MIDTERM EXAM - ANSWERS

Useful Information:

Avogadro's number: 6.02×10^{23} molecules / mole
1 Faraday = 96,494 Coulomb / mole = 96,494 Joules / Volt / mole
Gas constant (R) = 8.31 Joules K^{-1} mol^{-1} = 1.987 cal K^{-1} mol^{-1} = 0.082 liter atm K^{-1} mol^{-1}
1 calorie = 4.184 Joules

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QUESTION 1 (12 min)

The following table from your textbook lists a variety of coenzymes; some of which have not been covered in class. A series of structures are given below. Identify the structure by its name (from the Table 8-2), and give an example of a reaction in which the coenzyme is involved. The reaction requires only the names of substrates and products, not their structural formulae.

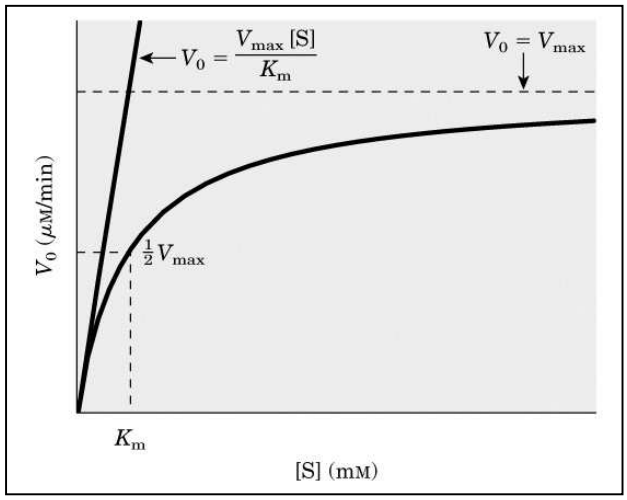
A) the cofactor shown in **thiamine pyrophosphate**; it is required in decarboxylation reactions, for example in the formation of ethanol and CO_2 from pyruvate.

B) the cofactor is **nicotinamide adenine dinucleotide** (NAD^+), used in a variety of oxidation/reduction reactions, for example the oxidation of glyceraldehyde 3-P to 1,3 bis-phosphoglycerate (with inorganic phosphate from the medium)
The lactate dehydrogenase or pyruvate dehydrogenase reactions are also acceptable.

C) the cofactor is **pyridoxal phoshate**; so far we have seen it appear in the glycogen phosphorylase reaction.

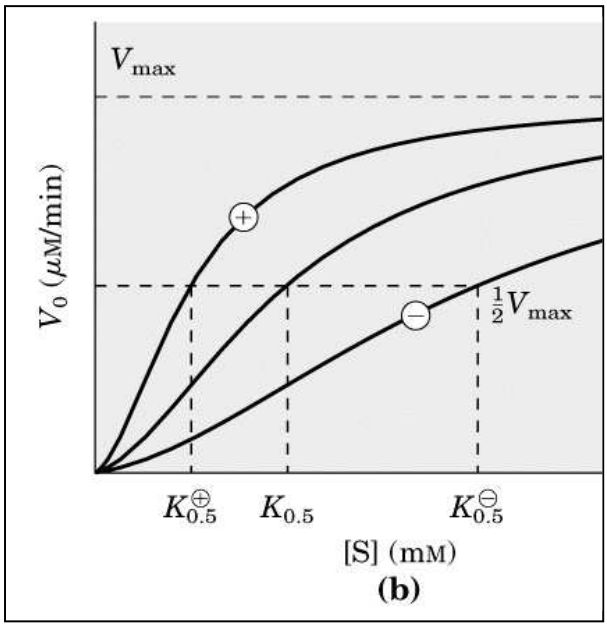
QUESTION 2 (8 min)

a) For an enzyme obeying Michaelis-Menten kinetics a **plot** of initial rate vs. substrate concentration yields a characteristic curve. Give the algebraic expression describing this curve (in terms of [S], K_M, v_{max}), and explain how such data allow one to estimate K_M quite readily.



