國立成功大學 105 學年度碩士班招生考試試題

系　　所：化學系

考試科目：生物化學

考試日期：0228，節次：3

※ 考生請注意：本試題不可使用計算機。　請於答案卷(卡)作答，於本試題紙上作答者，不予計分。

一、選擇題：(單選，每題 2 分，共 60 分)

Consider the following enzymatic transformation and answer the following questions (**1-4**):

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

(E: enzyme; S: substrate; P: product; K_S: [ES] dissociation constant; K_M: Michaelis-Menten constant)

1. Which of the following is true about steady-state kinetics?
(A) $k_{-1} << k_2$ (B) [S]<<[E] (C) [S]>K_M (D) $K_M < K_S$ (E) none of the above is true.

2. The K_M can be represented by the K_S when (A) [S]<<[E] (B) [S]>>[E] (C) $k_2 << k_{-1}$ (D) $k_1 >> k_2$ (E) $k_1 >> k_{-1}$.

3. The catalytic efficiency of the enzyme is (A) $(k_{-1} + k_2)/k_1$ (B) $<k_{-1}$ (C) $<k_1$ (D) $>k_2/K_S$ (E) k_2.

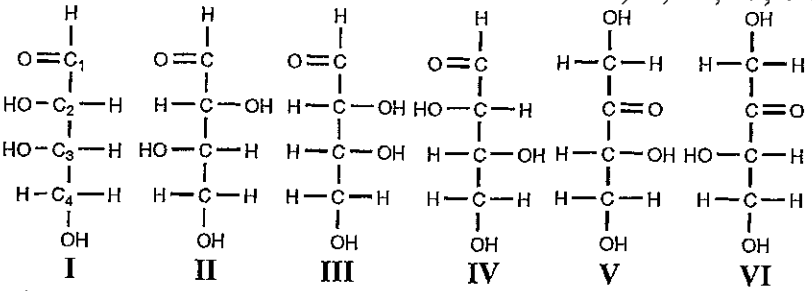
4. The catalytic efficiency of the enzyme can be best described as a function of k_{cat} and K_M when (A) [S]>>K_M (B) [S]<<K_M (C) [S]>>[E] (D) [S]<<[E] (E) K_M approaches K_S.

5. In kinetics, a mixed-type (noncompetitive) inhibitor must lead to (A) reduced V_{max} and increased K_M (B) unchanged V_{max} and reduced K_M (C) reduced V_{max} and unchanged K_M (D) unchanged V_{max} and increased K_M (E) none of above.

6. Which of the following is true about a serine protease that prefers to cleave a peptide bond adjoining a positively charged side chain? The protease (A) may be most related to chymotrypsin than other proteases (B) may be derived from trypsinogen (C) may possess a specificity pocket characterized with Lys and Thr (D) should have much higher catalytic rate than chymotrypsin (E) should have much lower catalytic rate than carboxypeptidase.

7. Which hydroxyl group of β-D-glucopyranose can be methylated to inhibit its interconversion with α-D-glucopyranose in solution?
(A) C5-OH (B) C4-OH (C) C3-OH (D) C2-OH (E) C1-OH.

This figure is used in questions **8-10**. The chemical structures of **I**, **II**, **III**, **IV**, **V** and **VI** are in Fischer projection.



8. Which ketose has an *L*-configuration? (A) **II** (B) **III** (C) **IV** (D) **V** (E) **VI**.

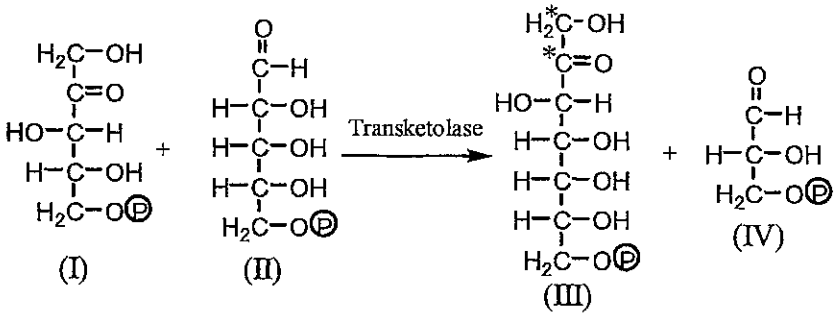
9. Which of the following can best describe the relationship between **I** and **III**.
(A) tautomers (B) isomers (C) diastereomers (D) enantiomers (E) conformational isomers.

