VI. GLYCOLYSIS

**Introduction:** During glycolysis glucose is converted to pyruvate. The series of ten reactions responsible for this conversion do not require oxygen, hence the process is also known as the anaerobic fermentation of glucose. There are Four Phases. Three are constant and do not depend upon the identity of the organism or the availability of oxygen, while the fourth is variable. The constant portion consists of 10 steps with an equilibrium constant of $10^5 \ (= \Delta G^o$ of -8.5 kcal/mole of pyruvate formed). The largest changes occur with hexokinase, phosphofructokinase and pyruvate kinase (*).

(1) **The preparative phase.** Overall the glycolytic pathway occurs with the net production of 2 moles of ATP. The metabolic sequence requires the fragmentation of a hexose into two trioses- and this is the key step in the rearrangement of the carbon skeleton. *A priori*, the most likely mechanism for breaking a C-C bond of a hydroxylated substrate is an aldol cleavage, a reaction which requires a β-hydroxycarbonyl functionality and results in cleavage between the α and β carbon atoms. Glucose in the open-chain form has such a group but, as an aldohexose, the products of cleavage would be a 2C plus a 4C unit; these are not the observed set of products!

![Chemical Reaction](image)

However if the carbonyl group were at C₂ and not at C₁ the aldol cleavage would yield 2 3C products. Thus one purpose of the initial steps of glycolysis is to convert glucose to fructose which, having the carbonyl at C₂, will produce 2 3C fragments after aldol cleavage.

The early intermediates are phosphorylated sugars--this sets up the hexose molecule for the ATP syntheses that occur in the terminal phases of glycolysis. However, as we shall see 2 ATPs are actually consumed in this preparatory phase. Phosphorylation also ensures that the intermediates cannot diffuse out of the cell compartment.

The products of the aldol cleavage are not identical, they are glyceraldehyde-3-P (G3P, also 3-PGA, for historical reasons) and dihydroxyacetone phosphate (DHAP). However these compounds are in very rapid, enzyme–catalyzed equilibrium and conceptually we can consider the products to be 2 moles of G3P.

(2) **The second phase** of glycolysis is the oxidation of G3P to 3–phosphoglyceric acid. This is the major energy conserving step, for not only is a mole of ATP synthesized but one NADH is also produced. Although we normally view NADH as a source of reducing equivalents we will later see that 1 NADH is the equivalent of 3 ATP - a relationship which will become obvious when we come to study oxidative phosphorylation.

(3) **In the third phase** of glycolysis each molecule of 3-phosphoglyceric acid is converted to a molecule of pyruvate, a sequence which produces the second ATP.

(4) Finally we have the fourth and variable phase of glycolysis, for the further metabolism of pyruvate depends upon whether or not oxygen is available and in which tissue glycolysis occurs (muscle vs. yeast etc.). However it must be emphasized that the first three phases are independent of the system and also do not depend on the presence of oxygen.

**The Individual Reactions.**

**HEXOKINASE.**

The first step in glycolysis is the nucleophilic attack of the C₆ hydroxyl of glucose on the γ-P of ATP leading to the production of G6P. This reaction proceeds via the formation of a ternary complex of the enzyme,
hexokinase, glucose and ATP, and the reaction is strongly in favor of the products ($\Delta G_\circ = -3.4$ kcal/mole). ATP reacts as the Mg salt.

$$\text{CH}_2\text{OH} + \text{ATP} \rightleftharpoons \text{CH}_2\text{OP} + \text{ADP}$$

The reaction catalyzed by hexokinase is typical of kinase reactions, with the OH at C-6 activated by a base that "removes" the proton thus creating a very strong nucleophile (-$O^-$); this nucleophile attacks the $\gamma$-P with the formation of a five-coordinate trigonal bipyramid at the P; subsequent departure of the ADP leads to inversion of configuration at the phosphorus.