By measuring the rates at various substrate concentrations up to very high values, one can obtain V_{max} from the asymptote. A horizontal line at 1/2 V_{max} intersects the experimental curve at Km, i.e. when [S] is equal to K_m we observe half of the maximal velocity.

b) In the same diagram contrast the behavior described in (a) with a curve one would obtain for an enzyme exhibiting allosteric behavior. (no algebra or formulae required)



The curves become distinctly S- shaped, and different curves are obtained with different concentrations of effector.

QUESTION 3 (12 min)

There are four boxes below labeled A - D.
a) match them in pairs, i.e. describe which kinetic curves correspond to which type of inhibition
b) characterize (name) the types of inhibition shown

A represents **competitive** inhibition and it matches the curves shown in **D**;
B represents **uncompetitive** inhibition and it matches the curves shown in **C**

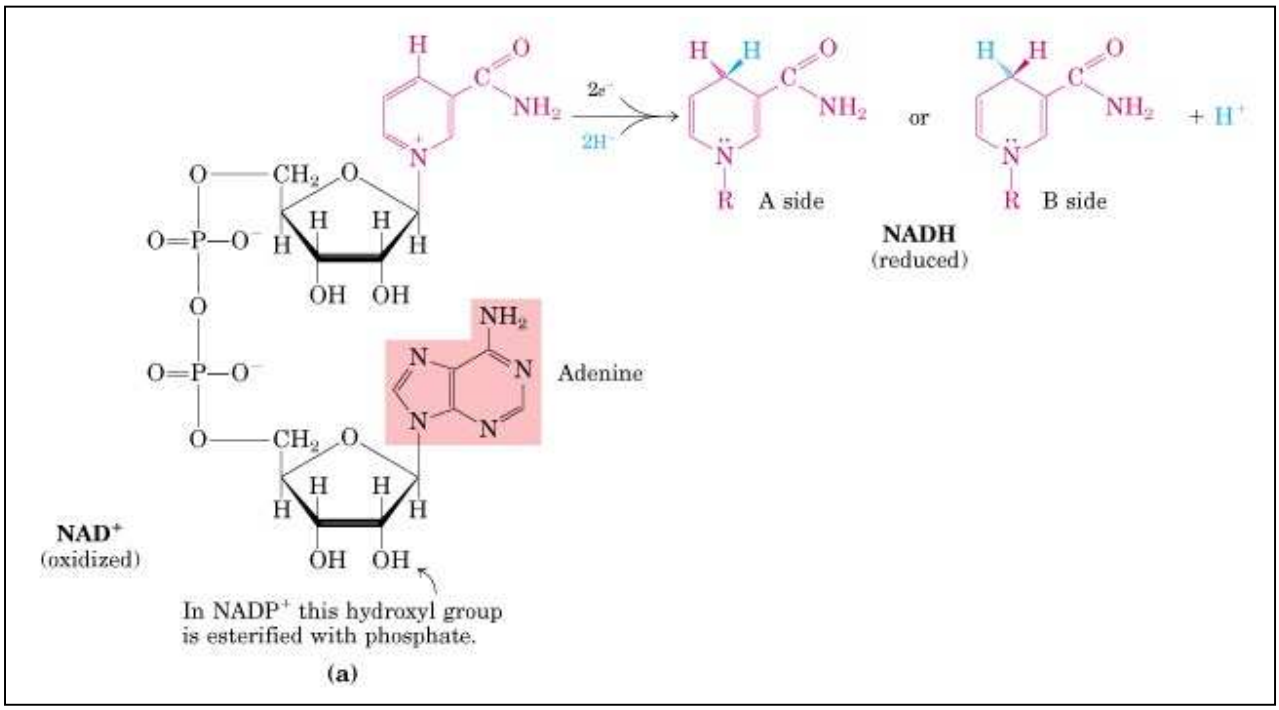
c) give a short (one or two sentences) explanation for the observation that all the lines intersect in one point on the Y-axis in figure D.

In competitive inhibition the inhibitor and the substrate bind to the same site. Thus, at infinite substrate concentrations (x = 0 in the double reciprocal plot) all rates are the same regardless of the inhibitor concentration (a single intercept with the y-axis).

(In uncompetitive inhibition substrate and inhibitor bind to different sites, and even at infinite substrate concentrations the inhibitor can still lower the rate, i.e. the intercept on the y-axis moves up as more and more inhibitor is present.)

