Chemical Engineering 170 October 11, 2004

Midterm Exam
Closed Book and Closed Notes
One 8.5 x 11 in. page of notes allowed

Section 1. Short Answers

1. (6 pts.) Recently site-directed mutagenesis was used to modify the cofactor specificity of the enzyme phosphite dehydrogenase (Woodyer et al., Biochemistry **2003**, 42, 11604). **Shown in Figure 1 on the last page** are interactions between the wildtype (WT), the E175A mutant, and the E175A, A176R double mutant enzymes and the nicotinamide cofactor NADP (the 2'-phosphate of NADP is shown in red and yellow). The numbers shown are distances of the dotted lines in angstroms.

a) Which of the above three enzymes has the highest K_m toward NADP?

WT (+1)

b) Briefly explain (1-2 sentences) your answer above.

Electrostatic repulsion between the two negative charges (+2)

c) Which of the above three enzymes has the lowest K_m toward NADP?

E175A, A176R double mutant (+1)

d) Briefly explain (1-2 sentences) your answer above.

Electrostatic attraction between NADP and R176 (+2)

2. (12 pts.) **As shown in Figure 2 on the last page,** the enzyme tRNA guanine transglycosylase (TGT) catalyzes replacement of guanine (G) by 7-aminomethyl-7-deazaguanine (PreQ1) at a specific position of four tRNAs. A proposed mechanism of the TGT-catalyzed reaction involves the following steps: (i) nucleophilic attack of the side chain of Asp102 on the C1' of the targeted nucleotide (tRNA-G) to assist the departure of the guanine base and form a TGT–tRNA covalent intermediate; (ii) replacement or exchange of guanine by PreQ1 in the guanine/PreQ1-binding pocket in TGT; and (iii) covalent linkage of PreQ1 to the tRNA through a nucleophilic attack of the N9 of PreQ1 (shown in blue on the last page) on the C1' of the targeted ribose to form the product.

a) Write a mechanistic equation (e.g., E + tRNA-G ? E·tRNA-G $\rightarrow$ E + tRNA-PreQ1) consistent with the mechanism described above.

E + tRNA-G ? (E•tRNA-G) ? E-tRNA ? (E-tRNA•PreQ1) ? E + tRNA-PreQ1 ? E + tRNA-PreQ1

or

E + tRNA-G ? (E•tRNA-G) ? E-tRNA ? (E-tRNA•PreQ1) ? E + tRNA-PreQ1 (+6)

b) Using site-directed mutagenesis, you propose to generate two different mutant enzymes, with each mutant containing a replacement for Asp in position 102. Assuming each mutant is to retain at least some catalytic activity, which two amino acids would you use to replace Asp102?

Glu and Ser

c) The above reaction is inhibited by 9-deazaguanine, in which the N9 of guanine is replaced by carbon. What type of inhibitor is 9-deazaguanine?

Competitive

d) Assume that the K_S value of the enzyme for tRNA-G is 10 μM, and that the enzyme-catalyzed reaction proceeds 10^7-times faster than the uncatalyzed reaction. What, then, is the theoretical lowest value of K_i, the inhibition constant for 9-deazaguanine?

$$\frac{k_e}{k_n} = 10^7 = \frac{K_S}{K_T} \longrightarrow \frac{10m\text{M}}{K_T}$$

(+1) (+1)

$$K_T \approx K_i = 10 \times 10^{-7}\, m\text{M} = 1\text{pM} \quad (+1)$$

3. (6 pts.) After mutagenizing a strain of *E. coli*, you identify a mutant with an interesting growth phenotype (specifically, the cells exhibit increased resistance to toxic heavy metals). You are interested in identifying the cause for this phenotype.