Why doesn't the ATP undergo hydrolysis? After all, water is much smaller than glucose and should have no trouble in fitting into the active center. The x-ray structure of the enzyme shows that the free enzyme contains two domains and in the absence of substrate the two domains comprise an open cleft. Upon binding the glucose the two domains come together and "wrap" themselves around the substrates effectively excluding water from the active center. (See V & V, Fig 16-5). As the reaction occurs within this hydrophobic pocket the removal of solvent lowers the dielectric within the active center and enhances both the nucleophilicity of the RO$^-$ and the electrophilicity of the $\gamma$-P. Hexokinase is subject to strong inhibition by G-6-P (end-product inhibition).

**PHOSPHOGLUCOMUTASE** (equivalent to phosphoglycerate mutase to follow; moves the P)

A second important entry point into the glycolytic pathway appears when glycogen is the source of the glucose units—as a result of the action of the enzyme called phosphorylase a; the product of the reaction is glucose–1–P. This G1P is converted into G6P by the enzyme known as phosphoglucomutase. This enzyme contains a bound Pi at the active center and the reaction proceeds by reaction of the $\alpha$-anomer of G1P with the intermediate formation of bound G-1,6-bisP:

$$\text{CH}_2\text{OH} \rightleftharpoons \text{CH}_2\text{OP} \rightleftharpoons \text{CH}_2\text{OP}$$

The reaction sequence is:

$$E\cdot P + G1P \rightleftharpoons E\cdot P.G1P \rightleftharpoons E.G-1,6\text{bisP}$$
$$E.G-1,6\text{bisP} \rightleftharpoons E\cdot P.G6P \rightleftharpoons E\cdot P + G6P$$

i.e. phosphate is transferred from a phosphorylated serine hydroxyl present at the active center of phosphoglucomutase to the incoming sugar to form a bound sugar bisphosphate; this subsequently returns the P to the enzyme. G-1,6-bisP occasionally dissociates to produce an unphosphorylated form of the enzyme which is inactive and this suggest that the reaction actually occurs with dissociation of the G-1,6-bisP (presumably with retention within the active center pocket), reorientation and subsequent rebinding before the sugar has a chance to escape to the solvent (see aconitase, in Ch. 8). Note that the P that ends up at C6 is not the P present at C1 but the P originally present in the enzyme.
**PHOSPHOGLUCOSE ISOMERASE** (hexose phosphate isomerase, formally equivalent to triose phosphate isomerase)

The second enzyme in the standard sequence catalyses an aldose-ketose isomerization (a 1,2 shift); this is the reaction that moves the carbonyl (cf. Lobry de Bruyn-van Eckenstein rearrangement in Ch. II). G6P binds and F6P is released in their respective ring forms with the enzyme catalyzing (using an active center lysine) ring opening and closing of hemi-acetals; thus the reaction actually proceeds with the sugars in the straight-chain form. The essence of the reaction is:

\[
\begin{align*}
\text{D-Glucose-6-P} & \quad \text{[Enediol]} \quad \text{Fructose-6-P} \\
\end{align*}
\]

A basic group (probably histidine) abstracts a proton at C₂ of the glucose with subsequent rearrangement, as shown, to form the enediol (the proton that is removed is α to a carbonyl and is therefore acidic). The enediol then loses a proton and the rearrangement is reversed. By moving the carbonyl in this way we have effected an internal oxidation-reduction with C₁ becoming reduced and C₂ oxidized. (Tritium exchange studies show that protons exchange with the medium which is good evidence for the formation of the carbanion.) The reaction is absolutely stereospecific; the uncatalyzed reaction converts fructose-6-P into glucose-6-P + mannose-6-P.

**PHOSPHOFRUCTOKINASE** - the committing reaction of glycolysis (K is large, k is small).

\[
\begin{align*}
\text{POH}_2\text{C} & \quad \text{CH}_2\text{OH} & \quad + \quad \text{ATP} & \quad \text{POH}_2\text{C} & \quad \text{CH}_2\text{OP} & \quad + \quad \text{ADP} \\
\end{align*}
\]

PFKase catalyses nucleophilic attack by the C₁ hydroxyl of -6P on the γ phosphorus of ATP to yield F–1,6-bisP; the reaction resembles that catalyzed by hexokinase!

