

Graduate Biochemistry
Exam 2 (Haas question)

Name: Key

Succinctly discuss how the glycolysis/gluconeogenesis pathways exemplify the principle strategies available to the cell in minimizing futile cycling. (10 points)

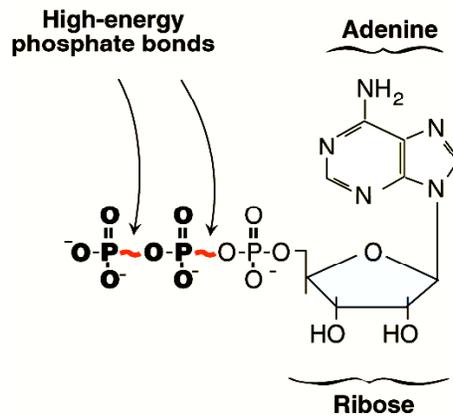
The strategies utilized to prevent futile cycling within glycolysis/gluconeogenesis include:

- a. Compartmentalization of the rate limiting steps in different cellular spaces: cytosolic for PFK1 in the glycolytic pathway and mitochondrial matrix for pyruvate carboxylase in gluconeogenesis.
- b. Evolution of irreversible steps for the committed steps of the pathways, requiring a separate enzyme for opposing step: PFK1 versus Bisphosphatase 1 and pyruvate kinase versus the series of enzymes and mitochondrial membrane transporters converting malate to PEP. Pyruvate kinase versus pyruvate carboxylase for gluconeogenesis.
- c. Differential regulation of the opposing committed step enzymes by the same allosteric effectors, particularly well demonstrated for PFK1 versus Bisphosphatase 1.
- d. Covalent regulation by phosphorylation: down regulation of pyruvate kinase by phosphorylation, differential regulation of PFK2 versus bisphosphatase 2 activities in controlling the steady state levels of Fructose-2,6 bisphosphate.
- e. Utilization of different intermediates in the two pathways: pyruvate in glycolysis versus the malate to PEP steps in gluconeogenesis.

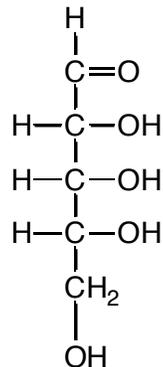
[The theme for the "Overview of Metabolism" lecture was that utilization of common metabolic intermediates results in the possibilities of futile cycling. In outline fashion, the lecture reviewed the different strategies the cell exploits to minimize futile cycling. This question required the synthesis of information (the lectures on the pathways as well as the overview lecture) to identify in two pathways how these different strategies are used in a "real situation".]

1. Draw one (1) of the following structures: (5 points)

ATP



D-ribose



2. Define two (2) of the following terms: (5 points each)

Enantiomers *stereoisomers that are mirror images of one another*

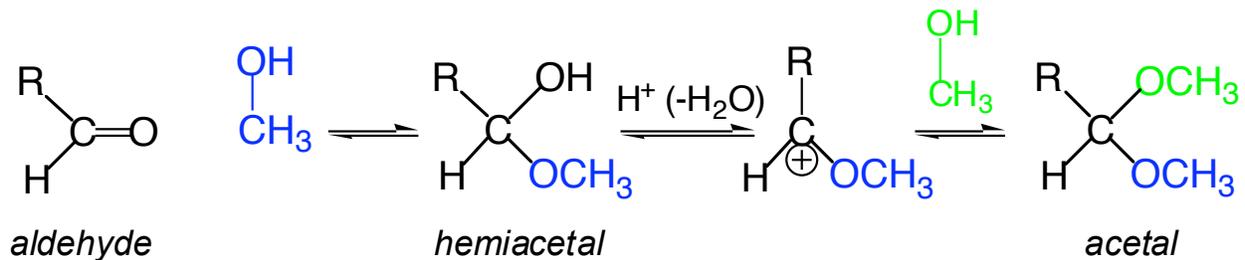
Anomers *stereoisomers of ring structures that differ in configuration about the C-1 carbon*

Conformational isomers *molecules with the same stereochemical configuration, but differing in three-dimensional conformation*

3. In living systems, an important class of reactions for sugars is reactions with an alcohol. Draw the reaction between an aldehyde and an alcohol to form a hemiacetal intermediate and an acetal product (5 points). Define whether these reaction steps are intramolecular or intermolecular (2 points). Explain the importance of these reactions in carbohydrate chemistry (3 points).

