyzed by Complex I from the redox couples of reactants and products and the Nernst equation ($\Delta G^{\circ} = -nF\Delta E_o'$, t is 37 C and R is 2.0 cal/mole K).
 b) If all of this standard state free energy were captured as a pH gradient, what ΔpH would be produced (assuming a constant ΔV of 0.14 V) if one mole of NADH reactant was converted to one mole of ubiquinol product? ($\Delta G^{\circ}_{\text{membrane}} = 2.303RT\Delta pH + F\Delta V$ where F is 23.06 kcal/mole)
2. In the presence of antimycin, $\text{cyt } b_H$ and b_L get reduced and the FeS protein remains oxidized. Where does this inhibitor bind and how does it act to block reduction of cytochrome c ?
3. Why did we say that an equivalent of 8 H^+ was pumped from the matrix to the intermembrane space by the cytochrome oxidase complex?
4. Discuss the role of the aspartic acid in the middle of the c subunit of the F_o complex of F_1F_o ATPase.

From Spring '99

I. Enzyme Catalysis 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.

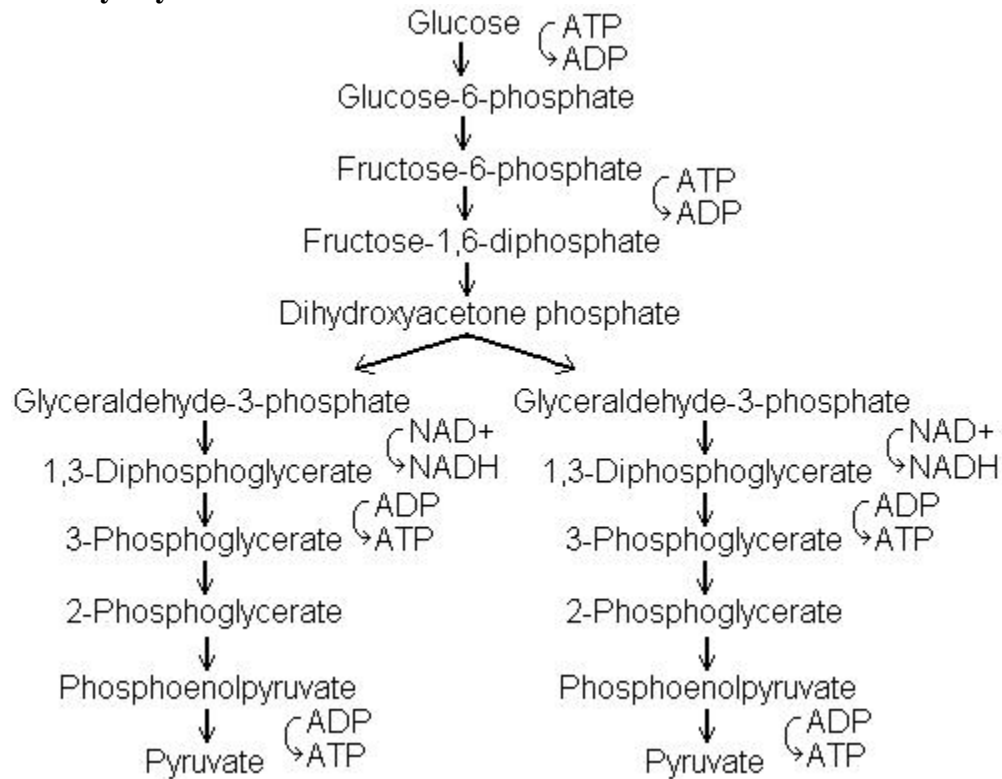


- b) The K_m 's for the three different substrates are 31 mM (Substrate A), 15 mM (Substrate B), and 25 mM (substrate C). If we assume that the ES complex is in equilibrium with the E and S starting material, we can calculate the binding energy directly from the K_m . What are the binding energies for each of the ES complexes at 37°C? What interactions might give rise to these differences?
- 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 activation barrier for each reaction at 37°C. Why do you suppose these barriers are so different?
- d) The k_{cat}/K_m values help to evaluate which substrate works "best" with an enzyme. Which of the substrates is the "best" substrate for chymotrypsin and why.

I. Michaelis Menten Kinetics

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.
2. a) Sketch the form of the Michaelis-Menten equation on a graph of initial rate vs. substrate concentration for a non-allosteric 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?
3. How would the graph you have drawn above change if a transition state analogue was added to the mixture of enzyme and substrate at the beginning of the reaction. Explain
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.