This is an extremely important enzyme because it is one of the major control sites for the glycolytic pathway. Consequently it is subject to activation and inhibition by a variety of metabolites:

**Activators:**
- a) ADP ....... a product of the reaction
- b) AMP
- c) F–2,6-bisP – this is the most potent.

**Inhibitors:**
- a) ATP ....... a substrate
- b) citrate ...... conveys information from another metabolic pathway.
At this point you should be saying "Wait a minute! ADP is a product of the reaction—it should be an inhibitor not an activator". If this enzyme were a conventional one then you would be correct. But the enzyme is a member of a class of enzymes that contains both a catalytic center and a regulatory center. Occupation of the regulatory center by the appropriate molecule modifies the behavior of the catalytic center. When ADP is in the regulatory center the catalytic center is made more efficient; when ATP is present it is made less efficient.

In the presence of the inhibitors the plot of velocity versus [F6P] changes from hyperbolic with a small \( K_m \) to sigmoidal with a large \( K_m \). The activators undo this transition. Whether these activity modifiers bind to a common or to separate regulatory sites on the enzyme is not established.

The specific role of these activators/inhibitors will be discussed later this semester when control of carbohydrate metabolism will be presented. However we can note now that when energy demands are high, the cell contains high amounts of ADP and AMP. Large amounts of the latter arise because the demands for energy lead to the reaction

\[
2 \text{ADP} \rightleftharpoons \text{ATP} + \text{AMP} \quad \text{(catalyzed by nucleoside bisphosphate kinase (myokinase))}
\]

A large ratio of ADP over ATP leads to an activation of FDPase. Conversely when energy is plentiful the ratio of ADP/ATP is small and this enzyme is inhibited. [Note that the ADP/ATP ratio is an important control quantity for many enzymes which participate in ATP metabolism].

**FRUCTOSE 1,6 BISPHOSPHATE ALDOLASE (Fig 16-9 of V&V).**

The reaction catalyzed by this enzyme might be considered as the characteristic reaction of glycolysis. This reaction, in which a C-C bond is cleaved, is called an ALDOL cleavage although you should note that it is frequently written in the reverse direction i.e. the condensation of two smaller fragments to yield a longer chain carbon compound \((K=10^{-4})\). This condensation reaction is, in fact, the spontaneous reaction. The aldol condensation/cleavage reaction is common in biochemical pathways although the identity of the reactants and products will differ in detail in the individual cases.
There are two classes of aldolases: In both kinds a cationic center within the enzyme is used to stabilize a carbanion which develops transiently as a catalytic intermediate. In the first class, Type I aldolases, found predominantly in mammals and plants, the reaction proceeds via the formation of a Schiff base between a lysine function at the active center and the carbonyl group of the substrate. There are three important amino acids at the active center, lysine, cysteine, and histidine.

The first step is the condensation of the carbonyl function of FDP with the active-center lysine. This reaction proceeds in several steps:

1a) **Addition** of an amino group (from lysine) to the carbonyl of FDP to form a carbinolamine.
1b) **Elimination** of water (dehydration) from the carbinolamine to yield the ketimine or Schiff’s base.\(^1\)
1c) **Protonation** of the Schiff base to yield the ketimine salt. This step is invoked to rationalize the facile reaction of the Schiff base with borohydride (BH\(^-\)). Normally the electron-rich double bond would be expected to repel this reagent.

\[
\begin{array}{c}
\text{CH}_2\text{OP} \\
\text{C}=\text{O} \\
\text{R}
\end{array} \quad \text{↔} \quad
\begin{array}{c}
\text{CH}_2\text{OP} \\
\text{HO-} \cdot \text{C-NH-} \\
\text{E}
\end{array}
\quad \begin{array}{c}
\text{CH}_2\text{OP} \\
\text{C}=\text{N-} \\
\text{E}
\end{array} \quad \begin{array}{c}
\text{CH}_2\text{OP} \\
\text{C}=\text{NH}^+\text{-} \\
\text{E}
\end{array}
\]

2) Then a base (cysteine) captures a proton 2 carbons away from the Schiffs’ base yielding an alcoholate. The positive charge on the N and the negative charge on the O are conducive to the electron flow shown and electron rearrangement occurs as indicated by the arrows, resulting in cleavage of the C-C bond adjacent to the alcoholate function and elimination of the aldo product, glyceraldehyde-3-P.