*Important hemiacetals and hemiketals have **intramolecular** linkages (1 pts)*
Intramolecular hemiacetals and hemiketals are essential for the formation of monosaccharide rings for simple sugars with ≥ 5 carbons (1.5 pts).

*Important acetals and ketals have **intermolecular** linkages (1 pt), and the linkage is called a glycosidic bond. The compound is a disaccharide (1.5 pts).*



4. What are the two major methods of sequestering monosaccharides in cells during catabolic carbohydrate metabolism? Please provide examples of each reaction, including substrate, product, and catalytic enzyme (5 pts total).

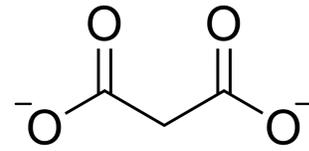
Phosphorylation – no specific transmembrane carriers for these compounds and too polar to diffuse through the cell membrane (2.5 pts)

- *Fructose is phosphorylated by hexokinase to form fructose 6-phosphate or by fructokinase to form fructose 1-phosphate.*
- *Galactose is phosphorylated by galactokinase to form galactose 1-phosphate*

Aldehyde reduction to form a polyol (2.5 pts)

- *Aldose reductase reduces glucose to form sorbitol*

5. Malonate, shown to the right, is a competitive inhibitor of succinate dehydrogenase in the citric acid cycle, because it is structurally similar to the substrate for that enzyme. How many *ATP equivalents* could be generated under aerobic conditions from one molecule of pyruvate if the inhibitor, malonate, is present (5 pts)? Provide detailed description of the reactions that you include in this answer (5 pts).



Malonate

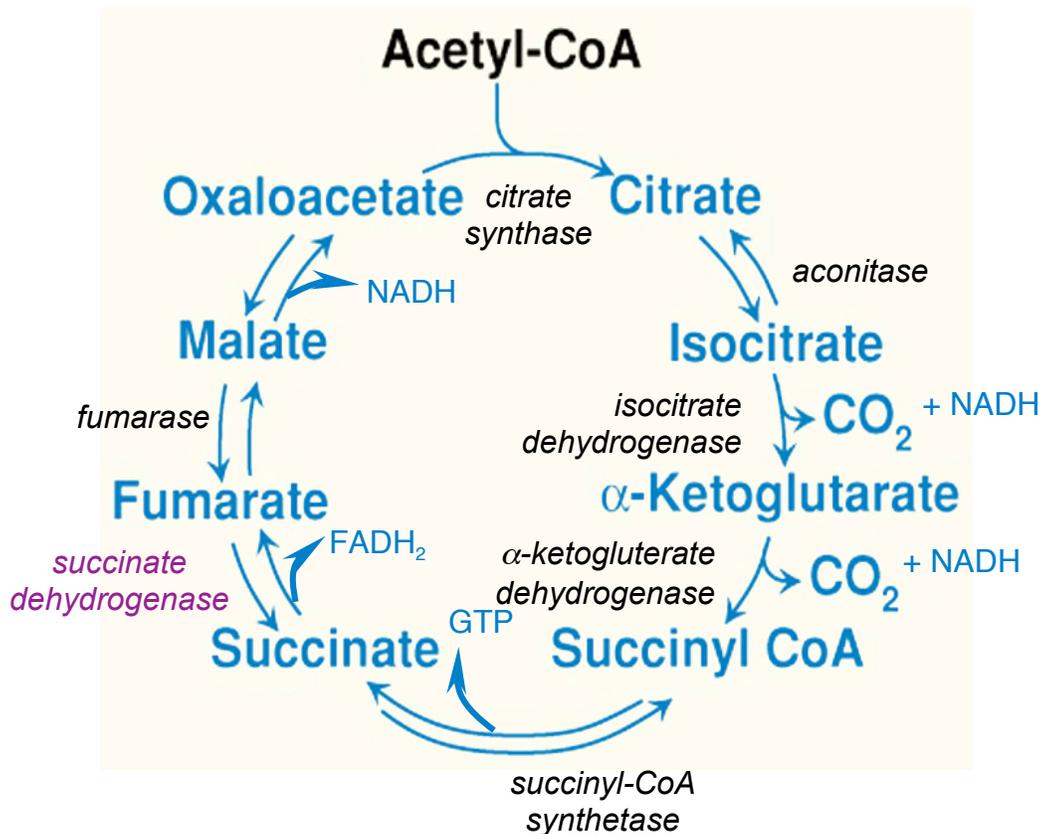
10 ATP equivalents are generated under aerobic conditions from one molecule of pyruvate if malonate is present (5 pts).

