Chemical Engineering 170 October 7, 2002

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

Section 1. Short Answers

1. (3 pts.) The serine protease subtilisin is the active enzyme in many household laundry detergents. This enzyme is inactivated readily by oxidation of Met 222, a residue that is conserved in all known subtilisin sequences and occupies a partially buried position next to the catalytic Ser 221. It should thus be possible to produce a more stable and useful enzyme by mutating this residue to one that is not susceptible to oxidation. Which amino acid do you believe would be best to use as a replacement for the Met residue, and why?

Valine and Leucine would be the best amino acids based on size and hydrophobicity. Cysteine is not a good answer because it has sulfur which is site of oxidation (if mention that cysteine would be a good substitute because of sulfur we gave partial credits)

2. (3 pts.) Name three interactions involved in maintaining the tertiary structure of proteins.

Any 3 of 5:

Hydrogen bond; Hydrophobic Interactions; Ionic Interactions (electrostatic); Van der Waals;

Disulfide bonds

3. a. (4 pts.) What are the definitions of the Dämkohler number (Da) and the Thiele Modulus (ϕ) for Michaelis-Menten kinetics?

$$\text{Da} = \frac{\text{Maximum Reaction Rate}}{\text{Maximum Transport Rate}};\ \text{Da} = \frac{V_{\max}}{K_S S_o}$$

$$\phi^2 = \frac{\text{Reaction rate}}{\text{Transport rate}}\ \phi = \frac{R}{3}\sqrt{\frac{V_{\max}}{K_m D_{eff}}}$$

b. (1 pt.) Which of the two deals with external mass transfer?

Damkohler number

c. (1 pt.) What does Da >>1 imply about the limiting rate of the process?

Da>>1 implies that the reaction rate is much greater than the transport rate, therefore the mass transfer rate is the limiting step of the process.

d. (1 pt.) What does $\phi << 1$ imply about the limiting rate of the process?

$\phi >> 1$ implies that the transport rate is much greater than the reaction rate, therefore the reaction rate is the limiting step of the process.

4. (3 pts.) Define the following terms:

a. DNA ligase

Covalently link strands of nucleic acid on a template, the oligonucleotide may have a blunt end or sticky end

b. Plasmid

Closed circular (supercoiled) piece of DNA, found in bacteria, usually a few thousand base pairs long; Plasmids are normally double-stranded

c. Restriction endonucleases

Recognize a particular sequence of nucleotides in a polynucleotide or nucleic acid, and cleave at a specific site.

5. (4 pts.) What are the two steps of protein synthesis? Give a brief description of each step.

1) Transcription: Synthesis of mRNA from DNA. RNA polymerase is directed to start site of transcription by one of its subunits affinity to a particular DNA sequence (promoter) that appears at the beginning of genes. The promoter is a unidirectional sequence on one strand of the DNA and it tells RNA polymerase where to start and in which direction to continue synthesis
2) Translation: Synthesis of polypeptide chain from mRNA. The mRNA has codons (triplets of bases) that specify the specific amino acid to be added to the protein. The amino acids are brought to the ribosome by tRNA, in the ribosome the amino acid is added to the growing protein chain. mRNA (messenger RNA); tRNA (transfer RNA)

6. (6 pts.) Describe a PCR cycle?

A PCR cycle consists of three steps:

1) Denaturation: Heat double stranded DNA (dsDNA) to cause separation of both strands of DNA. Use temperatures around 94^{o}C for 1 minute.
2) Annealing: Forward and reverse primers (oligonucleutide) anneal to a single strand DNA (ssDNA). This is achieved by using setting the temperature around 54^{o}C for approximately 45 seconds
3) Extension: dNTP's are added to the growing DNA strand. The addition is done by a thermostable DNA polymerase. Use temperatures around 72^{o}C for 2 minutes.

PCR cycle allows an exponential amplification of part of DNA. The use of a thermostable polymerase is essential as it avoids the need to add a DNA polymerase in each cycle, hence there is no addition of impurities in each cycle.

7. (1 pt.) What type of enzyme do you need to carry a PCR?

