

請依題號順序作答

1. A graduate student designs the purification strategy to purify a new discovered enzyme, generating the purification table below.

Procedure	Total protein (mg)	Activity (units)
1. Crude extract	40,000	5,000,000
2. Precipitation (salt)	10,000	4,000,000
3. Size-exclusion chromatography	5,000	800,000
4. Ion-exchange chromatography I	2,000	750,000
5. Affinity chromatography	100	700,000
6. Ion-exchange chromatography II	92	650,000

- (1) From the information given in the table, please calculate the specific activity of the enzyme and the percentage yield after the final procedure. (4 points)
 - (2) Which of the purification procedure used for this enzyme is most effective? Why? (4 points)
 - (3) The purification strategy is not optimal for the enzyme and has a serious flaw. If you were the student to purify the enzyme, which of the purification procedure you want to delete or change? Why? (5 points)
 - (4) Which of the purification procedure is not necessary? Why? (4 points) In addition, based on what characteristics of proteins, the procedure separates the enzyme from other proteins? (2 points)
 - (5) From the cDNA sequence of the enzyme, the enzyme is composed of 710 amino acid residues. What is the approximate molecular weight of the enzyme? (2 points)
 - (6) In order to check the purity of the enzyme after the step 6, the graduate student uses the SDS-PAGE to analyze the preparation. The student only observes one single protein band around the molecule weight of 85 kDa on the SDS-PAGE. Do you think the estimated molecular weight match the predicted molecular weight from (5)? If not, please explain the possible reason for causing difference of molecule weight of the enzyme. (4 points)
 - (7) From (6), what kinds of experiments can be done to verify your theory? (2 points)
 - (8) The student tries to predict the secondary structure of the enzyme. Parts of the amino acid sequence of the enzyme are LKAARAAEADEMSEA. What kind of the secondary structure is more likely to form at the region? Why? (4 points)
2. (1) Chorismate is an intermediate in the biosynthesis of several amino acids. What are these amino acids? (3 points)
- (2) Glyphosate, a weed killer sold under the trade name Roundup, is an analog of phosphoenolpyruvate that specifically inhibits 3-enolpyruvylshikimate 5-phosphate synthase, a key enzyme of the pathway for chorismate biosynthesis. This compound is a very effective plant herbicide, but has virtually no effect on mammals. Why? Please also explain the biochemical basis of glyphosate. (4 points)
3. (1) Gout occurs predominately in males and is a disease of the joints. The joints in patients become inflamed and painful. What molecule is found in high concentration in gout patients' blood? (2 points)
- (2) What molecules are metabolized into the (1) molecule? (2 points)
- (3) Allopurinol is the drug for treatment of chronic gout. Please explain the biochemical basis for the treatment. (4 points)

見背面

4. Cardiac muscle exhibits a high demand for oxygen, and its functioning is severely impaired when coronary circulation is blocked.
- (1) Considering the energy-generating substrates available to and used by the heart under normal circumstances, why is oxygen required by heart muscle? (2 points)
 - (2) If the supply of oxygen to heart tissue is reduced, what metabolite will be elevated in heart muscle that has an insufficient supply of oxygen? (2 points)
5. 請由結構的觀點解釋 starch 與 cellulose 兩者性質的差異。(4分)
6. 請寫出下列分子之基本結構 (每小題 2分)
- (1) ω -3 Fatty acid
 - (2) Steroid
 - (3) D-Hexose
7. 請回答下列有關 Adenine nucleotides 的問題
- (1) ATP 為何具有 high phosphoryl potential? (3分)
 - (2) ATP 除了提供代謝反應之能量外，還具有哪些功能？(3分)
 - (3) 請寫出另外兩種含有 adenine nucleotide 的 coenzymes 或 cofactors 的全名，並分別寫出它們所參與的反應（各舉一例即可）。(4分)
8. 請闡述 fructose 2,6-bisphosphate 在肝臟中的功能。(4分)
9. 請比較 photophosphorylation 與 oxidative phosphorylation 之異同 (6分)
10. 請閱讀以下文章 (取材自 Thomas-Chollier M, Watson LC, Cooper SB, et al. (2013) A naturally occurring insertion of a single amino acid rewires transcriptional regulation by glucocorticoid receptor isoforms. Proc. Natl. Acad. Sci. USA. 110: 17826-17831.)，並回答相關問題。
- In addition to guiding proteins to defined genomic loci, DNA can act as an allosteric ligand that influences protein structure and activity. Here we compared genome-wide binding, transcriptional regulation, and, using NMR, the conformation of two glucocorticoid receptor (GR) isoforms that differ by a single amino acid insertion in the lever arm, a domain that adopts DNA sequence specific conformations. We show that these isoforms differentially regulate gene expression levels through two mechanisms: differential DNA binding and altered communication between GR domains. Our studies suggest a versatile role for DNA in both modulating GR activity and also in directing the use of GR isoforms. We propose that the lever arm is a "fulcrum" for bidirectional allosteric signaling, conferring conformational changes in the DNA reading head that influence DNA sequence selectivity, as well as conferring changes in the dimerization domain that connect functionally with remote regulatory surfaces, thereby influencing which genes are regulated and the magnitude of their regulation.
- (1) 請將全文翻譯成中文 (6分)
 - (2) 解釋 allosteric ligand 的意義 (3分)
 - (3) 寫出 NMR 英文全名 (2分)。在此篇研究中，用 NMR 探討什麼問題？(2分)
 - (4) Glucocorticoids 為哪一類物質 (2分)？其功能為何 (3分)？如何發揮功能 (2分)？