

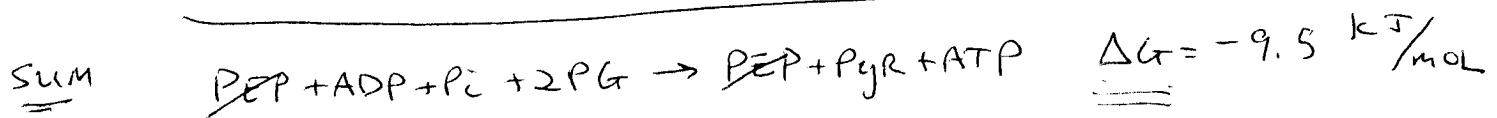
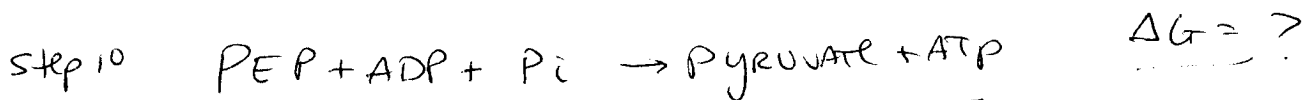
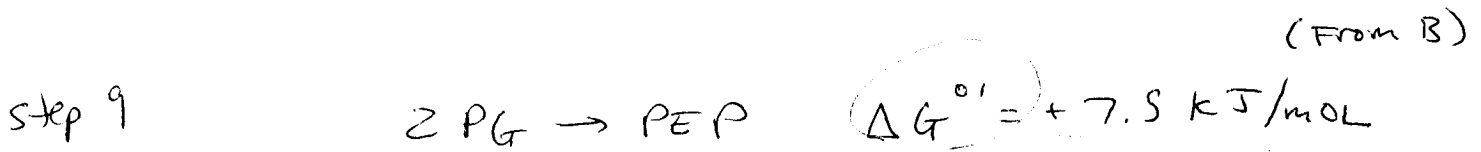
Continuation of problem 1.

(10) C. The tenth and final glycolytic reaction is catalyzed by pyruvate kinase, and generates both pyruvate and ATP.

In some cells, the free energy change (at cellular substrate and product concentrations) associated with the combined enolase and pyruvate kinase reactions (steps 9 and 10 of glycolysis) is -9.5 kcal/mol. Further, the mass action ratio (Q) for the enolase reaction in these cells is maintained at 1.0.

Calculate the free energy change, ΔG , for the pyruvate kinase reaction that occurs inside these cells. Show your work. Explain your reasoning.

Free energies of sequential reactions with a common intermediate are additive ✓



Q for the ENOLASE REACTION is given as 1.0

$$\Delta G = \Delta G^{\circ'} + RT \ln Q \rightarrow \Delta G = \Delta G^{\circ'} = +7.5 \frac{\text{kJ}}{\text{mol}}$$

$$\begin{aligned} \rightarrow \Delta G(\text{step 10}) &= \Delta G(\text{step 9} + \text{step 10}) - \Delta G(\text{step 9}) \\ &= -9.5 \frac{\text{kJ}}{\text{mol}} - 7.5 \frac{\text{kJ}}{\text{mol}} = -17 \frac{\text{kJ}}{\text{mol}} \end{aligned}$$

2. Answer each of the following in one clear and concise sentence each. Please make your answer as precise as you can. (20 points; 5 points each).

(a) In the aldolase reaction of glycolysis, an enzyme lysine forms a protonated Schiff base linkage at C2 of the fructose 1,6-bisphosphate substrate. How is this important to facilitating the subsequent carbon-carbon bond cleavage between C3 and C4?

The carbon-carbon bond cleavage proceeds through an unstable carbanion intermediate, and the positively-charged nitrogen of the Schiff linkage stabilizes this charge and acts as an electron sink to drive the reaction forward.

(b) Why is the lactate dehydrogenase reaction important in maintaining the ability of anaerobic cells to carry out glycolysis?

The LDH reaction oxidizes NADH to NAD⁺, which is necessary to replenish the NAD⁺ pool to allow the glyceraldehyde 3-phosphate dehydrogenase reaction to proceed.

(c) Adenylyl transfer reactions at the ATP α -phosphate (closest to the sugar) result in transfer of the adenylyl group to the attacking nucleophile. How does pyrophosphatase help to drive this reaction?