DNA polymerase

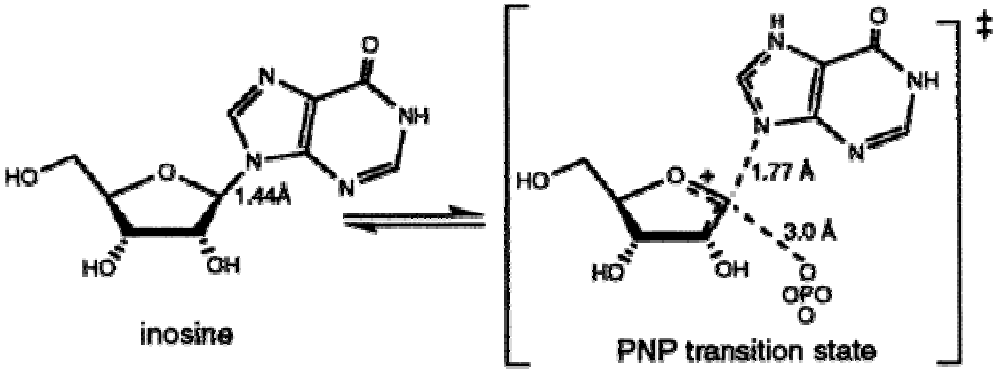
8. (2 pts.) What are two advantages for the cell to have an intermediate (mRNA) in protein synthesis?

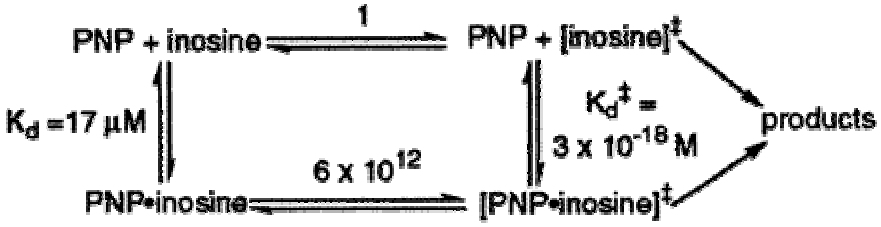
Any two of three:

1) The DNA can stay pristine and protected, away from the caustic chemistry of the cytoplasm
2) Gene information can be amplified by having many copies of an RNA made from one copy of DNA
3) Regulation of gene expression can be effected by having specific controls at each element of the pathway between DNA and proteins. The more elements there are in the pathway, the more opportunities there are to control it in different circumstances.

Section 2. Short Problems

1. (5 pts.) In class we discussed the design of inhibitors for the enzyme purine nucleoside phosphorylase (PNP), which has been identified as a potential target for inhibition by new drugs for the treatment of T-cell disorders. Shown below is the proposed structure of the transition-state for the reaction catalyzed by PNP, the phosphorylase of the inosine. Based on the information shown, estimate the rate enhancement of the enzyme-catalzyed reaction relative to the uncatalzyed reaction.





Rate enhancement can be estimated ratio of catalyzed reaction to uncatalyzed reaction:

$$\frac{k_e}{k_n}=\frac{\left(\frac{k_bT}{h}\right)k_e^{\neq}}{\left(\frac{k_bT}{h}\right)k_n^{\neq}}=\frac{k_e^{\neq}}{k_n^{\neq}}=\frac{[\mathrm{PNP}\cdot\mathrm{Inosine}]^{\neq}}{[\mathrm{PNP}\cdot\mathrm{Inosine}]}\times\frac{[\mathrm{PNP}][\mathrm{Inosine}]}{[\mathrm{PNP}][\mathrm{Inosine}]^{\neq}}=\frac{[\mathrm{PNP}\cdot\mathrm{Inosine}]^{\neq}}{[\mathrm{PNP}][\mathrm{Inosine}]}\times\frac{[\mathrm{PNP}][\mathrm{Inosine}]}{[\mathrm{PNP}\cdot\mathrm{Inosine}]}=\frac{K_d}{K_d^{\neq}}$$

