Chapter 16 - Glycolysis and Gluconeogenesis

Topics you’re not responsible for: A Regulated Bifunctional Enzyme...(p.445 - 446, Figure 16.20), mechanisms of triose phosphate isomerase (p.431 - 432), glucose transporters (pp 448 - 449)

I. Introduction
- Historical aspects
  - The metabolic goals of glycolysis are to extract the free energy released during catabolism of glucose and store it in the form of ATP, to extract and store the electrons released as NADH (the NADH will then be used to either make more ATP (oxidative phosphorylation) or provide reducing power for biosynthesis as NADPH), to provide intermediates for other pathways, and to make pyruvate:

\[
\text{NADH (reducing power), } \Delta G = -34.9 \text{ kcal/mole (only 5\% of total } \Delta G \text{ available)}
\]

The aerobic fate of pyruvate is to enter the citric acid cycle where it is degraded to \( \text{CO}_2 + \text{H}_2\text{O} \). Anaerobic fates are the formation of lactate or ethanol (yeast).

II. Reactions and mechanism of glycolysis
- There are ten reactions involved in glycolysis, each catalyzed by its own enzyme. The types of reactions that constitute glycolysis are, with the enzymes that catalyze these reactions in parentheses, phosphoryl transfers (kinase), phosphoryl shift (mutase), isomerization (isomerase), dehydration (dehydratase), aldol cleavage (aldolase - see p. 431. The metabolic strategy in ATP production is to synthesize high-energy, phosphorylated, intermediates with high phosphoryl group transfer potentials so that they can phosphorylate ADP. The problem in recitation 1 was an example of such a reaction, a substrate-level phosphorylation. A second substrate-level phosphorylation occurs in glycolysis
  - Both substrate-level phosphorylations involve phosphorylated metabolites. These substrate-level phosphorylations must therefore be preceded by reactions in which phosphate
groups are attached to form low-energy molecules (phosphate esters)

Note: This initial reaction of glycolysis serves not only to put a low energy phosphate on glucose, but also to lock glucose into the cell.

- The next two reactions of glycolysis result in the formation of a doubly phosphorylated intermediate, fructose 1,6 bisphosphate:

Note: The reaction catalyzed by phosphofructokinase is a key control point in glycolysis, since this step commits to glycolysis. Glycolytic intermediates “upstream” are common to other pathways (pentose phosphate pathway and glycogen metabolism). Also, fructose 1,6 bisphosphate is phosphorylated at both ends and can be split in two such that both products are phosphorylated and can be converted from low energy esters into high energy molecules.
- The second ATP-requiring step is followed by the cleavage of fructose-1,6-bisphosphate to form 2 3-carbon fragments, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. See p. 431.

\[
\begin{align*}
\text{Fructose 1,6 bisphosphate} & \quad \text{Dihydroxyacetone phosphate} \\
\text{Glyceraldehyde-3-phosphate} & \quad (\text{DHAP}) \\
\text{(G3P)} &
\end{align*}
\]

which is catalyzed by aldolase. An understanding of this mechanism is key to understanding the metabolic strategy of this and other pathways:

- Dihydroxyacetone phosphate is subsequently converted into glyceralde-3-phosphate (G3P) by another isomerase (compare to reaction 2).
- In the last phase of glycolysis, G3P is first oxidized to 1,3BPG. The first substrate-level phosphorylation follows in which 1,3BPG is converted into 3 phosphoglycerate (3PG):

- 3PG is then converted into 2PG (mutase), 2PG is converted into PEP (enolase), which is then converted to pyruvate via the second substrate level phosphorylation of glycolysis:

Note that in PEP the double bond is between carbon atoms, whereas in pyruvate it is between carbon and oxygen, where the electrons prefer to reside. The source of “high energy” in PEP is due to the favorable shift of electrons in this enol to keto tautomerism.

Note that all but three of the reactions of glycolysis are reversible and will be utilized when pyruvate is converted back into glucose in another chapter (gluconeogenesis).