a. In comparing your mutant to the non-mutagenized wild-type strain, what can you learn from an mRNA microarray experiment, proteomics experiment, and metabolomics experiment? Use only one sentence for each experiment.
mRNA microarray: transcript levels (mRNA levels) of the two strains relative to one another (+1)
proteomics: protein/enzyme levels of the two strains relative to one another; ideally the entire proteome of the cells (+1)
metabolomics: metabolite levels of the two strains relative to one another; again, ideally the entire metabolome of the cells (+1)

b. You identify a multi-step pathway capable of breaking down compounds containing heavy metals is up-regulated in the mutant strain. You hypothesize that a rate-limiting step still exists in the pathway, and you want to further increase the flux through this pathway. Which experiment from part (a) will give you the most direct information for best identifying a gene target for increased overexpression? Why?
The metabolomics experiment. (+1) This experiment will identify a buildup of intermediate (metabolite) at the rate limiting step. The enzyme responsible for metabolizing this substrate can then be over-expressed. (+2)

4. (3 pts.) Given two oligos of double stranded DNA:

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(I) GACTGGCAGGACGT          (II) GTTCATATGGAACT
    ||||||||||||||                ||||||||||||||
    CTGACCGTCCTGCA                CAAGTATACCTTGA
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a) Which sequence will separate into single strands at a lower temperature?

(II) (+1)

b) Why does this sequence dissociate at a lower temperature?

Strand II has a lower GC content. (+1)
GC pair with 3 hydrogen bonds; AT with only 2, making AT pairing easier to separate. (+1)

5. (6 pts.) Give brief definitions for the following:

a) Operon a group of prokaryotic genes under the control of a single operator (i.e. expressed under the control of one promoter and regulatory mechanism) (+2)

b) Codon a three-base sequence in mRNA that codes for a specific amino acid in a protein or causes the termination of translation (+2)

c) Diauxic growth growth occurring in two phases between which a temporary lag occurs; due to the switching in metabolism from one limiting growth substrate to another; generally carbon (+2)

6. (3 pts.) Name three components usually found on an *E. coli* expression vector (plasmid).
promoter, terminator, multiple cloning site (restriction enzyme sites), antibiotic resistance marker, origin of replication, gene of interest (each +1)

7. (6 pts.) Name three advantages and three disadvantages of immobilized enzymes.

Advantages:
can be reused continuously, easier product recovery, suitable for many reactor types, require less space (lower capital costs), often exhibit increased stability, introduce potential for manipulation of catalytic properties (each +1)

Disadvantages:
loss in activity, mass transfer limitations, additional cost (reagents, carrier), impractical for solid substrates (each +1)

8. (7 pts.) Write a complete expression for the rate of substrate consumption by a growing cell population. Label all terms discussed in class.

$$r_{s,i} = \frac{r_x}{Y_{x/s_i}} + m_i x + \sum_{j=1}^{n} \frac{r_{P_j}}{Y_{P_j/s_i}} \qquad \text{(+4)}$$

$r_{s,i} = q_{s,i} x = \text{rate of substrate } i \text{ consumed}$
$r_x = mx = \text{growth rate of cells}$
$Y_{x/s_i} = \text{yield coefficient on substrate } i$
$m_i = \text{maintenance coefficient}$
$x = \text{cell biomass}$
$r_{P_j} = \text{rate of formation of product } j$
$Y_{P_j/s_i} = \text{yield coefficient of product } j \text{ on substrate } i$

Is this an unstructured or structured model? Segregated or nonsegregated?

Unstructured (+1), Nonsegregated (+1)

An organism's doubling time is 40 minutes. Starting with a single cell, how many cells will be present after 6 hours?

$$6\,\text{hours} \times \frac{60\,\text{minutes}}{1\,\text{hour}} \times \frac{1\,\text{doubling time}}{40\,\text{minutes}} = 9\,\text{doubling times} \qquad 2^9 = 512\,\text{cells} \quad (+1)$$

3. (14 pts.) A peptide has four independently ionizable groups with these pK's:

pK_1 (COOH) = 2.0
pK_2 (ImidazoleNH^+) = 6.0
pK_3 (Phenolic OH) = 10.0
pK_4 (NH_3^+) = 10.0

a) What is the approximate (or exact) net + or – charge on each group at these pH's (complete the following table)?

