

系所組別： 製造資訊與系統研究所丙組

考試科目： 生物化學

考試日期：0226，節次：2

請將答案寫在答案卷上，並清楚註明題號。

一、選擇題（每小題 2 分，共 30 分）

- An  $\alpha$  helix would be destabilized most by:
  - an electric dipole spanning several peptide bonds throughout the  $\alpha$  helix.
  - interactions between neighboring Asp and Arg residues.
  - interactions between two adjacent hydrophobic Val residues.
  - the presence of an Arg residue near the carboxyl terminus of the  $\alpha$  helix.
  - the presence of two Lys residues near the amino terminus of the  $\alpha$  helix.
- Amino acid residues commonly found in the middle of  $\beta$  turn are:
  - Ala and Gly.
  - hydrophobic.
  - Pro and Gly.
  - two Cys.
  - those with ionized R-groups.
- Which of the following is *not* correct concerning cooperative binding of a ligand to a protein?
  - It is usually a form of allosteric interaction.
  - It rarely occurs in enzymes.
  - It results in a sigmoidal binding curve.
  - It results in a nonlinear Hill Plot.
  - It is usually associated with proteins with multiple subunits.
- The concept of "induced fit" refers to the fact that:
  - enzyme specificity is induced by enzyme-substrate binding.
  - enzyme-substrate binding induces an increase in the reaction entropy, thereby catalyzing the reaction.
  - enzyme-substrate binding induces movement along the reaction coordinate to the transition state.
  - substrate binding may induce a conformational change in the enzyme, which then brings catalytic groups into proper orientation.
  - when a substrate binds to an enzyme, the enzyme induces a loss of water (desolvation) from the substrate..
- In a plot of  $1/V$  against  $1/[S]$  for an enzyme-catalyzed reaction, the presence of a competitive inhibitor will alter the:
  - curvature of the plot.
  - intercept on the  $1/[S]$  axis.
  - intercept on the  $1/V$  axis.
  - $pK$  of the plot.
  - $V_{max}$ .
- The PCR reaction mixture does *not* include:
  - all four deoxynucleoside triphosphates.
  - DNA ligase.
  - DNA containing the sequence to be amplified.
  - heat-stable DNA polymerase.
  - oligonucleotide primer(s)..
- Which of the following does *not* involve cyclic AMP?
  - Signaling by acetylcholine
  - Regulation of glycolysis
  - Signaling by epinephrine
  - Signaling by glucagon
  - Regulation of glycogen synthesis and breakdown
- Which one of the following compounds does *not* have a large negative free-energy of hydrolysis?
  - 1,3-bis phosphoglycerate
  - 3-phosphoglycerate
  - ADP
  - Phosphoenolpyruvate
  - Thioesters (for example, acetyl-CoA)
- Which of the following statements is *incorrect*?
  - Aerobically, oxidative decarboxylation of pyruvate forms acetate that enters the citric acid cycle.
  - In anaerobic muscle, pyruvate is converted to lactate.
  - In yeast growing anaerobically, pyruvate is converted to ethanol.
  - Reduction of pyruvate to lactate regenerates a cofactor essential for glycolysis.
  - Under anaerobic conditions, pyruvate does not form because glycolysis does not occur.

(背面仍有題目,請繼續作答)