$\frac{k_e}{k_n}=\frac{K_d}{K_d^{\neq}}=\frac{17\times10^{-6}}{3\times10^{-18}}=5.67\times10^{12}$, the rate enhancement is $5.67\text{x}10^{12}$

An alternative solution is:

$$\frac{k_e}{k_n}=\frac{k_e^{\neq}}{k_n^{\neq}}=\frac{6\times10^{12}}{1}=6\times10^{12}$$

2. Consider the two-stage enzyme inactivation model we discussed in class:

$$N \underset{k_2}{\overset{k_1}{\leftrightarrow}} D \xrightarrow{k_3} I$$

where N represents the native enzyme, D the reversibly denatured enzyme, and I the irreversibly inactivated enzyme.

a) (6 pts.) Assuming the process occurs in a closed system, write unsteady-state mass balances for N, D, and I.

$$\frac{d[N]}{dt} = -k_1[N] + k_2[D]$$
$$\frac{d[D]}{dt} = -k_3[D] - k_2[D] + k_1[N]$$
$$\frac{d[I]}{dt} = k_3[D]$$

b) (3 pts.) Under what conditions is the loss of native enzyme, -dN/dt, equivalent to the appearance of irreversibly inactivated enzyme, dI/dt? Answer this question with both a mathematical expression and a brief statement of what it means.

$$\frac{d[N]}{dt} + \frac{d[D]}{dt} + \frac{d[I]}{dt} = 0$$

If [D] is constant with time then:

$$\frac{d[D]}{dt} = 0$$

Therefore

$$-\frac{d[N]}{dt} = \frac{d[I]}{dt}$$

The loss of native enzyme is equivalent to the appearance of irreversibly inactivated enzyme, if the reversibly denatured enzyme concentration does not change with time.

c) (6 pts.) Assuming the conditions in part ii) apply, derive an expression for the observed rate of inactivation, k_{obs}, that has the following form:

$$k_{obs} = \frac{\alpha}{\beta + 1}$$

what are the definitions of α and β in terms of the kinetic constants in the inactivation equation?

In class we had:

$$k_{obs}([N]+[D]) = k[D]$$

$$k_{obs} = \frac{k}{1+K} \qquad (1)$$

If $-\frac{d[N]}{dt} = \frac{d[I]}{dt}$ then

$$k_3[D] = k_1[N] - k_2[D] \Rightarrow k_3 = k_1\frac{[N]}{[D]} - k_2$$

$$K = \frac{[N]}{[D]} = \frac{k_3 + k_2}{k_1} \qquad (2)$$

Therefore, $\alpha = k_2$ and $\beta = K = \frac{k_3 + k_2}{k_1}$

It was also acceptable to have the following derivation (from part b), this would be harder to do in practice:

$$\frac{d[I]}{dt} = k_3[D] = -\frac{d[N]}{dt} = k_1[N] - k_2[D]$$

$$(k_3 + k_2)[D] = k_1[N]$$

$$[D] = \frac{k_1}{k_3 + k_2}[N]$$

$$v_{obs} = k_3[D] = k_{obs}[N] = \frac{k_3 k_1}{k_3 + k_2}[N]$$

$$k_{obs} = \frac{\alpha}{1+\beta} = \frac{k_1}{1+\frac{k_2}{k_3}} \Rightarrow \alpha = k_1 \text{ and } \beta = \frac{k_2}{k_3}$$

d) (2 pts.) What is the common name of the assumption you used in part b?

Pseudo steady state hypothesis, also known as quasi steady state approximation

3. (6 pts.) In class we considered the effect of internal diffusion on the apparent stability of immobilized enzymes, which required that we relate $d\ln\phi/dt$ to $d\ln E_a/dt$, where E_a is the concentration of active immobilized enzyme, and $dE_a/dt = -k_d E_a$. What would be the corresponding relationship between $d\ln\Phi/dt$ and $d\ln E_a/dt$, where Φ is the observable modulus?