10. Which of the following can best describe the relationship between **II** and **III**.
(A) tautomers (B) isomers (C) diastereomers (D) enantiomers (E) conformational isomers.

11. Which of the following techniques uses antibodies (or other specific ligands in related techniques) to identify target proteins among a number of unrelated protein species, where the proteins are typically separated by electrophoresis?
(A) ELISA (B) Western blotting (C) Coomassie Blue staining (D) Northern blotting (E) silverstaining.

12. What is the net charge on the tripeptide Gly-Arg-Lys at pH 7.0? The pK_as of the ionizable groups on the free amino acids are: Gly: 2.4 and 9.8; Arg: 1.8, 9.0 and 12.5; Lys: 2.2, 9.1 and 10.5.
(A) -2 (B) -1 (C) 0 (D) +1 (E) +2.

For the following enzymatic reaction, answer the following questions (**13** and **14**).



13. Which of the following cofactors/coenzymes is required for the above transketolase reaction?
(A) NADPH (B) NADH (C) TPP (D) $FADH_2$ (E) Biotin.

14. In the transketolase reaction, the two labeled carbons (*C) are from: (A) Compound I (B) Compound II (C) Compounds I and II (D) 2 CO_2 (E) Acetyl-CoA.

15. Which of the following pairs of restriction enzymes are isocaudomers which allow fragments generated with one enzyme to anneal with fragments generated with the other?
SalI: G↓TCGAC; PvuII: CAG↓CTG; XhoI: C↓TCGAG; EcoRV: GAT↓ATC; PstI: CTGCA↓G.
(A) PvuII and EcoRV (B) SalI and EcoRV (C) SalI and XhoI (D) XhoI and PstI (E) PvuII and PstI.

16. Lactate dehydrogenase catalyzes the reaction of pyruvate with_____ to yield lactate and _____.
(A) FAD; $FADH_2$ (B) $NADP^+$; NADPH (C) NAD^+; NADH (D) NADH; NAD^+ (E) NADPH; $NADP^+$.

17. Which of the following is <u>false</u> about UDP-galactose 4-epimerase?
(A) the enzyme catalyzes the reversible conversion of UDP-galactose to UDP-glucose (B) the enzyme catalyzes oxidation and reduction (C) the enzyme contains NADH in the active site (D) the enzyme performs the final step in the Leloir pathway of galactose metabolism (E) none of above.

18. Which carbon of pyruvate will be radiolabeled when the cells are fed with the C1-^{14}C-glucose?
(A) 3 (B) 2 (C) 1 (D) all (E) none.

19. Which type of the following transports is most sensitive to membrane fluidity?
(A) passive (B) pore-facilitated (C) carrier-facilitated (D) active (E) none of above.

20. An enzyme can be inactivated by iodoacetamide and covalently labelled in the active site, suggesting that the enzyme active site most likely contain (A) alanine (B) aspartate (C) cysteine (D) methionine (E) lysine.

21. NADH from cytosol enters the mitochondrion for oxidative phosphorylation in the form of (A) malate (B) succinate (C) oxaloacetate (D) aspartate (E) dihydroxyacetone phosphate.

22. The activity of pyruvate dehydrogenase complex increases when (A) [ATP] increases (B) [Mg^{2+}] increases (C) [AMP] decreases (D) [NADH] increases (E) all of above.

23. Which of the following metabolic pathways utilizes intermediates of the citric acid cycle?
(A) fatty acid oxidation (B) glycogen biosynthesis (C) amino acid biosynthesis (D) glycolysis (E) none of above.

24. In fatty acid biosynthesis, the two carbon units for repeating chain elongation are derived from?
(A) ceramides (B) palmitic acid (C) propionyl CoA (D) acetyl CoA (E) malonyl CoA (as an enzyme substrate).

25. In fatty acid biosynthesis mechanism, the β-ketoacyl-ACP synthase or ketosynthase (KS) domain of fatty acid synthase involves (A) a histidine for covalent catalysis (B) a cysteine for acid catalysis (C) repetitive condensation (D) a glutamate for base catalysis (E) repetitive reduction.

