UNIVERSITY OF THE WESTERN CAPE
DEPARTMENT OF BIOTECHNOLOGY

BIOTECHNOLOGY 223 EXAM (2004)

TIME: 90 MINUTES **MARKS: 90**

READ THE INSTRUCTIONS CAREFULY

THERE ARE 2 SECTIONS
ANSWER 2 QUESTIONS FROM SECTION A
ANSWER 4 QUESTIONS FROM SECTION B

ALL ANSWERS SHOULD BE WRITTEN IN THE EXAM BOOK PROVIDED

SECTION A [30 marks in total]

Question 1.

Penicillin is hydrolysed and made inactive by an enzyme, penicillinase, present in some resistant bacteria. The molecular mass of this enzyme is 29,600 Da. The amount of penicillin hydrolysed in 1 minute in a 10ml solution containing 10^{-9} g of purified penicillinase was measured at different starting concentrations of penicillin.

[penicillin] (μM)	Amount of penicillin hydrolysed (nmol)
1.0	0.11
3.0	0.25
5.0	0.34
10.0	0.45
30.0	0.58
50.0	0.61

Assuming that the concentration of penicillin does not change significantly during the assay

a) Make a plot of $1/V$ against 1/[S] from these data. Does penicillinase appear to obey Michaelis-Menton kinetics? If it does, what is the value of K_M? [5]

b) What is the value of V_{max}? [5]

c) Assuming one active site per enzyme molecule what is the value of k_{cat} under these experimental conditions? [5]

Question 2.
The steady state kinetics of an enzyme are studied in the presence of two different concentrations of inhibitor **B**. The initial rates at different starting substrate concentrations were found to be as follows:

[S] (mM)	Initial rate (mM min^{-1}) (3mM inhibitor **B**)	Initial rate (mM min^{-1}) (5mM inhibitor **B**)
1.25	1.25	1.01
1.67	1.54	1.26
2.50	2.00	1.72
5.00	2.86	2.56
10.00	3.70	3.49

From these data

a) Determine the apparent K_M (K_M^{app}) and apparent V_{max} (V_{ma}^{app}) at each inhibitor concentration [7]

b) What type of inhibitor is substance **B**? [2]

c) Using your data for K_M^{app} at the different inhibitor concentrations and the equation $K_M^{app} = (K_M + [\mathbf{B}]K_M/K_I)$, determine K_I and K_M [6]

Question 3.
Briefly describe the meaning of the following terms when applied to enzyme catalysed reactions:

a) an 'induced fit' model [3]
b) the kinetic parameter k_{cat}/K_M [3]
c) heteroallosteric regulation [3]
d) the 'activation energy' for a reaction [3]
e) non-competitive inhibitor [3]

SECTION B [60 marks in total]

Question 4
In a thermodynamic sense, the biological oxidation of organic substrates is comparable to non-biological oxidations such as the burning of wood. However, biological oxidations are far more complex. Discuss. [15]

Question 5.
a) The enzyme hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate, the first reaction in the glycolytic pathway. What are the characteristics of this enzyme? How does it differ from the special form found in vertebrate hepatic cells? [10]

b) What is substrate-level phosphorylation? Contrast it with oxidative phosphorylation. [5]

Question 6.
Chemiosmotic coupling refers to the establishment of a transmembrane proton gradient to drive endergonic processes. Discuss the experimental evidence that supports chemiosmotic coupling. [15]

Question 7.
How does the proton gradient result in the synthesis of ATP? Include a description of the model of ATP synthase in your answer. [15]

Question 8.
Discuss the metabolic fate of pyruvate under anaerobic conditions in eukaryotes. [15]

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BIOTECHNOLOGY 223 SUPPLEMENTARY EXAM (2004)

TIME: 90 MINUTES **MARKS: 90**

READ THE INSTRUCTIONS CAREFULY

THERE ARE 2 SECTIONS
ANSWER 2 QUESTIONS FROM SECTION A
ANSWER 4 QUESTIONS FROM SECTION B

ALL ANSWERS SHOULD BE WRITTEN IN THE EXAM BOOK PROVIDED

SECTION A [30 marks in total]

Question 1.

The kinetics of an enzyme are measured as a function of substrate concentration in the presence and absence of 2 mM inhibitor (**I**).

[S] (μM)	Initial rate (μmol min^{-1}) (No inhibitor)	Initial rate (μmol min^{-1}) (Plus inhibitor)
3.0	10.4	4.1
5.0	14.5	6.4
10.0	22.5	11.3
30.0	33.8	22.6
90.0	40.5	33.8

a) What are the values of V_{max} and K_M in the absence and presence of the inhibitor? [8]
b) What type of inhibitor is substance **I**? [2]
c) What is the binding constant, K_I, for substance **I**? [5]

Question 2.

The hydrolysis of sucrose at 25°C

Sucrose + H_20 -> glucose + fructose

was found to have the following time course.