QUESTION 4 (12 min)

a) (5 points) Give the structural formula for **NAD$^+$**; show also the reduced form with only the "business end" of the molecule.



b) (4 points) Illustrate the use of this cofactor in the final reactions of fermentation (production of alcohol) (structural formulae required for the substrates, intermediates and products, but not the co-factors).

The answer should show the decarboxylation of pyruvate to acetaldehyde and CO_2, and the reduction of acetaldehyde to ethanol (with NADH as cofactor). Structural formulae are required.

c) (3 points) If [1-^{14}C] pyruvate is given to a yeast extract, would you expect to be able to obtain radioactive ethanol? Explain your answer with the help of the structural formulae in the answer for (b)

In the structural formula for pyruvate the carbons should be numbered; the carboxyl group is the #1 carbon. The carboxyl group becomes the carbon dioxide; hence no radioactive ethanol can be produced

The following tables give the standard free energies of hydrolysis for a variety of phosphorylated compounds and the standard reduction potentials for biologically important half-reactions. They are to be used for Problems 5 and 6

QUESTION 5 (12 min)
Consider the following three reactions and determine which (if any) of these reactions could take place spontaneously as written from left to right (with reagents present at standard conditions). The reaction that could occur (as determined from thermodynamics), nevertheless does not occur in cells. What could be a reason?

a) ATP + creatine ➔ ADP + phosphocreatine
b) Phosphoenolpyruvate + glucose ➔ pyruvate + glucose 6-phosphate
c) AMP + PP_i ➔ ATP

a) reaction would not be spontaneous under standard conditions, since the free energy of hydrolysis of phosphocreatine in higher than the energy of hydrolysis of ATP; phosphocreatine has the higher "high energy phosphate".
b) thermodynamically this reaction is highly favored ($\Delta G_o' << 0$); however, there is no enzyme in the cell (that we have discussed or that I know) that can catalyze such a reaction
c) this reaction is highly unfavorable (($\Delta G_o'$ = +10.9 kcal/mole as written)

QUESTION 6 (8 min)
The following are some reactions involving the oxidation and reduction of carbon compounds. Which of the following would proceed in the direction shown under standard conditions (provided the appropriate enzymes were available)?

a) Malate + NAD+ ➔ oxaloacetate + NADH + H+
b) Malate + pyruvate ➔ oxaloacetate + lactate

a) the half reactions are:

i) oxaloacetate + $2H^+$ + 2e ➔ malate E'^o = -0.166 volts
ii) NAD^+ + H^+ + 2e ➔ NADH E'^o = -0.320 volts

if the first reaction is reversed and added to the second we obtain the overall reaction in (a). **$\Delta E'_o$ would still be negative**, and hence this reaction would not proceed under standard conditions; however,, if the NADH is quickly removed and its concentration is kept low we can see this reaction (as shown for the Krebs cycle)

b) the half reactions are:

i) oxaloacetate + $2H^+$ + 2e ➔ malate E'^o = -0.166 volts
ii) pyruvate + 2H+ + 2e ➔ lactate E'^o = -0.185 volt

again, reversing the first reaction and adding it to the second yields the overall reaction. $\Delta E'^o$ would still be slightly negative, and hence the reaction would not go under standard conditions, but it could be pushed either way by slight changes in the concentrations of the reactants and products.

Both answers require a calculation of $\Delta E'_o$ for the reactions as written and a judgement of what the sign tells us.

QUESTION 7 (8 min)

In the glycolysis pathway we encounter a variety of organic reactions. With names (using standard abbreviations if desired) give **one** example for each of the following types of reactions:

a) Group transfer reaction
b) Isomerization reaction
c) Kinase reaction
d) Oxidation-reduction

group transfer reactions:

Glucose + ATP ➔ Glu 6-P + ADP
Glu 6-P + ATP ➔ Fru 1,6 bisP
PEP + ADP ➔ pyruvate + ATP

isomerization reactions:

Glu 6-P ➔ Fru 6-P
DHAP ➔ 3-P glyceraldehyde
3-P glycerate ➔ 2-P glycerate

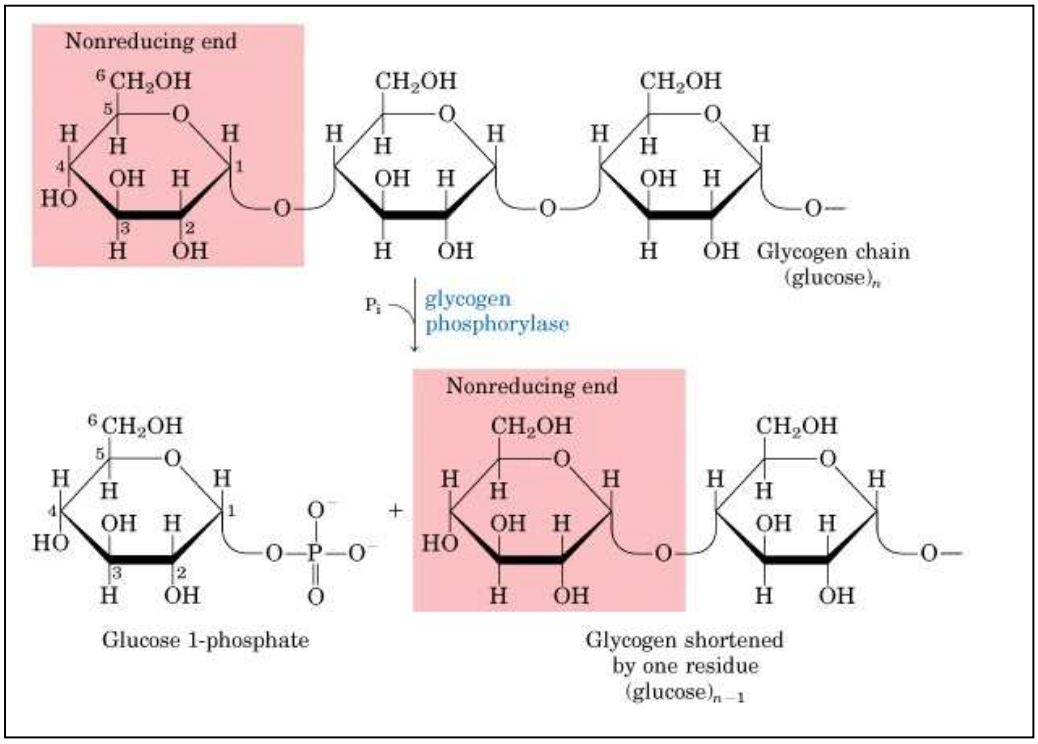
kinase reaction (is also a group transfer reaction);
other example: 1,3 bisphosphoglycerate + ADP ➔ ATP + 3-phosphoglycerate

oxidation reduction:

3-phosphoglyceraldehyde + NAD^+ + P_i ➔ 1,3 bisphosphoglycerate + NADH

QUESTION 8 (12 min)