26. Which of the following cofactors is utilized for biosyntheses of several neurotransmitters by decarboxylations of amino acids?
(A) tetrahydrofolate (B) thiamine pyrophosphate (C) biotin (D) pyridoxal-5'-phosphate (E) vitamin B_{12}.

27. Which of the following bioactive agents does not function by targeting and inhibiting protein synthesis?
(A) tetracycline (B) chloramphenicol (C) streptomycin (D) ethidium bromide (E) none of above.

28. Which of the following *E. coli* DNA polymerases possesses both 3'→5' and 5'→3' exonuclease activities?
(A) Pol I (B) Pol II (C) Pol III (D) all of above (E) none of above.

29. By ion exchange chromatography, Arg (R), Val (V) and Glu (E) can be eluted and separated from a negatively charged carboxymethyl column at pH 6.0 in the order of (A) R, V and E (B) E, V and R (C) R, E and V (D) E, R and V (E) V, E and R.

30. Which of the following amino acids is not converted to succinyl-CoA subsequently entering the TCA cycle? (A) Isoleucine (B) methionine (C) alanine (D) threonine (E) valine.

二、簡答題：(共 40 分)

1. Draw the major structure of aspartic acid at pH 7.0. (2%)

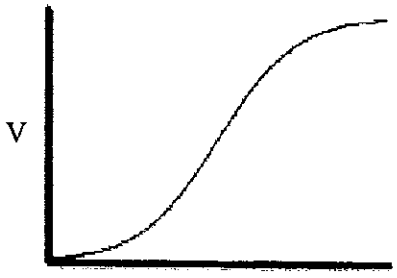
2. Describe and explain the following terms with an example:
(1) proximity effect. (2%)
(2) affinity labelling. (2%)

3. Hexokinase catalyzes ATP-dependent phosphorylation of glucose and is proposed to undergo conformational change in the mode of induced fit. Provide a valid experimental design and a solid evidence to validate the conformational change occurring during the catalysis of hexokinase. (4%)

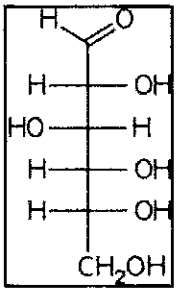
4. Sketch out the graphs that would be obtained at 3 different inhibitor concentrations for the following types of inhibition kinetics. For each type, indicate the slope and the mathematical values at x- and y-intercepts. (S: substrate; I: inhibitor; V: velocity, K_I: inhibition constant)
(1) competitive inhibition (plotting 1/V versus 1/[S]) (2%)
(2) uncompetitive inhibition (plotting 1/V versus 1/[S]) (2%)

5. Draw a complete reaction mechanism of the chymotrypsin which catalyzes hydrolysis of a peptide substrate. Please also indicate structures of the triad and all the tetrahedral intermediates. (6%)

6. When allosteric effectors are not present, the enzyme glycogen phosphorylase displays reaction velocity (V) as a function of the inorganic phosphate substrate (P_i) as shown in the graph below.
(1) Add and draw a curve representing the T state. (2%) **(2)** Add and draw a curve representing the R state. (2%) **(3)** Which of the three curves (original, T and R) may look like the case when ATP is present? (2%)



7. Please answer the questions below.
   (1) Provide a Fischer projection of D-mannose as an epimeric form of glucose at C2. (2%)
   (2) Provide a Haworth projection of β-D-mannose in pyranose form. (2%)
   (3) Draw and name the type of glycosidic linkage involved in amylopectin branches in polymeric form of glucose. (2%)
   (4) Draw and name the type of glycosidic linkage involved in cellulose in polymeric form of glucose. (2%)



(D-glucose)

8. A research fellow wants to purify a metal-binding protein (MBP) from the other three proteins (I, II, and III) in crude material. Based on the protein information shown below, please assign a type of separation technique to separate MBP from **(1)** protein I (2%) **(2)** protein II (2%) **(3)** protein III (2%). Please also provide a rational explanation (principle) to each separation technique assigned to answer each of the three questions **(1)~(3)**.

Protein Name	Molecular Weight (kDa)	Binding Affinity to Metal Ion	p*I* value
MBP	27	strong	7.8
protein I	82	strong	7.5
protein II	28	strong	3.0
protein III	30	none	7.7