The other product of this reaction is pyrophosphate; cleavage of pyrophosphate to yield 2x P_i lowers the P_i concentration, providing a thermodynamic driving force in the direction of adenylyl transfer.

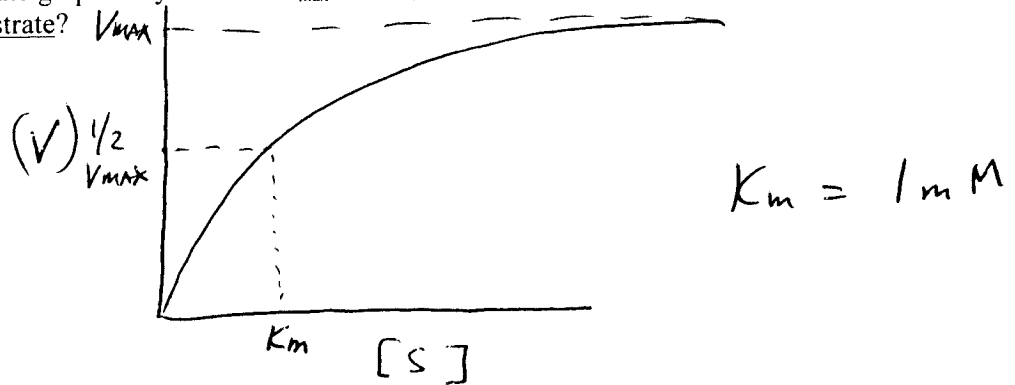
(d) How does induced fit in hexokinase help to ensure specificity of the enzyme for phosphorylation of glucose? [Hint - what is the competing "noncognate" substrate?]

Induced fit here shields the active site from solvent, keeping the "noncognate" water out of the active site, and preventing hydrolysis of ATP.

3. (20 points) Your biochemistry lab partner has disappeared, leaving you alone to complete the previous weeks' experiment on steady-state kinetics. Unfortunately, you missed lab last week in the wake of the first Biochemistry midterm, and your partner did not keep an accurate notebook. The only information that you can be sure of is: (i) the V vs. S plot is hyperbolic; (ii) the substrate concentration at $\frac{1}{2} V_{\max}$ is 1 mM ; (iii) the enzyme concentration used was 100 nM ($1 \times 10^{-7} \text{ M}$).

(a) Draw and label the V vs. S plot, showing how the reaction velocity varies with the concentration of substrate and indicate graphically how the V_{\max} and K_m are determined. $V_0 = V_{\max}[S]/K_m + [S]$. What is the K_m for this substrate?

(5)



(d) Is K_m a relevant derived parameter for all enzyme reactions in which a V vs. S plot shows substrate saturation? Explain your answer.

(5)

No. Allosteric enzymes show sigmoidal V vs S plots in which substrate saturation is still observed. These enzymes do not follow Michaelis-Menten kinetics, so the substrate concentration at half-maximal velocity should not be considered as K_m .

(b) Describe the experiments that you would do to determine the numerical value of V_{\max} .

(5)

Measure the accumulation of product with time using substrate concentrations that are greater than 1 mM ; continue increasing $[S]$ until V no longer increases; or make measurements both above and below $[S] = 1 \text{ mM}$, and ~~fit~~ fit the data to:

$$V_0 = \frac{V_{\max}[S]}{K_m + [S]}$$

(c) What is the value of k_{cat}/K_m for $V_{\max} = 50 \mu\text{M}/\text{min}$? Give the correct units.

