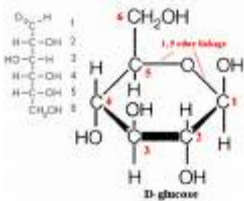


CHEM-643 Intermediary Metabolism

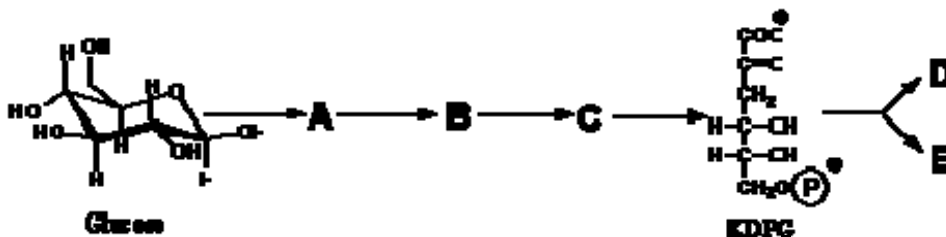
Problem No. 2

Review of Glucose Metabolism



Note from Dr. White: My goal is your understanding. The best way to achieve that understanding is to first work on the problem alone to see if you can solve it. Don't give up without making a serious effort. Remember, your text book is a resource. If you can't solve a problem, please feel free to work and talk through it with other students in the class. However, **write up your answer on your own** to convince yourself *and me* that you really understand the problem and its resolution. If you do work with others, please acknowledge their participation. There is no penalty for such collaboration unless answers are copied and explanations paraphrased from another student's work. You may find it useful to printout pathway work sheets from the course web-site.

1. Variations on Themes. (10 Points) The gram negative bacteria *Pseudomonas fluorescens* ferments glucose by a pathway that resembles the pentose phosphate pathway more than glycolysis in its initial steps. A key intermediate in this pathway is 2-keto-3-deoxy-6-phosphogluconate (KDPG) which is cleaved by KDPG aldolase in a reaction analogous to that of fructose 1,6-diphosphate aldolase in glycolysis. The products of this reaction are metabolized by reactions familiar to you. The structure of Glucose and KDPG are given below.



- (2 points) Give the structures for compounds A through E. (Reaction A to B is a hydrolysis reaction.) Show how D and E of the KDPG-aldolase reaction are metabolized. How are D and E formed mechanistically by analogy to fructose biphosphate aldolase?
- What amino acid is likely to be at the active site of KDPG-aldolase and react covalently with the substrate? If you had purified enzyme to study, how could this residue, among all the others of its type, be labeled *specifically* and *irreversibly* (chemically modified with a radioactive substituent)?

- c. If $l\text{-}^{14}\text{C}$ -glucose forms carboxyl-labeled KDPG, where would the label appear in the end products of fermentation, ethanol and CO_2 ? Compare this pattern to the glycolytic fermentation of $l\text{-}^{14}\text{C}$ -glucose to ethanol and CO_2 . Draw a diagram that compares glycolysis with the pathway involving KDPG. Why are both considered fermentations?
- d. One ATP is used up in the formation of KDPG from glucose. How many net ATPs are generated in the conversion of glucose to ethanol and CO_2 by this pathway? Compare this to the formation ethanol and CO_2 by glycolysis (Embden-Meyerhoff Pathway). Which is more efficient?
- e. Consider the conversion of glucose to KDPG and make an intelligent guess at the intermediates A, B, and C between glucose and KDPG. In so far as is possible, use well-known reactions. Indicate the coenzymes, if any, involved in the three reactions.

2. Paying Attention to Details: (10 Points) The process of gluconeogenesis from pyruvate in liver does not involve the net incorporation of HCO_3^- ; however, when liver tissue is incubated with pyruvate and $\text{H}^{14}\text{CO}_3^-$, the glucose formed is radioactive. (If you think the answer is simple, you haven't paid attention to details and analyzed the problem or you have a superb understanding of intermediary metabolism. Working through this problem on your own initially will help you review metabolism that you need to understand to do well in this course.)

- a. Explain this result given that carbons 3 and 4 of glucose are labeled.
- b. What carbons of glucose would be labeled starting with $3\text{-}^{14}\text{C}$ -pyruvate?

This assignment has several purposes:

1. To review and consolidate your understanding of Glycolysis, Gluconeogenesis, the Hexose Monophosphate/Pentose Phosphate Pathway, and the TCA Cycle.
2. To illustrate the consequences of enzyme specificity and stereochemistry on metabolism.
3. To show how useful radioisotopes are in metabolic studies.
4. To apply chemical principles to metabolism.
5. To exercise your biochemical problem-solving skills.

This assignment will be evaluated on the following elements:

1. A clear analysis of the problems.
2. Answers that display an understanding of the problems.
3. Handed in on time.