

Chemistry 431b

Test #1 Guidelines

The first Midterm exam (April 25, 2005, Monday) will include lecture materials and chapters covering enzyme kinetics, general metabolism and thermodynamics and the glycolysis of different sugars covered in class. Review the lecture notes, the homework, the quizzes and read up on your own the entire chapters.

Typically, the midterm may contain a variety of types of questions including: fill in the blanks, multiple choice, true or false, essay questions, chemical structures, calculational and graphical problems. The BEST WAY TO PREPARE is to UNDERSTAND the material THOROUGHLY not superficially.

Below are some topics to consider in starting your review. Do not limit yourself to these questions.

1) Enzymes & enzyme kinetics: : Go over the homework problems.

Know the classification of enzymes, the features and roles of enzymes, the Michaelis Menten equations and graphs, the 3 different ways to graph kinetic data. Be able to interpret the graphs. Discuss the various parameters (like K_m and V_{max}) used, know how to determine them.

Be prepared to graph data from raw data of v_o and $[S]$. BRING YOUR OWN RULER (we'll supply the graphing paper). Know the effect of inhibitors and activators, on the graphs. Know the different types of inhibition and how they can be distinguished by graphical analysis.

Determine catalytic rate constants, enzyme efficiencies, turn over numbers, etc.

What models are used to explain enzyme allostery. What are the distinguishing features of heteroallostery and homoallostery?

2) Metabolism: Go over the homework problems.

Be able to explain the general principles of metabolism, or illustrate them by examples. Know how to do calculations using thermodynamic relationships involving free energy, enthalpy, entropy, equilibrium constant, RQ , etc. Draw the chemical structures of ATP, NADH and NADPH. Be able to show discuss their chemical properties- such as high energy bond character, etc. Know how the sequence of intermediates in a hypothetical pathway is determined using mutants.

Be able to know the principles in enough detail that you can answer questions that distinguish between different properties. For instance, does an actual cellular reaction with a small ΔG° get affected much by changes in substrate concentration? What happens if it involves formation of H^+ ? (explain).

2) Glycolysis: Go over the homework problems.

a) Pathway: Describe in detail the STRUCTURES & FULL names of the metabolites, FULL names of ENZYMES, coenzymes, cofactors and regulation sites (& effectors) involved in all the steps of glycolysis. Know the "biochemical logic" of the steps- especially as discussed in our lectures. Be able to locate the sites of substrate level phosphorylation. Know which steps are irreversible vs those that are reversible. Know about unique or interesting roles of F2,6BP, 2,3-BPG, F1,6BPG (in Pyr Kinase) etc. Trace the carbons from glucose to pyruvate. (for example, if C_1 is radiolabelled in glucose, which carbon(s) will it be in pyruvate? Examples of feedback inhibition? Feedforward activation?

b) Entry of other sugars: Know the steps in the glycolysis of the following dietary sugars: lactose, maltose, mannose, fructose, galactose etc. What are some disorders associated with defects in these steps? For example, what step is skipped in muscle fructose glycolysis?

- c) Fermentation: Describe in detail the structures & names of the metabolites, names of enzymes, coenzymes, cofactors and regulation sites involved in all the steps of fermentation - alcoholic and homolactic. What is the purpose of fermentation? Read about LDH isozymes. Describe the Cori cycle.
- d) Discuss the metabolism of glycogen. Discuss the cascade of steps which give rise to the quick hydrolysis of glycogen into glucose.

Here are a few sample questions from previous midterms: No answers will be provided with these questions. The purpose is to give you an idea of the types of questions and the level of detail you are expected to know the material. It is suggested that you review first before you attempt these so they are used to the maximum of their value to you.

Part I True or False

- _____ 1. The enzyme hexokinase is an example of a transferase enzyme.
- _____ 2. The MWC enzyme model can account for allosteric effects observed in the first committed step of glycolysis.
- _____ 3. The C4 carbon of glucose ends up as CO₂ in ethanolic fermentation.

Part II. Fill in the blanks [1 pt each, total = 5 pts] Give all substrate, enzyme names in full for credit.

- 1) In glycolysis, the enzyme _____ catalyzes the first substrate-level phosphorylation step.
- 2) In galactosemia, a possible enzyme that may be defective is _____ (give name of enzyme).
- 3) Suppose a 2.5×10^{-10} M enzyme preparation ($K_M = 1.0 \times 10^{-3}$ M) has a V_{max} of 5.0×10^{-3} M/s, then the efficiency of the enzyme will be given by _____ (calculate the most appropriate quantity).

Part III. Multiple choice:

- _____ (1) Choose the incorrect statement about the glycolytic pathway:
- a) Glycolysis has 3 irreversible steps all of which have regulated enzymes.
- b) The sole purpose of phase I of glycolysis is to prepare glucose for phase II by adding two phosphates to C₁ and C₆ respectively.
- c) Glycolysis has 3 distinct steps in which a high energy phosphoryl group is added to the substrate
- d) The Pasteur effect refers to the inhibition of glycolysis (through inhibition of the PFK step) when oxygen levels are high..
- e) Inhibition of the last irreversible step in glycolysis will eventually cause a build up of fructose-1,6 bisphosphate.

Part IV. Problems

- 1) Write down the structures of the substrates, enzymes and products of the first step in glycolysis. in which an oxidoreductase is involved. Name all substrates, enzyme and coenzymes. State if this step is irreversible or not.

2) A Lineweaver-Burk plot is constructed for an ATP-utilizing enzyme in the glycolytic pathway. The data is collected under 2 conditions: i) no phosphoenol pyruvate, PEP and ii) 10mM PEP. Two straight lines are obtained both of which intersect in the y-axis at $.025 \text{ mM}^{-1}\text{s}$. There are two different x-intercepts: one at

- 0.050 mM^{-1} (for the case of no PEP) and another at $-.0375 \text{ mM}^{-1}$. From this data, determine the following:

a) K_M and V_{\max} for the enzyme and the type of inhibition:

$K_M =$ _____, $V_{\max} =$ _____, type of inhibition = _____

b) The dissociation constant for the PEP-enzyme complex. _____

c) Which enzyme is being studied? _____ . Name one other (+) and (-) effector for this enzyme: (+): _____, (-): _____