Isocitrate → α-ketoglutarate yields one molecule of NADH (1 pt).

α-ketoglutarate → succinyl coA yields one molecule of NADH (1 pt).

succinyl coA → succinate yields one molecule of GTP (1 pt).

In oxidative phosphorylation, electron transfer through complex I, III, and IV (1 pt) provides the proton gradient sufficient to produce 3 equivalents of ATP per NADH that is oxidized (1 pt).



6. A male patient with acute hemolytic anemia was diagnosed with Class I G6PD deficiency. Explain what biochemical products in the pentose phosphate pathway will be compromised in this patient and whether these compounds are produced in the oxidative or non-oxidative stages of the pathway (6 pts). From the crystal structure of G6PD and the single nucleotide polymorphism data collected from patients, what region of the enzyme would be the likely site of mutation (5 pts)? How would this putative alteration affect enzyme function (5 pts)?

Primary products of the PPP pathway are (2 pts apiece)

- *To provide ribose 5-phosphate (R5P) for synthesis of nucleotides and nucleic acids (non-oxidative stage)*
- *Generate NADPH for reductive biosynthesis reactions within cells (oxidative stage)*
- *Rearrange the carbon skeletons of dietary carbohydrates into glycolytic/gluconeogenic intermediates (fructose 6-phosphate and glyceraldehyde 3-phosphate) in the non-oxidative stage*

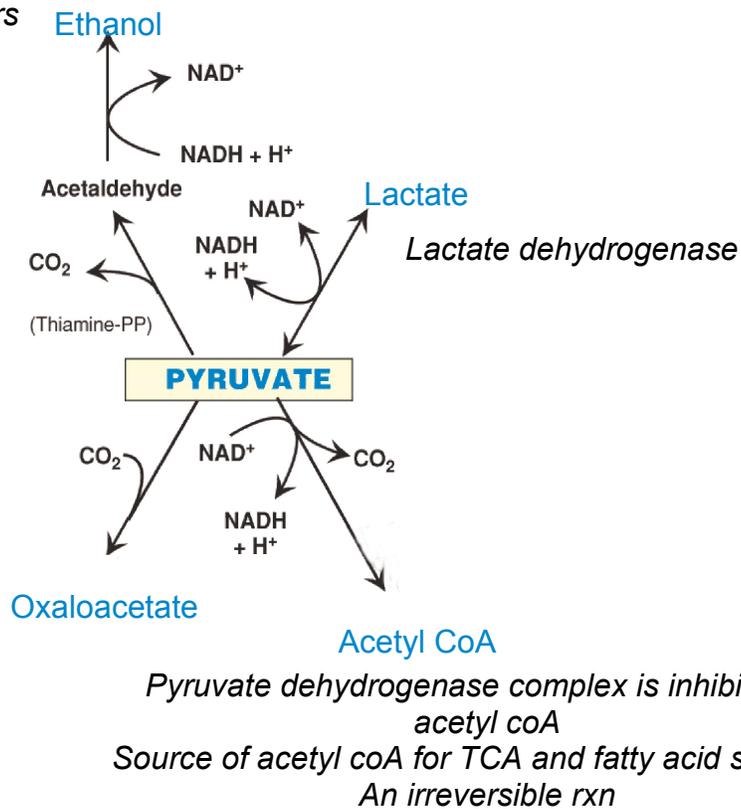
Region of G6PD most likely targeted in class I patients (most severe) is between residues 362-446 at the carboxy end, that contains the cofactor (NADP+) binding site and the dimerization interface. (5 pts)

These amino acid substitutions typically result in < 2% activity in the red blood cells of G6PD patients in the class I category. This loss of enzymatic activity could result from allosteric inhibition from loss of NADP+ binding or from lack of quaternary structure organization (5 pts).

7. Pyruvate is a key intermediate between different metabolic pathways. Describe how pyruvate is formed (4 pts) and the four metabolic fates of pyruvate (10 pts). Include enzymes responsible for these reactions, required cofactors, and additional products formed for each of these reactions.

Glycolysis is the catabolic process in which glucose is broken down to pyruvate with formation of ATP (4 pts).