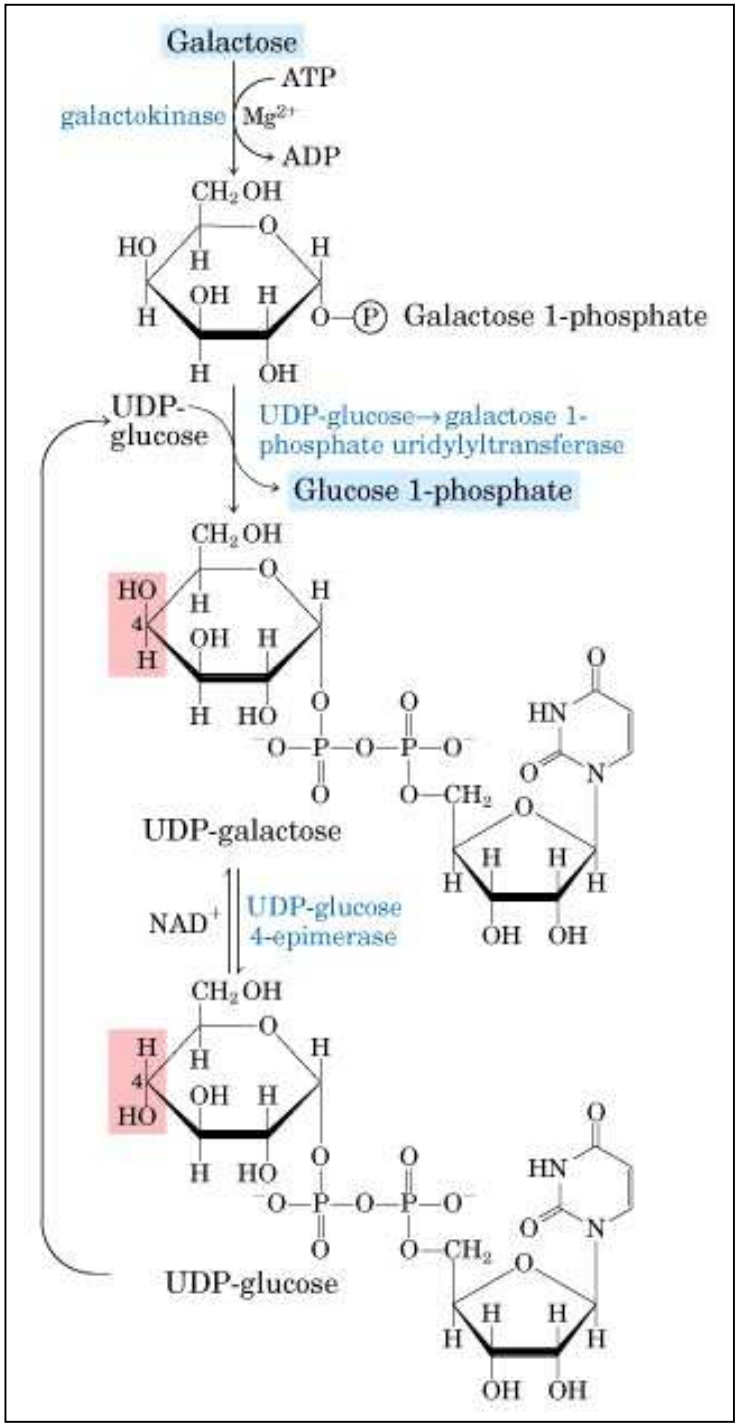
Give the complete structural formulae for a short, unbranched glycogen oligosaccharide. (4 points)
Indicate the reducing and the nonreducing end. (2 points)
What happens when glycogen is broken down by the enzyme glycogen phosphorylase? What are the low molecular weight substrate and product (structural formulae). (4 points)
The product is not immediately available for glycolysis but requires phosphoglucomutase for another reaction. What is the reaction (structures required)?



The Glu 1-P has to be isomerized to Glu 6-P by the enzyme phosphoglucomutase. The structures with the two different positions for the phosphate should be given explicitly with the carbons numbered (2 points).

QUESTION 9 (12 min)

We have encountered **UDP-Glucose** in two important reactions. Give the complete structural formula for UDP-Glu.
What are the two reactions in which it is used in carbohydrate metabolism? The substrates and products can be described by names without explicit structural formulae.



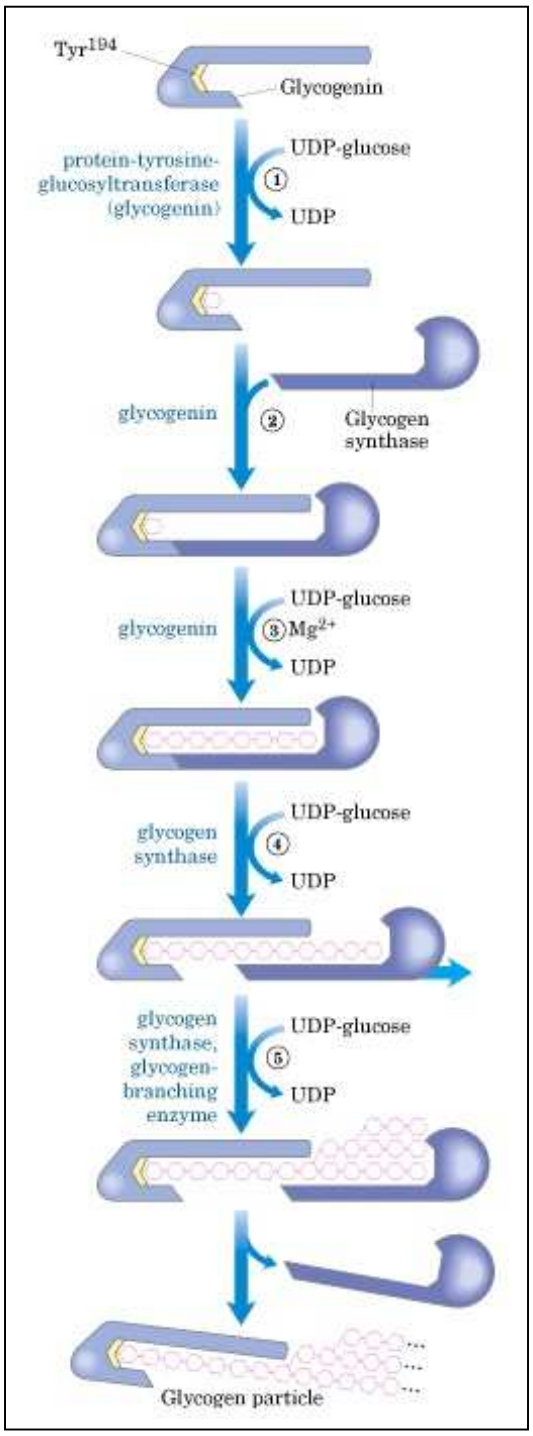
i) UDP-Glu is used in the Leloir pathway for utilizing galactose (interconverting galactose sugar to glucose). (Galactose is derived from lactose, a disaccharide found abundantly in milk.)