$$v_{obs} = \eta_I \frac{k_{cat} E_a S_o}{K_m + S_o}$$

$$\frac{d\ln v_{obs}}{dt} = \frac{d\ln \eta_I}{dt} + \frac{d\ln E_a}{dt} \text{ (assuming } k_{cat}, K_m \text{ and } S_o \text{ are constant with time)} \qquad (1)$$

$$\frac{d\ln \eta_I}{dt} = \frac{d\ln \eta_I}{d\ln \Phi}\frac{d\ln \Phi}{dt}$$

$$\Phi = \left(\frac{R}{3}\right)^2 \frac{v_{obs}}{S_o D_{eff}}$$

$$\frac{d\ln \Phi}{dt} = 2\frac{d\ln R/3}{dt} - \frac{d\ln\left(S_o D_{eff}\right)}{dt} + \frac{d\ln v_{obs}}{dt} \text{ (assuming } D_{eff} \text{ and } S_o \text{ are constant with time)}$$

$$\frac{d\ln \Phi}{dt} = \frac{d\ln v_{obs}}{dt}$$

$$\frac{d\ln v_{obs}}{dt} = \frac{d\ln \eta_I}{d\ln \Phi} \times \frac{d\ln \Phi}{dt} + \frac{d\ln E_a}{dt} = \frac{d\ln \eta_I}{d\ln \Phi} \times \frac{d\ln v_{obs}}{dt} + \frac{d\ln E_a}{dt}$$

$$\frac{d\ln v_{obs}}{dt} = \frac{d\ln \Phi}{dt} = \frac{\dfrac{d\ln E_a}{dt}}{\left(1 - \dfrac{d\ln \eta_I}{d\ln \Phi}\right)}$$

An alternative way to get to (1) is:

$$\Phi = \eta_I \left(\frac{\phi^2}{1+\beta}\right)$$

$$\frac{d\ln \Phi}{dt} = 2\frac{d\ln \phi}{dt} - \frac{d\ln(1+\beta)}{dt} + \frac{d\ln \eta_I}{dt} \text{ (assuming } \beta \text{ is constant with time)}$$

$$\frac{d\ln \Phi}{dt} = 2\frac{d\ln \phi}{dt} + \frac{d\ln \eta_I}{dt}$$

From class: $\dfrac{d\ln \phi}{dt} = \dfrac{1}{2}\dfrac{d\ln E_a}{dt}$

$$\frac{d\ln \Phi}{dt} = \frac{d\ln E_a}{dt} + \frac{d\ln \eta_I}{dt}$$

4. (23 pts.) In class we considered the carbon and energy requirements for growth of an organism on hexadecane, $C_{16}H_{34}$. In this problem we shall consider the growth of a yeast, *Candida utilis*, on ethanol, which may be simply described by the following equation:

$\alpha C_2H_6O + \beta O_2 + \gamma NH_3 \rightarrow CH_{1.82}N_{0.19}O_{0.46}$ (cells) + κCO_2 + $1.60H_2O$

a) (4 pts.) Assuming the yield coefficient, $Y_{X/S}$, is equal to 0.664, calculate the value of α.

$$Y_{X/S} = \frac{\text{mass cell produced}}{\text{mass substrate consumed}} = 0.664 = \frac{12 + 1.82 + 14 \times 0.19 + 0.46 \times 16}{\alpha(2 \times 12 + 6 + 16)}$$

$$\alpha = \frac{23.84}{0.664 \times 46} = \frac{23.84}{30.544} = 0.781$$

b) (3 pts.) What is the value of κ?

A balance on carbon: $2\alpha = 1 + \kappa$

$$\kappa = 2\alpha - 1 = 2 \times 0.781 - 1 = 0.561$$

c) (6 pts.) What is the respiratory quotient (RQ) for the above process?

$$RQ = \frac{\text{rate of } CO_2 \text{ production}}{\text{rate of } O_2 \text{ consumption}} = \frac{\kappa}{\beta}$$

In order to determine RQ, we need to determine β, this can be done through an oxygen balance:

$$\alpha + 2\beta = 0.46 + 2\kappa + 1.6$$

$$\beta = \frac{0.46 + 2\kappa + 1.6 - \alpha}{2} = \frac{0.46 + 2(0.561) + 1.6 - 0.781}{2} = \frac{2.4}{2}$$

$\beta = 1.2$, thus:

$$RQ = \frac{\kappa}{\beta} = \frac{0.561}{1.2} = 0.467$$

d) (3 pts.) Write an overall electron balance in terms of degrees of reductance, where, as usual, the degrees of reductance are defined per *g-atom of carbon*?