- The energy yield of glycolysis is 2 ATP and 2 NADH. See Figure 16 - 3, p. 429, for the complete pathway.
III. Entry of fructose and galactose into glycolysis - These sugars, also occurring frequently in the diet (fructose in table sugar (i.e., sucrose) and high-fructose corn syrup, galactose as lactose in milk), enter glycolysis via mini-pathways:

- Fructose: In extra-hepatic tissues (liver, muscle) fructose is phosphorylated by hexokinase to form fructose-6-phosphate, a glycolytic intermediate. In the liver, however, the abundance of glucose keeps hepatic hexokinase busy with all the glucose present, hence another mini-pathway is necessary (see Figure 16 - 15, p. 441):

![Fructose Pathway Diagram]

Note: When fructose is catabolized in the liver according to this pathway the products, Dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, occur below the main regulatory reaction in glycolysis. Thus, the catabolism of glucose to acetyl CoA in the liver is missing a critical element of regulation and a diet high in fructose can lead to elevated serum cholesterol and triglycerides (see regulation below).

- Galactose: A kinase phosphorylates Gal, a transferase activates it by attaching a UDP group (see p. 443) which is transferred from an activated Glu, an epimerase epimerases the activated Gal, and a mutase converts Glu-1-phosphate to Glu-6-phosphate:

\[
\text{Galactose} \xrightarrow{\text{kinase}} \text{Gal-1-phosphate} \xrightarrow{\text{transferase}} \text{Glu-1-phosphate} \xrightarrow{\text{mutase}} \text{Glu-6-phosphate}
\]

Note that galactosemia, a severe genetic disease (autosomal recessive), results from a defective gene encoding the transferase, leading to inability to metabolize galactose and subsequent death if galactose is not excluded from the diet.

IV. Regulation - Three reactions serve as regulatory points in glycolysis, those catalyzed by hexokinase (first reaction), phosphofructokinase (PFK-1) and pyruvate kinase (last step). PFK-1 is the key regulatory enzyme because it catalyzes the committed step. If the hexokinase-catalyzed reaction were the key point of regulation, the pentose phosphate pathway and glycogen metabolism could be compromised, and if pyruvate kinase catalyzed the key step, glycolytic intermediates beyond the committed step (i.e., those common only to glycolysis), could potentially build up. In general, glycolysis is inhibited allosterically by a high energy charge ([ATP]/[ADP]) and by the presence of building blocks in other pathways provided by glycolysis (i.e.,
citrate and alanine)
V. Anaerobic vs. Aerobic fates of pyruvate - In aerobic organisms pyruvate enters the mitochondria where it is further catabolized to CO$_2$ in the citric acid cycle, during which several oxidations, hence, several NADH (and FADH$_2$) are produced. The reducing power in these reduced cofactors produces several more ATP during oxidative phosphorylation. NAD$^+$ is produced during oxidative phosphorylation, which is needed for glycolysis. In the absence of these aerobic events, NAD$^+$ must be generated by alternate means, lactate production and alcoholic fermentation in yeast. Lactate, or lactic acid, is responsible for the muscle-burning we experience when we exercise vigorously.
VI. Cancer and glycolysis: Cancer cells grow more rapidly than the blood vessels that nourish them. Since blood supplies oxygen, cancer cells metabolize glucose anaerobically (in order to regenerate NAD\(^+\)) until sufficient vascularization occurs. In the absence of oxygen a transcription factor, HIF-1 (hypoxia-induces transcription factor) stimulates expression of glucose transporters which transport glucose into the cell. HIF-1 also stimulates the growth of new tumors by enhancing vascularization. Efforts are underway that inhibit vascularization of tumors.
Gluconeogenesis

1. Introduction
   - Converts pyruvate and other precursors into glucose to maintain constant serum glucose level to supply brain, blood cells, etc., with fuel
   - Most gluconeogenesis occurs in the liver, the major exporter of glucose (also in cortex of kidney)
   - 7 of 10 glycolytic reactions are utilized by gluconeogenesis:
II. Individual reactions:

Comment: In both cases, a (different) phosphatase cleaves a low-energy phosphate ester because to synthesize an ATP via the exact reversal of the corresponding glycolytic reactions is energetically unfeasible.