Net + or - Charge

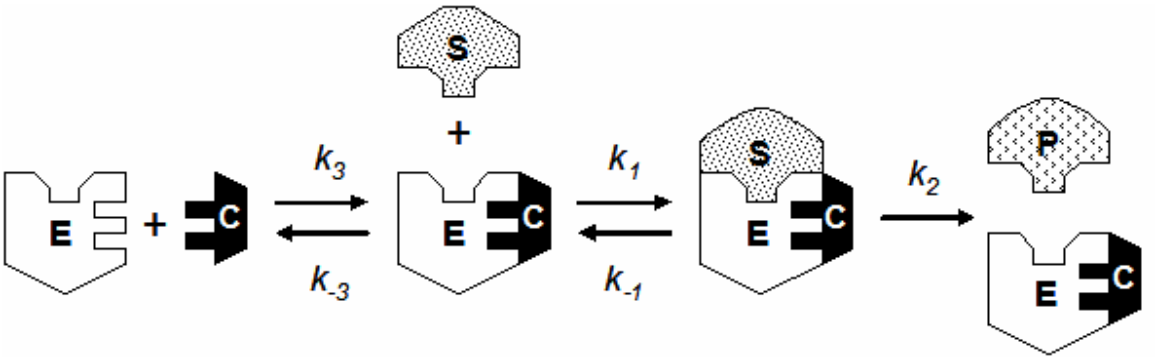
	COOH of pK_1	Imid. of pK_2	OH of pK_3	NH_3^+ of pK_4
pH 3	-0.90	+1.0	0	+1.0
pH 7	-1.0	+0.10	0	+1.0
pH 10	-1.0	0	-0.5	+0.5

b) What is the net + or – charge on the peptide at

pH 3: +1.1 pH 7: +0.10 pH 10: -1.0

Section 2. Short Problems

1. (18 pts.) Consider the following reaction scheme:



For this reaction a cofactor, C, is required to activate the apoenzyme, E, for binding and conversion of substrate S.

(a) Write the non-steady-state mass balances for the enzyme complexes EC and ECS.

$$\frac{d[EC]}{dt} = k_3[E][C] - k_{-3}[EC] - k_1[EC][S] + k_{-1}[ECS] + k_2[ECS] \quad (+2)$$

$$\frac{d[ECS]}{dt} = k_1[EC][S] - k_{-1}[ECS] - k_2[ECS] \quad (+2)$$

(b) Assume rapid equilibrium between the cofactor and apoenzyme as well as between the cofactor-enzyme complex and the substrate. Use these assumptions with the following definitions to write a rate equation for the formation of product. Your final answer should look as much like the Michealis-Menten equation as possible.

$K_S = k_{-1} / k_1$ $\quad K_C = k_{-3} / k_3$

$$\frac{d[P]}{dt} = k_2[ECS] \quad (+1)$$

$$[E_0] = [E] + [EC] + [ECS] \quad (+1)$$

$$K_S = \frac{[EC][S]}{[ECS]} \quad (+1)$$

$$[EC] = \frac{K_S[ECS]}{[S]} \quad (+1)$$

$$K_C = \frac{[E][C]}{[EC]} \quad (+1)$$

$$[E] = \frac{K_C[EC]}{[C]} = \frac{K_S K_C[ECS]}{[S][C]} \quad (+1)$$

$$[E_0]=[E]+[EC]+[ECS]=\frac{K_S K_C[ECS]}{[S][C]}+\frac{K_S[ECS]}{[S]}+[ECS]$$

$$[ECS]=\frac{[E_0]}{\frac{K_S K_C}{[S][C]}+\frac{K_S}{[S]}+1}$$

$$\frac{d[P]}{dt}=k_2[ECS]=k_2\frac{[E_0]}{\frac{K_S K_C}{[S][C]}+\frac{K_S}{[S]}+1} \quad (+2)$$

$$\frac{d[P]}{dt}=\frac{k_2[E_0][S][C]}{K_S K_C+K_S[C]+[S][C]}=\frac{k_2[E_0][S][C]}{K_S(K_C+[C])+[S][C]} \quad (+2)$$

$$\frac{d[P]}{dt}=\frac{k_2[E_0][S]}{K_S\left(\frac{K_C}{[C]}+1\right)+[S]} \quad \text{(this form is also acceptable)}$$