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10. Gluconeogenesis must use "bypass reactions" to circumvent three reactions in the glycolytic pathway that are highly exergonic and essentially irreversible. Reactions carried out by which three of the enzymes listed must be bypassed in the gluconeogenic pathway?  
 1) Hexokinase      2) Phosphoglycerate kinase      3) Phosphofructokinase-1  
 4) Pyruvate kinase      5) Triosephosphate isomerase  
 A) 1, 2, 3      B) 1, 2, 4      C) 1, 4, 5      D) 1, 3, 4      E) 2, 3, 4
11. Transport of fatty acids from the cytoplasm to the mitochondrial matrix requires:  
 A) ATP, carnitine, and coenzyme A.  
 B) ATP, carnitine, and pyruvate dehydrogenase.  
 C) ATP, coenzyme A, and hexokinase.  
 D) ATP, coenzyme A, and pyruvate dehydrogenase.  
 E) carnitine, coenzyme A, and hexokinase.
12. Uncoupling of mitochondrial oxidative phosphorylation:  
 A) allows continued mitochondrial ATP formation, but halts O<sub>2</sub> consumption.  
 B) halts all mitochondrial metabolism.  
 C) halts mitochondrial ATP formation, but allows continued O<sub>2</sub> consumption.  
 D) slows down the citric acid cycle.  
 E) slows the conversion of glucose to pyruvate by glycolysis.
13. The most precise modern definition of a gene is a segment of genetic material that:  
 A) codes for one polypeptide.      B) codes for one polypeptide or RNA product.  
 C) determines one phenotype.      D) determines one trait.  
 E) that codes for one protein.
14. Which one of the following is true about the genetic code?  
 A) All codons recognized by a given tRNA encode different amino acids.  
 B) It is absolutely identical in all living things.  
 C) Several different codons may encode the same amino acid.  
 D) The base in the middle position of the tRNA anticodon sometimes permits "wobble" base pairing with 2 or 3 different codons.  
 E) The first position of the tRNA anticodon is always adenosine.
15. Protein amino acid side chains can hydrogen bond in the major groove of DNA, and discriminate between each of the four possible base pairs. In which one of the following groups of amino acids can all three members potentially be used in such DNA-protein recognition?  
 A) Ala, Asn, Glu      B) Arg, Gln, Leu      C) Asn, Gln, Trp  
 D) Asn, Glu, Lys      E) Glu, Lys, Pro

二、非選擇題 (共70分)

1. A biochemist is attempting to separate a DNA-binding protein (protein X) from other proteins in a solution. Only three other proteins (A, B, and C) are present. The proteins have the following properties:

	pI (isoelectric point)	Size <i>M<sub>r</sub></i>	Bind to DNA?
protein A	7.4	82,000	yes
protein B	3.8	21,500	yes
protein C	7.9	23,000	no
protein X	7.8	22,000	yes

- What type of protein separation techniques might she use to separate  
 (a) protein X from protein A? (3%)      (b) protein X from protein B? (3%)  
 (c) protein X from protein C? (3%)

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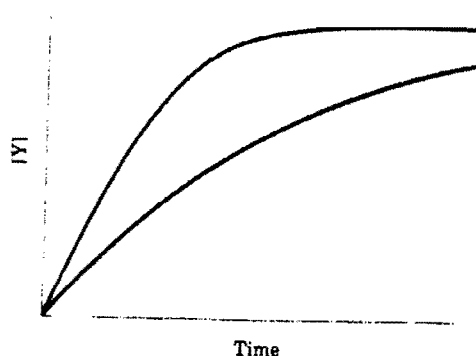
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2. A student was assigned to discover and purifies a new enzyme, generating the purification table below.

Procedure	Total protein (mg)	Activity (units)
1. Crude extract	20,000	4,000,000
2. Precipitation (salt)	5,000	3,000,000
3. Precipitation (pH)	4,000	1,000,000
4. Ion-exchange chromatography	200	800,000
5. Affinity chromatography	50	750,000
6. Size-exclusion chromatography	45	675,000

- Based on the data in the table, calculate the specific activity of enzyme after each purification step. (6%)
  - Which of the purification steps used for this enzyme is most effective. (2%)
  - Is there any indication based on the results shown in the table that the enzyme after step 6 is now pure? What else could be done to estimate the purity of the enzyme preparation. (4%)
3. a). At what substrate concentration would an enzyme with  $K_{cat}$  of  $30.0 \text{ s}^{-1}$  and  $K_m$  of  $0.0050 \text{ M}$  operate at  $1/4$  of its maximum rate? (4%)
- b). An enzyme that catalyses the reaction  $X \rightleftharpoons Y$  is isolated from two bacterial species. The enzymes have the same  $V_{max}$ , but different  $K_m$  values for the substrate. Enzyme A has a  $K_m$  of  $2.0 \mu\text{M}$ , while enzyme B has a  $K_m$  of  $0.5 \mu\text{M}$ . The plot below shows the kinetics of reaction carried out with the same concentration of each enzyme and with  $[X] = 1 \mu\text{M}$ . Which curve corresponds to which enzyme? Account for your answer. (5%)