Time (minutes)	[Sucrose] (M)
0	0.5011
30	0.4511
60	0.4038
90	0.3626
130	0.3148
180	0.2674

a) Determine the order of this reaction with respect to sucrose. [5]
b) Determine the half-life ($t_{1/2}$) and rate constant (k) for this reaction [5]
c) How long will it take to hydrolyse 87.5% of the sucrose initially present? [2]
d) This reaction is zero-th (0^{th}) order with respect to H_20. Why might this be the case? [3]

Question 3.

a) The enzyme triosephosphate isomerase catalyses the interconversion of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Briefly describe the roles played by residue Glutamate165 in the active site of the enzyme and by the 'flexible loop' in speeding up this reaction. [10]

b) Briefly describe how the coenzyme NADH is able to transfer a hydride ion (H^-) to one specific side of a symmetrical substrate [5]

SECTION B [60 marks in total]

Question 4

Phosphofructokinase (PFK) is an allosteric enzyme whose activity is acutely sensitive to the energy status of the cell, as well as to the levels of various other intermediates. Describe how PFK serves as a regulatory target in glycolysis. [15]

Question 5.

Discuss the role of the enzyme pyruvate dehydrogenase in the regulation of the citric acid cycle. [15]

Question 6.

Second messengers transmit information from hormones bound at the cell surface, thereby controlling intracellular metabolic processes. Discuss the role of these second messengers in glycogenolysis. (Please include a diagram in your discussion) [15]

Question 7.

Briefly describe the biological rationale for each of the following allosteric phenomena:

a) activation of pyruvate decarboxylase by acetyl-CoA [3]

b) activation of pyruvate dehydrogenase kinase by NADH [3]

c) inhibition of isocitrate dehydrogenase by NADH [3]

d) activation of isocitrate dehydrogenase by ADP [3]

e) inhibition of α-ketoglutarate dehydrogenase by succinyl-CoA [3]

Question 8.

How does the dissaccharide lactose enter the glycolytic pathway? [15]

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BIOCHEMISTRY 221 SPECIAL EXAM (2004)

TIME: 90 MINUTES **MARKS: 60**

READ THE INSTRUCTIONS CAREFULLY

THERE ARE 2 SECTIONS
SECTION A (MULTIPLE CHOICE SECTION) IS COMPULSORY
ANSWER TWO QUESTIONS IN SECTION B
ANSWER SECTION A (QUESTIONS 1 TO 25) ON THE MCQ FORM PROVIDED ANSWER SECTION A (QUESTIONS 26 TO 30) AND TWO QUESTIONS OUT OF SECTION B IN THE EXAM BOOK PROVIDED.

SECTION A: (COMPULSORY)

ANSWER QUESTIONS 1 TO 25 ON THE PROVIDED MCQ FORM [25]

QUESTIONS 1 TO 5

ANSWER TRUE OR FALSE. TRUE WILL BE OPTION A AND FALSE WILL BE OPTION B

1. Catalysts change the rates of processes and thus affect the state of equilibrium of the reaction.
2. The Michaelis Constant K_m, is a measure of the substrate concentration required for effective catalysis to occur.
3. Heterotrophs synthesise their organic metabolites only from inorganic compounds which they must therefore consume.
4. The last five reactions of glycolysis represent an energy generation phase in which the triose phosphates are converted to energy-rich compounds.
5. The Citric Acid Cycle is the central oxidative pathway is respiration, by which all metabolic fuels are anabolised in anaerobic organisms and tissues.

[5]

QUESTIONS 6 TO 10

MATCH THE CORRECT ANSWERS WITH THE CORRECT NUMBERS

6._____is the metabolic fate of pyruvate in aerobic glycolysis.
7._____is a terminal electron acceptor.
8._____is the coenzyme used in biosynthetic reactions.
9._____is the tendency of an acid to lose a proton.
10._____is a carrier of both electrons and acyl groups.

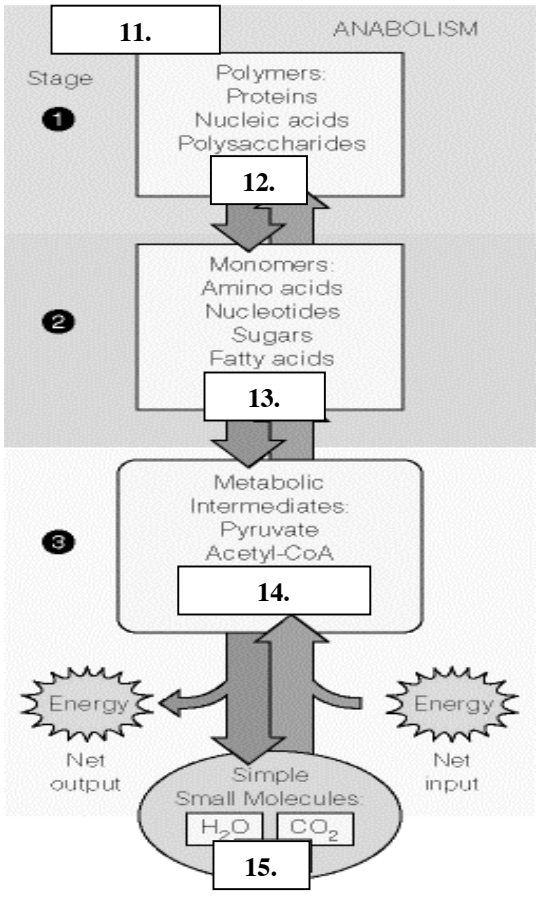
A. pK_a B. Acetyl-CoenzymeA C. NADPH
D. Lipoamide E. Oxygen