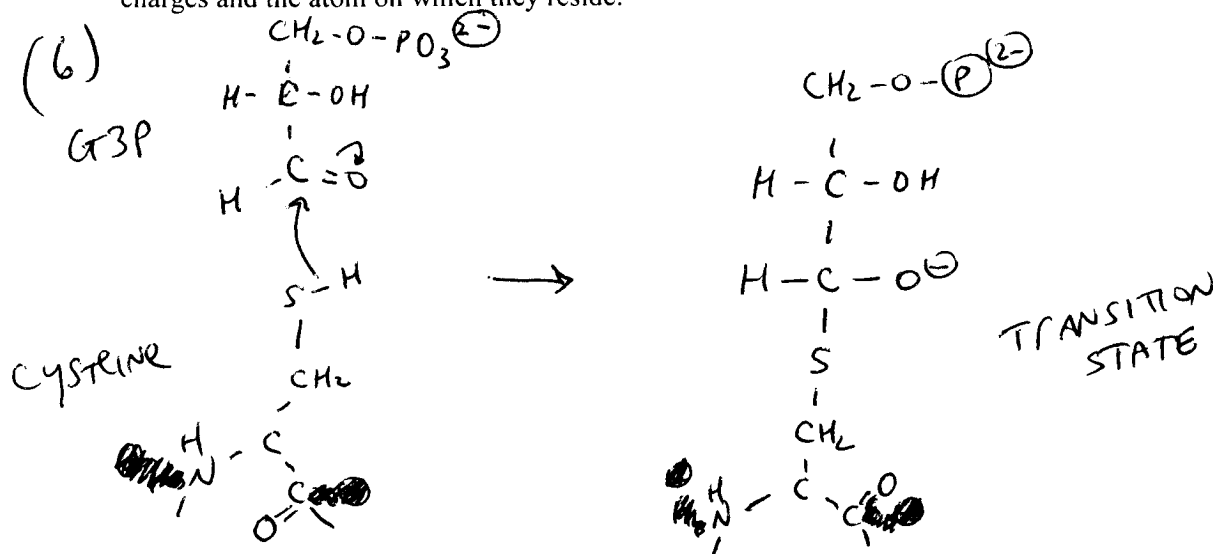
(5)

$$k_{\text{cat}} = \frac{50 \mu\text{M}/\text{min}}{0.1 \mu\text{M}} = 500 \text{ min}^{-1}$$

$$\frac{k_{\text{cat}}}{K_m} = \frac{500 \text{ min}^{-1}}{1 \text{ mM}} = 3 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$$

4. (20 points) In the glycolytic reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase (G3PDH), a cysteine sulfhydryl group functions as a nucleophile to attack the aldehyde carbon of glyceraldehyde-3-phosphate (G3P).

(a) Draw the structures of the cysteine and the G3P substrate, pushing electrons to show how the transition state of the reaction is approached. Draw the transition state as well. Indicate any electric charges and the atom on which they reside.



(b) Describe the geometry about the aldehyde carbon of G3P in both the ground and transition states.

(2)

ground state - trigonal planar

transition state - tetrahedral

(c) Considering this portion of the reaction only, what two features would you expect to see in the enzyme active site, that would lower the activation energy barrier for the reaction?

- (4)
- (1) A general base to abstract the proton from the cysteine sulfhydryl group
 - (2) A positively charged or electrophilic group to stabilize the negative charge on the carbonyl oxygen in the transition state

(d) Describe how the overall G3PDH-catalyzed reaction provides an example of a double-displacement mechanism (ping-pong kinetics). (Name all the substrates and products, and describe the order of addition and leaving).

(8)

First, $\text{NAD}^{(+)}$ and G3P bind to the enzyme,

Next, the thioester intermediate is formed after passing thru the tetrahedral transition state depicted above, with formation as well of NADH

The NADH (product 1) dissociates, and the second substrate, P_i , then binds.

Another $\text{NAD}^{(+)}$ rebinds at this stage, but does not undergo any reaction

The phosphate substrate attacks at the carbon of the thioester, resulting in the formation of the second product, 1,3 bisphosphoglycerate, which then dissociates to generate the resting enzyme, which retains $\text{NAD}^{(+)}$ for the next catalytic cycle

5. Give the best answer to each of the following (2 points each)

A. Nondiffusible redox cofactor that can accept either one or two electrons

FAD (FMN) ~~FMN~~

B. Identity of the substrate group that acquires a negative charge in the transition state for peptide cleavage by chymotrypsin

Carbonyl Oxygen in the scissile peptide bond

C. Name of the enzyme that interconverts 3-carbon sugars at the midpoint of the glycolytic pathway

Triose phosphate isomerase

D. Reduced product of fermentation in organisms that lack lactate dehydrogenase

Ethanol

E. General name given to the unstable compound of highest energy on the path from substrate to product

Transition state

F. For the reaction interconverting Substrate and Product at standard state conditions, the direction of the reaction when $K'_{eq} = 0.5$

In the direction of substrate

G. Considering the reaction in F., what is the effect on the equilibrium of adding enzyme at a molar quantity in excess of substrate?

No effect

H. Crucial byproduct generated by organisms that reduce CO_2 to sugars using light photons.

O_2

I. Common enzyme cofactor required for effective catalysis in all steps of glycolysis

Mg^{2+} ions

J. Rationale for why thioester compounds are at higher energy than the corresponding oxygen esters.

Thioesters are not stabilized by resonance