Comment: To synthesize PEP from pyruvate requires the energy equivalent of 2 ATP and requires 2 distinct steps, each of which requires an ATP (GTP in the second case). The carboxylase-catalyzed reaction is the anaplerotic reaction of the CAC. Thus, OAA is at an intersection involving gluconeogenesis, the CAC, and also the glyoxalate cycle in plants. Since gluconeogenesis occurs in the cytoplasm whereas the CAC occurs in the mitochondria, and since the inner mitochondrial membrane is impermeable to OAA, export of OAA intended for gluconeogenesis occurs via the malate shuttle:
Note that during this process mitochondrial levels of NADH decrease whereas cytoplasmic levels increase. In other words, reducing power is being exported from the mitochondria which is desirable under conditions which favor biosynthesis (which gluconeogenesis is an example of).

Note also that the last enzyme in gluconeogenesis, glucose-6-phosphatase, is present only in those cells whose metabolic duty is to export glucose, i.e., the liver and to a lesser extent the kidney. Otherwise, glucose-6-phosphate cannot leave the cell.

III. Energy yield of gluconeogenesis:

Total energy cost per glucose = \(\frac{2\text{ ATP} + 1\text{ GTP}}{\text{pyruvate X 2 pyruvate/glucose}} = 6\)

IV. Regulation - There are two aspects of regulation. Entry of pyruvate into gluconeogenesis is regulated because pyruvate carboxylase requires acetyl CoA. Note that this is the anaplerotic reaction of the CAC so production of OAA can either refill depleted CAC levels or be converted into glucose. The choice here is determined by ATP levels. As an indicator of an energy-rich condition high levels of ATP will inhibit the CAC and direct OAA into gluconeogenesis and vice versa. Thus, an energy-rich condition diverts OAA into biosynthesis, and energy-poor condition increases CAC activity and the
production of energy.

A second regulatory requirement, not necessary until now, is the need for reciprocal regulation of glycolysis and gluconeogenesis. Both cannot be going simultaneously as no metabolic goal would be accomplished other than the net expenditure of 4 ATP per cycle from glucose → pyruvate → glucose. Such a cycle is called a *substrate cycle* (or futile cycle) and can be used to generate heat (bumblebees and hibernating bears). Reciprocal regulation is achieved by 1) having the key regulatory elements occurring between the same pair of intermediates, and 2) the presence of allosteric modulators that will have opposing effects on glycolysis and gluconeogenesis:

Regulation at transcriptional level also occurs: Insulin, which stimulates uptake of glucose after a meal, stimulates the production of glycolytic enzymes involved in the three control points. Glucagon, a pancreatic hormone, signals a need for glucose (starvation) and thus inhibits production of these glycolytic enzymes and stimulates production of key regulatory enzymes involved in gluconeogenesis.

V. Cori Cycle - During rigorous exercise ATP demand by contracting muscle is great and cannot be supplied by the CAC, which occurs more slowly than glycolysis. As we have seen, pyruvate is reduced to lactate to replenish NAD\(^+\) to allow glycolysis to continue. During recovery, lactate, a dead-end metabolite must be re-oxidized to pyruvate and recycled back into glucose via the *Cori Cycle*:

LDH (lactate dehydrogenase) is an enzyme consisting of four copies of two types of subunits, M (muscle) and H (heart), which can combine in any fashion, i.e., M\(_4\), M\(_3\)H, M\(_2\)H\(_2\), ..., H\(_4\). It is likely the M and H forms evolved from a single ancestral gene and diverged into the M and H forms via gene duplication. M\(_4\) predominates in the muscle and is designed to convert pyruvate to lactate. H\(_4\) is allosterically inhibited and is designed to convert lactate to pyruvate in heart muscle which uses the pyruvate as a fuel (CAC). Recall similarity to hemoglobin story.
Problems: 8, 9a), b), d), 11, 14, 16