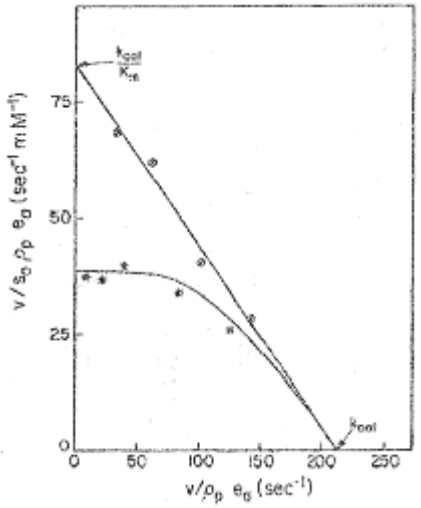
(c) Under what condition would the reaction velocity to be first order in C?

$$[C] << K_C \quad (+2)$$

(d) Under what condition would the rate be independent of cofactor concentration?

$$[C] >> K_C \quad (+2)$$

2. (19 pts.) As we discussed in class, Eadie-Hofstee plots are useful for determining kinetic parameters of enzyme-catalyzed reactions. Shown below are two such plots (note the axes have been switched from what we discussed in class): one for the enzyme chymotrypsin immobilized to small (~ 10 μm) catalyst particles (O), and one for chymotrypsin immobilized to large spherical (~ 120 μm) porous particles (*). The reaction velocity, v, is defined per unit catalyst volume; s_0 is the bulk substrate concentration, e_a is the active enzyme loading per weight of catalyst, and ρ_p is the particle density.



a) Why does the plot for the large catalyst particles curve downward near the y-intercept, and approach the straight line for the small catalyst particles at large values of v? Please limit your answer to a few sentences.

b) Now we will derive an expression for the y-intercept of the immobilized enzyme data in terms of the Thiele modulus of the catalyst. If ϕ is sufficiently large, as we will assume it is for the large particles, the effect of curvature can be neglected and **the particle can be treated as a slab.**

i) Write a steady state material balance on substrate through the particle in terms of d^2S/dr^2.

$$D_c \frac{d^2S}{dr^2} = v(r) = \frac{v_{\max} \cdot S(r)}{S(r) + K_m} \quad (+3)$$

ii) Use the following identity

$$\frac{d^2S}{dr^2} = \frac{1}{2}\frac{d}{dS}\left(\frac{dS}{dr}\right)^2$$

and the material balance from (i) to show that, if $S(r) << K_m$,

$$\left.\frac{dS}{dr}\right|_{r=R} \cong \left(\frac{n_{max}}{K_m D_{eff}}\right)^{1/2} S(R)$$

where S(R) is the substrate concentration at the particle surface.

$$\frac{d^2S}{dr^2} = \frac{1}{2}\frac{d}{dS}\left(\frac{dS}{dr}\right)^2 \longrightarrow \frac{D_c}{2}\frac{d}{dS}\left(\frac{dS}{dr}\right)^2 = v(r)$$

$$\frac{1}{2}\int_{dS/dr|_R}^{0} d\left(\frac{dS}{dr}\right)^2 = \int_{S=S_R}^{s(r=0)} \frac{v(r)ds}{D_c} \quad (+6)$$

$$\frac{1}{2}\left(\left.\frac{dS}{dr}\right|_R\right) = \sqrt{\frac{v_{\max}}{D_c K_m}}\frac{S(R)}{1} \quad (+2)$$

(iii) At steady state, the overall reaction rate, v_{obs}, must equal the rate of substrate uptake through the outer surface of the particles, that is,

$$n_{obs} = D_{eff}\left(\frac{3}{R}\right)\left.\frac{dS}{dr}\right|_{r=R}$$

Combine this expression with the expression in part (ii) to obtain the y-intercept of the Eadie-Hofstee plot of diffusion-limited rate data. Your answer should include the Thiele modulus, ϕ.