*Ethanol synthesis occurs in yeast and some bacteria (including intestinal flora)
Thiamine pyrophosphate-dependent pathway*



*Pyruvate carboxylate is activated by acetyl coA
Replenishes intermediates of the TCA cycle
Provides substrates for gluconeogenesis
An irreversible rxn*

*Pyruvate dehydrogenase complex is inhibited by acetyl coA
Source of acetyl coA for TCA and fatty acid synthesis
An irreversible rxn*

BONUS QUESTION:

Regarding question 5, if you were informed that malonate was also an inhibitor of complex II, how many *ATP equivalents* could be generated under aerobic conditions from one molecule of pyruvate if malonate is present?

Still 10 ATP equivalents are generated under aerobic conditions from one molecule of pyruvate if malonate is present (5 pts).

Complex II does not participate in the proton gradient formation across the mitochondrial inner membrane.

Dr. Wojcik's portion of Exam 2 [answer key]

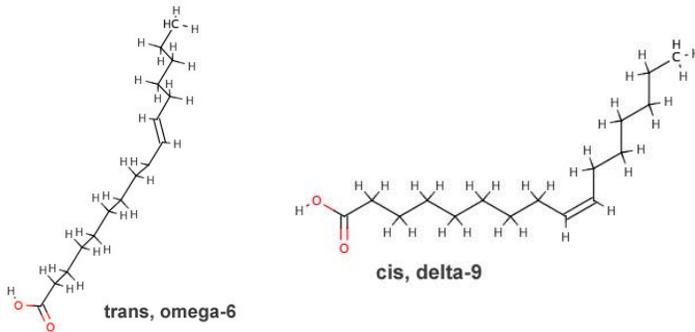
Describe what happens to a fatty acid chain component of a TAG molecule during its journey from the lumen of the intestine to its destination in an adipose cell.

Broken down to free fatty acids and glycerol monophosphate within intestinal lumen by pancreatic lipases

Reassembled into TAG within intestinal mucosal cells, and packaged into chylomicrons

Chylomicrons circulate and encounter lipases that break them down into free fatty acids and glycerol to facilitate transport in to cells

Draw a wire diagram of (i) a 16 carbon fatty acid with a *cis* double bond at the delta-9 position and (ii) a 16 carbon fatty acid chain with a *trans* double bond at omega-6. Label the double bonds and discuss the physical and chemical differences between the two.



trans fatty acid will be more linear, pack tightly, and more likely to be a solid at RT, while cis fatty acid will be kinked, pack loosely, and more likely to be a liquid at RT.

What is the role of the ACP domain in the function of fatty acid synthase?

Accept acyl chain from acyl CoA (via its own CoA prosthetic group) and move it between the 6 catalytic domains of fatty acid synthase during chain elongation reactions

Describe a structural characteristic that we often observed of the substrate binding sites of enzymes that act on fatty acids, such as carnitine acyltransferase.

The active site contains a tunnel that sequesters the (hydrophobic) acyl chain from the aqueous cytoplasm

Where does the arachidonic acid in your body come from? Suppose some of the

Dr. Wojcik's portion of Exam 2 [answer key]

arachidonic acid in your cell membranes had one of its double bonds in the trans configuration. Compare and contrast the affect this modified arachidonate would have on the major roles for this fatty acid.

It comes from the diet, either directly or via linoleic acid precursor.

Trans configuration would competitively inhibit prostaglandin synthesis

Describe how aspirin reduces pain, swelling and blood clotting at the molecular level.

Aspirin enters the active site of Cox 1 and acetylates a Serine residue (ser530) causing irreversible inhibition of Cox 1 by occluding the active site. Inhibition of Cox 2 is thought to proceed by the same mechanism. In both cases, prostaglandin synthesis is reduced, resulting in reduces sensation of pain, reduced swelling, etc.

Describe how statin drugs lower serum cholesterol.

Statins are competitive inhibitors of HMG CoA reductase (due to their chemical similarity to HMG CoA). Because HMG CoA reductase catalyzes the rate limiting step of cholesterol biosynthesis, the effect of inhibition is to lower serum cholesterol.

What is thought to be responsible for the formation of lipid rafts, and how do they potentially affect the efficiency of cell signaling events?

Spontaneous segregation of DHA related lipids from sphingolipids, which are subsequently stabilized by cholesterol and protein interactions.

They may directly alter the conformation of associated proteins or favor certain protein/protein interactions as compared with the bulk membrane lipids.