Student Name : _____ 2013-11-21
Student ID : _____

Instructions:

Write neatly and clearly. Cross out with a single line any material you do not wish to have marked. Marks will be deducted for incorrect statements. Students must work independently and may not knowingly utilize resource materials or share resource materials with other students. Students may use pens, pencils, erasers and calculators only.

Electronic devices including cell phones, personal information managers and audio devices are prohibited.

Question	Mark	Total Marks
1		11
2		8
3		8
4		5
5		4
6		6
7		9
8		4
Total		**55**

(1) In the space provided, give brief definitions or unique descriptions of the following terms: (11 marks)

(a) Allosteric inhibitory modulator

Inhibitor that differs from the ligand, does not binds at the active site and induces a conformational change that decreases the ligand binding affinity ($K_{eq,a}$)

(b) Temperature Jump experiment

Rapid (<0.1 ms) heating of sample induces folding, unfolding or a conformational change that can be followed in real time using a detector (eg. UV-Visible, Intrinsic Fluorescence, etc).

(c) Mixed inhibition

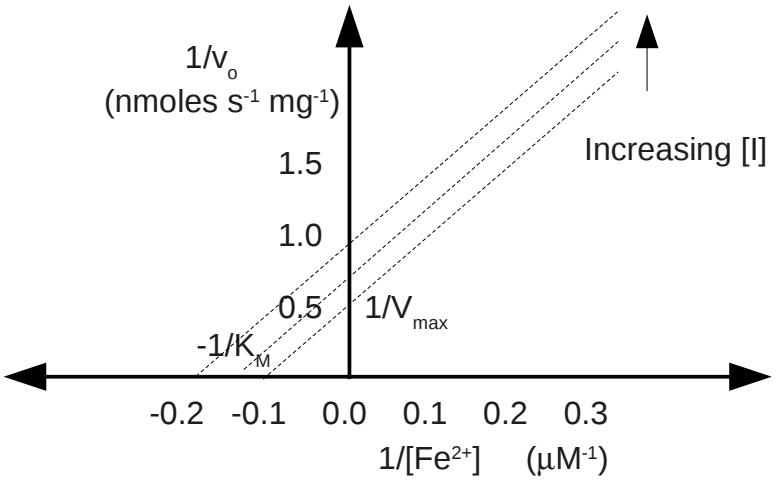
Inhibitor that binds both E (affecting K_M^{app}), like a competitive inhibitor, and ES (affecting K_M^{app} and V_{max}^{app}) like an uncompetitive inhibitor. (1 mark for kinetic parameters affected)

(d) Limited hydrolysis

Hydrolysis reaction that is stopped before all substrates are converted to products. In a mixture, the most susceptible substrates will be preferrentially hydrolysed.

(2) You have isolated an uncharacterized enzyme from a plant source for study. Answer the following questions. (8 marks)

a) You have determined the K_M^{app} (10 µM) and V_{max}^{app} (2 nmoles s^{-1} mg^{-1}) from a Lineweaver-Burk (Double Reciprocal) plot. Draw a reasonable of representation of your plot below. Include all necessary labels (axis and significant features) and show how the K_M^{app} and V_{max}^{app} were calculated from the plot.



$-1/K_M = -0.1\ \mu M^{-1}$ and

$K_M = 10\ \mu M$

$1/V_{max} = 0.5$ (nmoles s^{-1} $mg^{-1})^{-1}$ and

$V_{max} = 2$ nmoles s^{-1} mg^{-1}

1 mark each for labels/intercepts

1 mark each for K_M / Vmax labels

1 mark each for K_M / Vmax calc

b) On the same plot (part a, above), draw two additional curves representing the effect of increasing concentrations of an uncompetitive inhibitor.

1 mark each for parallel and increasing [I]

(3) Globular protein folding is primarily driven by hydrophobic forces and its initial stage involves the rapid formation of most secondary structures. (8 marks)

(a) Why or how is the rapid formation of secondary structures consistent with hydrophobic forces driving the folding of globular proteins?

Rapid mixing techniques and temperature jump experiments indicate globular proteins rapidly adopt a compact structure formed when non-polar residues aggregate in a hydrophobic enviroment. In known protein structures, the hydrophobic core is almost exclusively formed by residues that are parts of secondary structures. The formation of secondary structures is required for non-polar residues to aggregate as these structures satisfy the main-chain hydrogen bonding potential of the polypeptide.

(b) Globular proteins typically fold via a common mechanism (*i.e.* molten globule intermediate). However, a significant fraction of proteins in a cell are 'integral' membrane proteins that cannot be isolated without disrupting membrane integrity. Assuming integral membrane proteins fold by a similar mechanism, how might the folding of an integral membrane protein differ from the folding of a globular protein?

The formation of a hydrophobic core would only make sense for domains that extend into the aqueous phase. This may promote formation of the membrane domain. Given, the driving force for membrane domain aggregation is relatively smaller, folding of membrane domains may take correspondingly longer. Alternatively, hydrophilic residues in the membrane domain may aggregate into a hydrophilic core as seen in many transporters.

