**Glycolysis and carbon-carbon bond chemistry**

I. Intro to Glycolysis

**Role in energy production** If you trace the oxygen content of the earth’s atmosphere over the past several thousand million years, it appears that there was almost no oxygen in the air until about 2000 million years ago, when it suddenly started to rise. However, organisms had been photosynthesizing since at least a thousand million years before that. What happened to the $\text{O}_2$ those early organisms produced? One idea holds that the evolved oxygen was complexed with iron to form ferric oxide; when the iron was saturated with oxygen, it began to appear in the air. During this time the principal source of ATP formation was **glycolysis**.

Compared to **oxidative phosphorylation** (our current primary, oxygen-dependent method for extracting energy from food), glycolysis is far less efficient - about 20 times less energy is produced per molecule of glucose. But with abundant food, (as well as the lack of oxygen and thus no choice in the matter), glycolysis worked perfectly well for the early cells. When oxidative phosphorylation arose later, it caught on due to its higher efficiency. Nevertheless, under certain conditions glycolysis remains the metabolic path of choice due to its faster energy production and its lack of an oxygen requirement. For example, people who don’t have time to wait for their energy, such as those sprinting 100 meters, rely entirely on glycolysis. Specifically, the **fast-twitch muscles**, which appear white and contain few mitochondria, run on glycolysis alone.

II. Carbon-carbon bond breaking

A. Cleavage of glucose The overall glycolysis reaction consists of the breakdown of glucose to form two lactate molecules and two ATP:

$$\text{glucose} + 2\text{ADP} + 2\text{Pi} \leftrightarrow 2\text{lactate} + 2\text{H}^+ + 2\text{ATP} + 2\text{H}_2\text{O} \quad \Delta G^\circ = -32 \text{ kcal/mol}$$

An important part of this reaction is the breaking of a carbon-carbon bond to split glucose in half:

![Glucose glycolysis reaction diagram]

In the absence of ATP (as in the figure), this reaction has a $\Delta G^\circ = -47 \text{ kcal/mol}$. How could the free energy change be so large, especially in the absence of any net change in the oxidation-reduction state? Two aspects of entropy explain this large free energy
change: 1) the number of molecules goes from one to two, and 2) due to the carboxylic acid groups on each lactate, the products have more resonance structures than the reactant and are thus more stable.

So why did nature stop with just two fragments? Why not cut two C-C bonds and end up with three products and more resonance structures, or all C-C bonds to yield six products (although no increase in resonance)?

The answer is because carbon-carbon bond breaking is hard. Nature manages, but it takes many enzymes. The following diagram shows how many enzymes are required to break each C-C bond in the complete oxidation of glucose.

Unlike the other steps, converting pyruvate to AcetylCoA only requires one enzyme because pyruvate is an $\alpha$-keto acid and is therefore easy to decarboxylate. The advantage of a nearby carbonyl group turns out to be key to nature’s ability to break a given C-C bond and explains why only one of the C-C bonds in glucose is broken.

**B. Atomic structure of C-C bond breaking**

When a C-C bond is broken, the two electrons from the bond can be distributed either of two ways. One electron might go with each C, forming two radicals:

\[
\begin{align*}
\text{C} & \quad \bullet \\
\text{C} & \quad \bullet
\end{align*}
\]

However, if radicals formed, they would be so reactive that they would immediately collapse back together. Nature almost never uses this pathway.

The other possibility is that both electrons from the bond go with one carbon, leading to a negatively charged carbanion and a positively charged carbocation:

\[
\begin{align*}
\text{C}^+ & \quad \cdot \\
\cdot & \quad \text{C}^-
\end{align*}
\]

This is preferred pathway, but only under conditions which stabilize the two charges. **Stabilization of either carbon ion requires a nearby carbonyl group:** to form a carbocation the carbon must be part of the carbonyl group, while to form a carbanion the charged carbon must be alpha to a carbonyl group:
Thus, in order to cut a carbon chain in a certain place, the carbonyl groups have to be arranged appropriately. This requirement explains why glucose is only cut once.

II. Enzymatic mechanisms in glycolysis

A. Aldolase

Aldolase catalyzes the overall reaction:

\[
\text{fructose 1,6-bisphosphate} \longrightarrow \text{dihydroxyacetone phosphate} + \text{glyceraldehyde 3-phosphate}
\]

(for structures, see below and V&V p. 450.) Aldolase gets its name from the fact that it catalyzes an aldol condensation, a reaction in which two carbonyl compounds are joined to form an aldol, which is a $\beta$-hydroxy carbonyl compound. Although an aldol condensation is the opposite of what happens when fructose 1,6-bisphosphate is broken down, remember that an enzyme always catalyzes both the forward and reverse reactions - hence it also catalyzes the reverse of an aldol condensation which is its natural function.

The catalytic mechanism for this reaction is exactly the reverse of that shown in V&V p. 452, and key steps in the mechanism are illustrated below:
Hydrolyses with water
Note that the bond between C3 and C4 is to be broken and that the hydroxyls at C3 and C4 are on opposite sides from each other. Also note that a carbonyl group is located at C2 - that is, alpha to the carbon which is to become a carbanion, as required. Note also that both ends of the chain are phosphorylated. As will be discussed in more detail later, one of the phosphate groups is added as soon as glucose enters the cell and serves to keep it from escaping, while the other is thought to serve as a second handle for the enzyme to grab onto.

The key residue in the active site of aldolase is a lysine, which reacts with C2 in the first step to form a Schiff base. The Schiff base (as shown in V&V p. 452) consists of a double bond between the lysine N and C2 of the substrate. The N acquires a positive charge, and C2 becomes electron deficient. To help compensate for the electron deficient state of C2, the electrons in the bond between C3 and C4 collapse to form a bond between C2 and C3. The OH of a tyrosine residue participates as a base and as an acid in the reaction. At the end of the reaction, the Schiff base is hydrolyzed, and the lysine and tyrosine residues are recovered unchanged.

B. Hexokinase - induced fit  The aldolase reaction starts with fructose 1,6-bisphosphate, but prior steps are required for the cell to convert its original glucose source into fructose 1,6-bisphosphate. As mentioned above, the first phosphorylation step occurs immediately after glucose enters the cell. The reaction,

\[
\text{ATP} \quad \text{glucose} \quad \text{------------------> glucose 6-phosphate} + \text{ADP} \\
\text{hexokinase}
\]

involves the transfer of a phosphate group from ATP to C6 of glucose and is catalyzed by hexokinase (structures for reaction are shown in V&V p. 447).

As shown in V&V Fig. 16-5, binding of glucose induces a conformational change in the active site of hexokinase. Since the substrate is required for this change, the new conformation is known as the induced fit. As the figure shows, the enzyme active site looks like an open mouth when empty, but as soon as the substrate enters, the mouth closes, leaving no empty space inside the active site.

The advantage of closing the active site upon substrate binding is that when substrate and ATP enter the active site, the induced conformation excludes water molecules and thus eliminates the possibility of ATP hydrolysis, which is energetically more favourable than the phosphorylation of glucose.