III Glycolysis



- Next to each of the reactions above, write one of the following letters which best describes the type of chemical reaction catalyzed:
 - Phosphoryl transfer
 - Phosphoryl shift
 - Isomerization
 - Dehydration
 - Aldol Cleavage
 - Phosphorylation coupled to oxidation

2. In 8/10 of the reactions cited below, 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?

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

Note: ΔG° and ΔG are expressed in kcal/mol. ΔG , the actual free-energy change, has been calculated from ΔG° and known concentrations of reactants under typical physiological conditions.

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

- binds a six carbon sugar keto in its open form
- binds to many different hexose sugars
- is inhibited by high levels of 2,3 Diphosphoglycerate
- needs to bind a second molecule of oxidized substrate before it can release product
- incorporates radioactive oxygen from solvent into one of its products
- would have its activity destroyed by an inhibitor that reacted with thiols
- Has as its main job recycling of its second product which would otherwise go to waste
- Dehydrates its substrate

IV. Regulation/Control/ k_{cat}/S

Substrate	Concentrations		Change as a result of ischemia (%)	mM	Km
	Initial	Ischemia			
Glucose	2560	1930	-25		
Glucose-6-P	224	91	-59	0.1	gly1
Fructose-6-P	50	27	-46	0.7	gly2
Fructose-1,6-bis-P	27	153	+467	depends	gly3
Dihydroxyacetone-P	13	39	+200	0.1	gly4
Glyceraldehyde-P	0.9	3.3 ^a	+267	0.87	gly5
1,3-bis-P-glycerate	<1	<1	0	0.07	gly6
3-P-glycerate	25	85	+240	1.2	gly7
2,3-bis-P-glycerate	29	29	0	5.0	gly8
2-P-glycerate	2.8	8.8	+214	.07	gly9
P-pyruvate	3.5	8.5	+151	0.6	gly10
Pyruvate	39	72	+85		

Shown above are data for concentrations in mM of glycolytic intermediates in a resting animal (initial) and in one deprived of oxygen for 25 seconds (ischemia). Old data for Km is also shown. Your handouts may have different values more recently determined.

- 1) Given the concentrations of the glycolytic intermediates, and the Kms above, identify the degree of saturation of each of the enzymes of glycolysis.
 - a) in the resting animal
 - b) in the ischemic animal

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

From old exams

Enzymes of Glycolysis and Citric Acid Cycle

1. a) 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:	<u>dehydrogenases</u>	Gly-6 or GAP dehydrogenase
phosphorylation:		
isomerization:		
dehydration:		
aldol condensation:		

- b) 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.

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 Schiff 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 electrophilic 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:

(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
- b) uses a lysine side group to form a Schiff Base intermediate which binds and activates its substrate
- c) uses a metal ion to activate a water molecule and form an acylenzyme intermediate
- d) uses a histidine side group in catalysis and forms a phosphorylated enzyme intermediate

- e) uses a cysteine side group as a nucleophile and forms a thioester intermediate
 - d) turns an achiral substrate into a chiral product
 - e) scrambles the C¹³ label on acetyl-CoA: H₃C^{*}-C-CoA
 - f) is a transmembrane protein in the mitochondrial membrane which binds ATP but is not a kinase
 - g) under normal cellular conditions operates below its V_{max}
 - h) under normal cellular conditions operates at or near its V_{max}
 - i) exists in at least two different isozymes
 - j) is not really an enzyme but a protein which acts as a mobile carrier of electrons in the electron transport chain
 - k) needs to reduce its substrate via a four electron transfer
 - l) incorporates a radiolabeled oxygen from O¹⁸ water into its substrate in glycolysis
 - m) incorporates a radiolabeled oxygen from O¹⁸ water into its substrate in the TCA cycle
4. In trying to elucidate the mechanism of TIM, experiments were conducted with glyceraldehyde 3-phosphate (GAP) tritiated at C2. The dihydroxyacetone phosphate (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 tritium with the solvent.
 - c) This experiment suggests that two basic groups in close proximity must shuttle the tritium 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. K_m/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) K_m/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:
 - b) K_m/S values (greater or less) than one mean that the enzyme is working far below its V_{max} and that the rate of the reaction will change drastically with small changes in the enzyme concentration.
enzyme:

8. Enzyme Catalysis

The enzyme HIV-1 protease has been 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	K _m (mM)	k _{cat} (s ⁻¹)	k _{cat} /K _m (mM ⁻¹ s ⁻¹)
GNY-PVQ	0.60	2.4	4.0
RNF-PVA	1.25	0.8	0.6
LAA-PQF	0.13	1.9	14.6
LNL-PVA	0.02	2.2	110.0

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



1. Sketch a reaction coordinate diagram indicating the heights of the activation barriers for the four substrates from the data above.

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

3. How would you interpret the k_{cat}/K_m values in the last column?

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 K_m , R_{cat}
" K_m " increased
in presence
of
inhibitor

L-689,502
 $K_i = 0.051 \text{ nM}$
MW = 672.64 Da