$$v_{obs} = D_{eff}\,\frac{3}{R}\left(\frac{v_{\max}}{K_m \cdot D_{eff}}\right)^{1/2} S(R)$$

$$\frac{v_{obs}}{S(R)} = D_{eff}\,\frac{3}{R}\left(\frac{v_{\max}}{K_m \cdot D_{eff}}\right)^{1/2} = \frac{v_{\max}}{K_m}\,\frac{3}{R}\left(\frac{K_m \cdot D_{eff}}{v_{\max}}\right)^{1/2} = \frac{v_{\max}/r_p e_a}{K_m \cdot f}$$

(+2) (+1) (+2)

Bonus Questions

Because of improvements in technology and advances in our understanding of biology, the cost of developing new drugs is decreasing. True of false?

Several biotechnology companies now rank among the top ten sellers of prescription drugs worldwide. True of false?

In 2003, the leading biotherapeutic protein in US sales was _____

The biotechnology company that produces the leading biotherapeutic protein in US sales is _____

The 2003 US sales of the top selling biotherapeutic protein fell within which range:

$10-100 million $100 million – 1 billion $1-5 billion >$5 billion

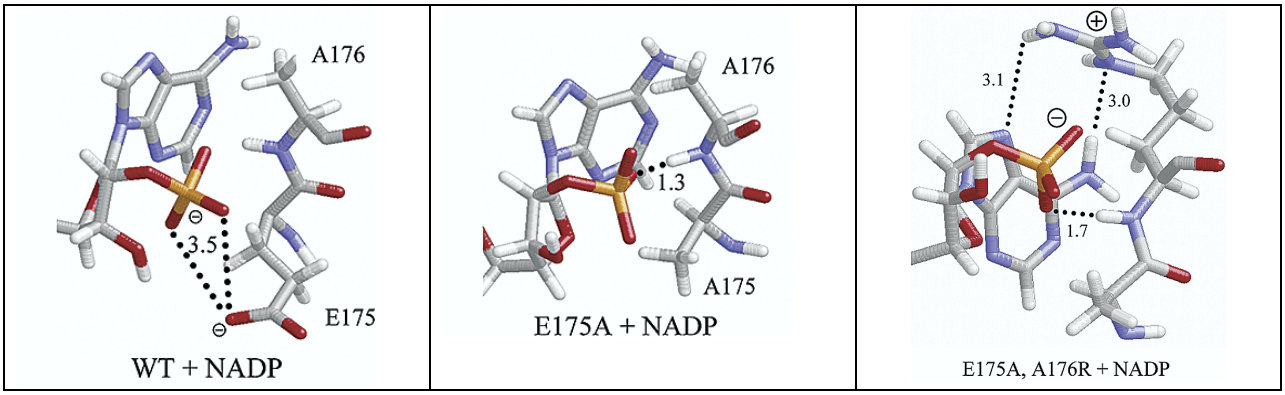


Figure 1 (for Problem 1)

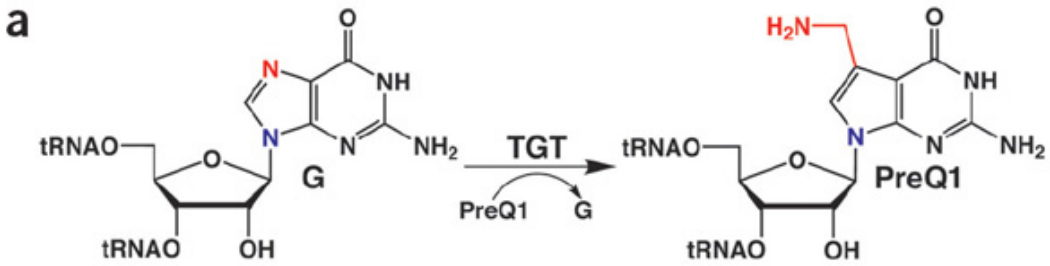


Figure 2 (for Problem 2)