[5]

QUESTIONS 11 TO 15

MATCH THE LETTERS WITH THE NUMBERS IN THE DIAGRAM

A Brief overview of metabolism



A. Citric Acid Cycle B. NH_3 C. Catabolism
D. Glycerol E. Lipids

[5]

QUESTIONS 16 TO 25
SELECT THE CORRECT ANSWER

16. The reaction, Rate (Velocity) = $k[A]^1[B]^1$ exhibits _____ with respect to B

a. First-order kinetics
b. Zero-order kinetics
c. Second-order kinetics
d. All of the above

17. A typical example of an irreversible inhibitor is _____

a. Niacin
b. Serine Proteases
c. Diisopropyl Flourophosphate
d. Acetylcholinesterase

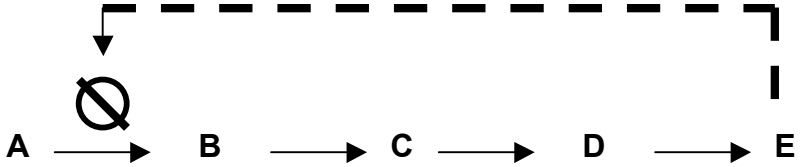
18. Enzymes which catalyse reactions in which two molecules are joined are called _____

a. Lyases
b. Hydrolases
c. Transferases
d. Ligases

19. Molecules also referred to as abzymes.

a. Catalytic Antigens
b. Catalytic Antibodies
c. Non-protein Biocatalysts
d. Catalytic Ribosomes

20. Type of Feedback control involved in the reaction below:



a. Inhibition
b. Heteroallostery
c. Activation
d. Homoallostery

21. The major carbon input to glycolysis usually derived from either energy storage polysaccharides or dietary carbohydrates is _____

a. Galactose
b. Maltose
c. Glucose
d. Fructose

22. In the catabolism of glucose about 40% of the released energy is used to drive the synthesis of _____

a. Pyruvate
b. ATP
c. NADH
d. All of the above

23. Pyruvate oxidation to acetyl-CoA is a virtually irreversible reaction that involves _____

a. Three enzymes and five coenzymes
b. Three enzymes and three coenzymes
c. Five enzymes and five coenzymes
d. Five enzymes and three coenzymes

24. Oxidation of 1 mole of NADH provides sufficient energy for synthesis of about _____ moles of ATP from ADP

a. 2
b. 6
c. 3
d. 8

25. Electrons are transported into mitochondria by _____

a. Hormones
b. Diffusion
c. Selective permeability
d. Metabolic shuttles

[10]

ANSWER QUESTIONS 26-30 IN THE EXAM BOOK PROVIDED

BRIEFLY DEFINE THE FOLLOWING TERMS

26. Induced Fit Model
27. Compartmentation
28. Irreversible Inhibitor
29. Hexokinase
30. Reductant

[5]

SECTION B:

ANSWER <u>TWO</u> QUESTIONS FROM SECTION B IN THE EXAM BOOK PROVIDED

QUESTION 1

a) Sucrose (common table sugar) is hydrolysed to glucose and fructose in a classic experiment in kinetics. The reaction is catalysed by the enzyme invertase. Using the following data, determine by the Lineweaver-Burke method whether the inhibition of this reaction by 2M urea is competitive or non-competitive. Determine V_{max} and K_m in the absence and presence of the inhibitor.

(10)

Sucrose Concentration (mM)	V, No Inhibitor (mM sec^{-1})	V, Inhibitor Present (mM sec^{-1})
0.0292	0.182	0.083
0.0584	0.265	0.119
0.0876	0.311	0.154
0.117	0.330	0.167
0.175	0.372	0.192

b) Name three proteins that are subject to the control mechanism of zymogen activation.

(3)

c) Why is it necessary or advantageous for the body to make zymogens? (2)

[15]

QUESTION 2

a) Diagrammatically illustrate the possible metabolic fates of pyruvate in anaerobic glycolysis. (Hint: Enzymatic Reaction) (4)

b) Name two reactions, which are control points in glycolysis. (4)

c) Explain (with an example) how you would experimentally determine an enzymes function. (3)

d) Give a detailed description of one type of metabolic response mechanism to hormones. (4)

[15]

QUESTION 3

a) The predominant pathway for glucose catabolism is glycolysis. An alternative process is the pentose phosphate pathway. Name the two primary functions of this pathway. (2)

b) Briefly describe the dual role of lipoic acid in the pyruvate dehydrogenase complex. (2)

c) Distinguish between oxidases and dehydrogenases. (2)

d) Discuss cytochromes as respiratory electron carriers. (3)

e) Briefly discuss the concept of chemiosmotic coupling, naming one type of experimental evidence for this concept. (4)

f) Name two ways in which oxygen can be used as a substrate for other metabolic reactions. (2)

[15]