(4) Chemical sequencing methods are often able to determine the number and nature of disulfide bonds within proteins. Assuming you have a monomeric protein containing three cysteine residues (C20, C40 and C75), explain how to determine if there is a disulfide bond present, and the specific residues involved. (5 marks)

Requires we identify an enzyme or reagent that can fragment the polypeptide chains between both residues 20 and 40 and between residues 40 and 75.

Fragmenting the protein in the presence and absence of reducing agents and then separating all fragments (as done during sequencing) is sufficient to identify the disulfide bond location.

In the presence of reducing agents we will have one more fragment than in the absence of reducing agents. A careful comparison of the purified fragments will reveal two fragment (reducing conditions) have become a larger single fragment (oxidizing conditions). From the amino acid sequences of the purified sequences in the presence of reducing agents, the disulfide is identified.

(5) A fundamental problem in all ligand binding studies is the detection of the amount of protein-ligand complex. Assume you wish to quantify the *in vitro* binding of fructose to a bacterial activator of transcription. Describe an experiment(s) that would determine the usefulness of UV-Visible spectroscopy for quantifying the binding interaction. (4 marks)

A wavescan across the UV-Visible spectrum in the presence and absence of saturating concentrations of substrate will answer the question. If the wavescans are identical in the presence and absence of substrate we cannot determine the binding affinity using these methods. If the wavescans show significant differences (ie. Differences that can be reliably measured) theses methods can be used to assess ligand binding. In particular, we would typically choose the single wavelength with greatest difference to monitor substrate binding.

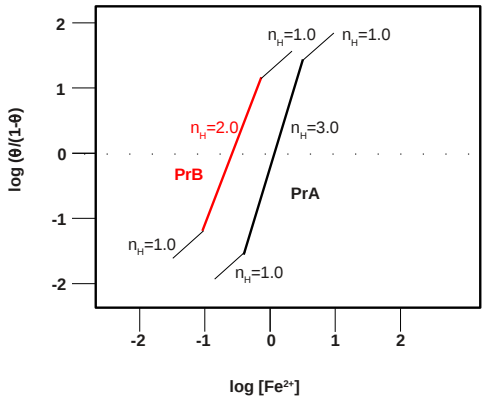
(6) Consider the reaction catalyzed by hexokinase (a glycolytic enzyme) in which a phosphoryl group is transferred from ATP to glucose. Briefly describe and explain a simple kinetics experiment(s) to distinguish between the common bisubstrate catalytic mechanisms. (6 marks)

Initially, we must distinguish between Ping-Pong and Ordered mechanisms. As Ping-Pong mechanisms produce some product in the absence of all substrates, we can incubate hexokinase with saturating concentration of ATP and with saturating concentrations of glucose in separate reactions and then test for the presence of product. If product is present in either reaction, the enzyme mechanism is Ping-Pong.

If product is not present in the above reactions, the mechanism is Ordered and we must determine whether the reaction is Random or Sequential. As Sequential Ordered mechanism bind substrates in a particular order, we can incubate hexokinase with excess ATP followed by glucose in one reaction and in a second reaction we can incubate hexokinase with excess glucose followed by ATP. If we obtain product in only one reaction, order is important and the mechansim is Ordered Sequential; otherwise the mechanism is Random Sequential.

(7) Consider two homologous, cooperative proteins (PrA and PrB) that bind Fe^{2+} ions. (9 marks)

(a) Draw and label a Hill Plot indicating PrA has a Hill coefficient of 3.
(a) Draw and label a Hill Plot indicating cHC has a Hill coefficient of 3.0.



1 each for axis labels and numbers
1 for n_H 3
1 for n_H outside of transition
1 for midpoint of transition
1 each for n_H of 2 and higher affinity

(b) On the same plot, draw a second 'curve' indicating PrB has a Hill coefficient of 2 AND has a larger binding affinity (K_a).

(c) If PrA is a homotetramer and PrB is a homodimer, what can we conclude regarding the stochiometry of Fe^{2+} binding?
As PrA and PrB are homologs their individual subunits have similar structure and function. Knowing PrA is a homotetramer with n_H=3, each subunit can bind 1 or more ions. However, since PrB is a homodimer with n_H=2, idicates each subunit must bind more than 1 ion as no protein is perfectly cooperative.

(8) Mutations that affect GroEL-GroES function strongly affect the cellular concentrations of roughly 300 *E. coli* proteins. At the same time, different experiments show that native GroEL-GroES interacts with most *E. coli* proteins. Explain these results? (4 marks)

Protein concentrations strongly affected by GroEL-ES mutations indicate these ~300 proteins require GroEL-ES to properly fold. Experiments indicating GroEL-ES interacts with most proteins suggest GroEL-ES aids the folding efficiency and/or rate of these proteins without being essential for folding.