The degree of reductance of NH_3, CO_2 and H_2O is zero thus the electron balance is:

$$2\alpha\gamma_S - 4\beta = \gamma_b$$

e) (3 pts) What is the value of γ_b, the degree of reductance of the biomass?

The degree of reductance of biomass can be found from above electron balance if the degree of reductance of substrate is known. γ_S can be calculated from its composition:

$$\gamma_S = \frac{2\times 4 + 6 - 2}{2} = 6$$

$$\gamma_b = 2\alpha\gamma_S - 4\beta = 2(0.781)(6) - 4(1.2) = 4.57$$

Alternatively the degree of biomass could be found directly from biomass composition:

$$\gamma_b = 4 + 1.82 - 3(0.19) - 2(0.46) = 4.33$$

f) (4 pts.) What fraction of energy obtained from the substrate is used for the production of biomass?

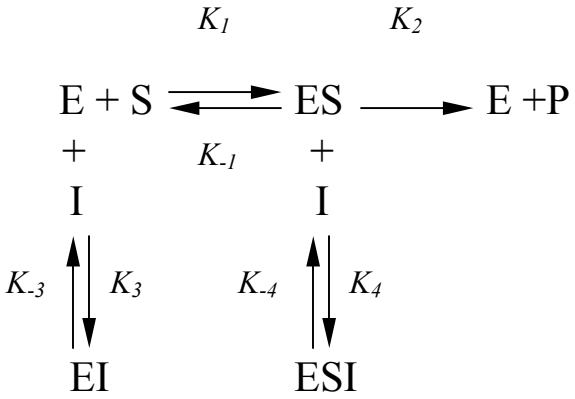
FEB - fraction of energy obtained from substrate used for production of biomass

Energy obtained from substrate: $2\gamma_S\alpha$

Energy used for production of biomas: γ_b

$$FEB = \frac{\gamma_b}{2\gamma_S\alpha} = \frac{4.57}{2(6)(0.781)} = 0.488 \text{ or } FEB = \frac{\gamma_b}{2\gamma_S\alpha} = \frac{4.33}{2(6)(0.781)} = 0.462$$

5. a) (10 pts.) Derive the rate equation for the following scheme:



Use the following definitions of:

$$\overline{K_3} = \frac{K_{-3}}{K_3};\ \overline{K_4} = \frac{K_{-4}}{K_4};\ K_m = \frac{K_{-1} + K_2}{K_1}$$

State all your assumptions

Enzyme balance:

$$[E_o] = [E] + [ES] + [EI] + [ESI] \qquad (1)$$

The reaction rate, v, can be written as:

$$v = \frac{d[P]}{dt} = k_2[ES] \qquad (2)$$

Write a balance for the enzyme complexes (*ES*, *EI* and *ESI*):

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] \qquad (3)$$

$$\frac{d[EI]}{dt} = k_3[E][I] - k_{-3}[EI] \qquad (4)$$

$$\frac{d[ESI]}{dt} = k_4[ES][I] - k_{-4}[ESI] \qquad (5)$$

Assume that the concentration of enzyme complexes is constant with time, hence:

$$\frac{d[ES]}{dt} = 0 \Rightarrow k_1[E][S] = (k_{-1} + k_2)[ES] \Rightarrow [E][S] = K_m[ES]$$

$$[E] = \frac{K_m}{[S]}[ES] \qquad (6)$$

$$\frac{d[EI]}{dt} = 0 \Rightarrow k_3[E][I] = k_{-3}[EI] \Rightarrow [E][I] = \overline{K_3}[EI]$$

$$[EI] = \frac{[E][I]}{\overline{K_3}} = \frac{K_m}{[S]}\frac{[I]}{\overline{K_3}}[ES] \qquad (7)$$

$$\frac{d[ESI]}{dt} = 0 \Rightarrow k_4[ES][I] = k_{-4}[ESI] \Rightarrow [ES][I] = \overline{K_4}[ESI]$$