ii) UDP-Glu represents the activated from of glucose in the biosynthesis of glycogen. Glucose from UDP-Glu is transferred to the nonreducing end of a growing glycogen molecule making a 1,4 glycosidic bond

QUESTION 10 (12 min)

A protein called **glycogenin** plays an essential role in glycogen synthesis. Explain briefly in words and with the help of a simple schematic diagram (clearly labeled).

A glycogen molecule cannot be started by glycogen synthase alone. Glycogenin is a protein that can catalyze the transfer of a glucose from UDP-Glu to one of its tyrosine residues (side chains). Glycogenin can also catalyze the extension from the first covalently linked glucose to form a short oligosaccharide from more glucoses derived from UDP-Glu. When the chain has a minimal length, glycogen synthase takes over in extending the nascent glycogen chain to make a long, unbranched molecule. (A branching enzyme is required to generate branches and hence multiple nonreducing ends for further extensions.)



QUESTION 11 (12 min)

In the diagram several reactions are presented. Focus on the reactions with the numbered arrows (1-4).

a) give the generic names for each of the four enzymes; can you be more specific?

Enzymes (1) and (3) are kinases and enzymes (2 and (4) are phosphatases; specifically, enzyme 1 is phophofructokinase-1 with specificity for transferring the phosphate to the #6 hydroxyl group, while enzyme 3 is phosphofructokinase-2 with a specificity for transfer to the #2 hydroxyl group.
The phosphatases are also specific for each substrate. (4 points)

b) You have learned about the control of the enzymes (1), (3), and (4). What can you say about the multiple mechanisms for the control of enzyme (1)?

Phosphofructokinase-1 is the key control enzyme regulating the flux through the glycolytic pathway. Depending on the energy state of the cell (relative levels of ATP, ADP, AMP) and the demands made by cellular activity, glycolysis can be speeded up or reduced by modulating the activity of this enzyme. The enzyme is a typical allosteric enzyme with multiple (4) identical subunits. In addition to the substrate binding sites each subunit can bind small molecules such as ATP or AMP at a different site. Binding of ATP at this regulatory site slows down the enzyme, while binding of AMP at this site makes the enzyme more active. A second key regulatory effector molecule is Fru 2,6-bisphosphate. At high concentrations of Fru 2,6-bisphosphate it also binds to the PFK-1 and stimulates the activity of this enzyme. (4 points)

c) There are some particularly unusual and interesting aspects in the control of enzymes (3) and (4).

Enzymes (3) and (4) are not distinct proteins, but are formed by two domains of a single polypeptide chain; one domain has kinase activity, while the other domain has the phosphatase activity. These two opposing activities are regulated in a coordinated fashion: when the kinase is high, the phosphatase is low, and when the kinase is low, the phosphatase is high. This switch is achieved by phosphorylating a key serine residue on this protein. This control by covalent modification requires another kinase which can be regulated by a pathway including cyclic AMP as a second messenger. (4 points)