- c). One of the enzyme isolated in b) has molecular weight of  $30,000 \text{ (g/mol)}$ , catalyzes  $0.3 \text{ g}$  of X, molecular weight  $44$ , in  $1 \text{ min}$  at  $37^\circ\text{C}$  at  $V_{max}$ , what is the turnover number ( $K_{cat}$ ) of this enzyme (in units of  $\text{min}^{-1}$ )? (5%)
4. a). Construct a diagram showing the sequence of electron transfer complexes and mobile electron carriers that are associated with the inner mitochondrial membrane. (6%)
- b). Based on the diagram you construct, indicate the final electron acceptor in each of the following case.
- Abundant NADH and  $\text{O}_2$ , but cyanide added. Explain. (3%)
  - Abundant NADH under anaerobic condition. Explain. (2%)

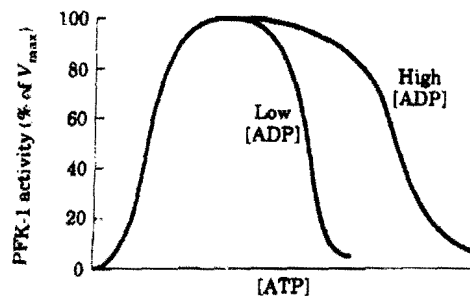
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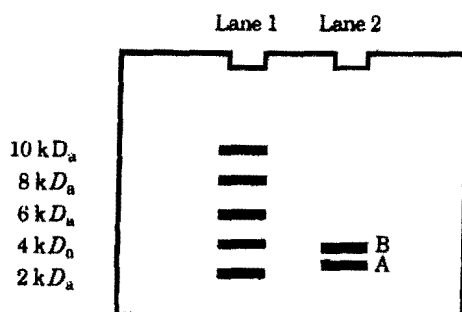
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5. The effect of ATP on the allosteric enzyme PFK-1 is shown below. For a given concentration of fructose 6-phosphate, the PFK-1 activity increases with increasing concentration of ATP, but a point is reached beyond which increasing concentration of ATP inhibits the enzyme.



- a). Explain how ATP can be both a substrate and an inhibitor of PFK-1. How is the enzyme regulated by ATP? (4%)
- b). The inhibition of PFK-1 by ATP is diminished when the ADP concentration is high, as shown in the plot. How can this observation be explained? (4%)
6. Assume you have isolated a relatively abundant protein, and you want to obtain the amino acid sequence. You perform the following experiments:
- a). Addition of dithiothreitol to the protein sample followed by gel electrophoresis results in the protein gel shown below. What can you conclude? (4%)



**Lane 1** contains molecular weight markers as indicated to the left of the figure.

**Lane 2** contains the DTT-treated, purified protein.

- b). i). Cleavage with chymotrypsin produces the following fragments:  
 Band A---- CN, NLQNY, and GIVEQCCHKRCSEY  
 Band B---- F, Y, DPTKM, IACGVRGF, and RTTGHLGKDLVNALY.
- ii). Cleavage with *Staphylococcus aureus* V8 protease produces the following fragments:  
 Band A---- GIVE, YNLQNYCN, and QCCHKRCSE  
 Band B---- PTKM, RTTGHLGKGD, and LVNALYIACGVRGFFYD
- What is the amino acid sequence of your isolated protein? (4%)

7. You wish to set up a system for *in vitro* protein translation. What components would the system require? Assume you are working with a prokaryotic system. (8%)