$$[ESI] = \frac{[ES][I]}{\overline{K_4}} \qquad (8)$$

Substitute (6), (7) and (8) into (1):

$$[E_o] = \frac{K_m}{[S]}[ES] + [ES] + \frac{K_m[I]}{\overline{K_3}[S]}[ES] + \frac{[I]}{\overline{K_4}}[ES]$$

$$[E_o] = \left(\frac{K_m}{[S]} + 1 + \frac{K_m[I]}{\overline{K_3}[S]} + \frac{[I]}{\overline{K_4}}\right)[ES]$$

$$[E_o] = \left\{\frac{K_m}{[S]}\left(1 + \frac{[I]}{\overline{K_3}}\right) + \left(1 + \frac{[I]}{\overline{K_4}}\right)\right\}[ES]$$

$$[ES] = \frac{[E_o]}{\frac{K_m}{[S]}\left(1 + \frac{[I]}{\overline{K_3}}\right) + \left(1 + \frac{[I]}{\overline{K_4}}\right)} \qquad (9)$$

Substitute (9) into (2):

$$v = \frac{k_2[E_o]}{\frac{K_m}{[S]}\left(1+\frac{[I]}{\overline{K_3}}\right)+\left(1+\frac{[I]}{\overline{K_4}}\right)}$$

$$v = \frac{k_2[E_o][S]}{K_m\left(1+\frac{[I]}{\overline{K_3}}\right)+[S]\left(1+\frac{[I]}{\overline{K_4}}\right)} = \frac{V_{\max}[S]}{K_m\left(1+\frac{[I]}{\overline{K_3}}\right)+[S]\left(1+\frac{[I]}{\overline{K_4}}\right)} \qquad (10)$$

If you can't derive an expression for the rate equation you can use the following expression for parts b and c (note that this is not the expression you would find in part a)

$$v = \frac{V_{\max}S}{K_m\left(1+\frac{P}{\overline{K_3}}\right)+S\left(1+\frac{P}{\overline{K_4}}\right)}$$

b) (5 pts.) Based on the equation derived in part a. What it physically means if $\overline{K_4} = \infty$? What type of inhibition do you have? How does your reaction rate look like?

Based on the reaction rate derived in part a:
$\overline{K_4} = \infty$ means that enzyme-substrate complex has no affinity for inhibitor I, so no enzyme-substrate-inhibitor complex is formed. Therefore you have competitive inhibition and the reaction rate is:

$$v = \frac{V_{\max}[S]}{K_m\left(1+\frac{[I]}{\overline{K_3}}\right)+[S]} \text{ (1 point)}$$

Based on the reaction rate given above:
$\overline{K_4} = \infty$ means that enzyme-substrate complex has no affinity for inhibitor I, so no enzyme-substrate-inhibitor complex is formed. The product is the inhibitor in this case. Therefore you have competitive inhibition and the reaction rate is:

$$v = \frac{V_{\max}[S]}{K_m\left(1+\frac{[P]}{\overline{K_3}}\right)+[S]} \text{ (1 point)}$$

c) (5 pts.) Based on the equation derived in part a. What it physically means if $\overline{K_3} = \infty$? What type of inhibition do you have? How does your reaction rate look like?

Based on the reaction rate derived in part a:
$\overline{K_3} = \infty$ means that enzyme has no affinity for inhibitor I, so no enzyme-inhibitor complex is formed. Therefore you have uncompetitive inhibition and the reaction rate is:

$$v = \frac{V_{\max}[S]}{K_m + [S]\left(1 + \frac{[I]}{\overline{K_4}}\right)}$$ (1 point)

Based on the reaction rate given above:
$\overline{K_3} = \infty$ means that enzyme has no affinity for inhibitor I, so no enzyme-inhibitor complex is formed. The product is the inhibitor in this case. Therefore you have uncompetitive inhibition and the reaction rate is:

$$v = \frac{V_{\max}[S]}{K_m + [S]\left(1 + \frac{[P]}{\overline{K_4}}\right)}$$ (1 point)