3) The carbon fragment remaining attached to the lysine is present as a carbanion: it captures a proton from a nearby acid (a protonated histidine) yielding the ketimine salt which loses the proton on the N to become the ketimine Schiff base adduct of DHAP with the enzyme.

4) This then eliminates the DHAP by reversal of Schiff base formation detailed in step 1, above i.e. it rehydrates and dissociates into free DHAP and enzyme.

**Type II Aldolases:** In the second class, the Type II aldolases, found principally in micro-organisms, the active center contains the metal ion, Zn\(^{+2}\). Thus the Type II aldolases are metalloproteins. The function of the Zn is to stabilize the carbanion that develops during the reaction and possibly to assist in the activation of the electrophilic carbonyl.

The cleavage of F-1,6-bisP introduces a wrinkle in the use of equilibrium constants as covered in Ch. 4 (See BioEnergetics Problem Set (No 11)) showing how composition of mixture can change with concentration).

**Triose Phosphate Isomerase:** (3-carbon analog of phosphohexose isomerase, running in reverse)

The aldolase reaction yields two different products but both products are further metabolized by the same sequence of the glycolytic pathway. However whereas the 3-PGA is metabolized directly, the second product, DHAP, is first converted to G3P prior to further reaction. The enzyme that performs this reaction is called triose

\(^1\) The existence of this sugar-Enzyme Schiff base is well documented. Enzyme is incubated with radioactive DHAP to form the labeled ES complex (DHAP is used because it will not be converted to F-1,6-diP in the absence of the second reactant, 3-PGA). This complex is then treated with sodium borohydride (0 C, pH 6); the borohydride reduces the Schiff base to the secondary amine with the original carbonyl now covalently attached to the original amino group. Subsequent hydrolysis of the protein produces beta-glyceryllysine (the phosphate is lost on acid hydrolysis). Limited hydrolysis followed by peptide mapping and sequencing of the radio-active peptide establishes the point of attachment of the carbonyl to the enzyme: In this case the point of attachment was lys-227 in a chain containing 361 residues. This expt is the standard protocol testing for establishing the participation of a Schiff base in enzyme reactions.
phosphate isomerase (TIM). The reaction is formally equivalent to phosphoglucoisomerase (p. VI-2) the enzyme that converted G6P to F6P, except that (i) the R group is simply CH₂OP and (ii) the reaction proceeds in the reverse direction. The active center base is a carboxylate provided by a glutamic acid.

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**Triose Phosphate Dehydrogenase**

At this point the original 6C sugar has been converted to 2 moles of the 3C aldehyde, G3P. This conversion has consumed 2 moles of ATP and has thus been an energy drain on the cell. The glyceraldehyde-3-P is now oxidized to the corresponding acid. This reaction is one of the best understood examples of so-called substrate level phosphorylation (i.e. the synthesis of ATP which does not occur by means of oxidative phosphorylation). The reaction proceeds in two steps each with its respective enzyme

1) Glyceraldehyde-3-P is oxidized and then phosphorylated to produce 1,3-bisphosphoglyceric acid; a reaction catalyzed by the enzyme *glyceraldehyde-3-P dehydrogenase* (triose phosphate dehydrogenase). The steps of the reaction are:

   a) In the active enzyme a sulfhydryl group is present at the active center tied up in an internal complex with enzyme bound NAD. This SH group adds to the carbonyl of glyceraldehyde-3-P to form an adduct called a thiohemiacetal. Note that this is the site of action of IODOACETATE.

   b) This adduct is then oxidized to the corresponding thioester with concomitant reduction of NAD⁺ to NADH (B side). The thioester so-formed is a high-energy compound. Textbooks are a little misleading at this point because it is stated that the bound NADH does not dissociate but transfers the hydride to NAD⁺ free in solution. The evidence for this is not convincing and it has not been established whether-or-not this bound NADH exchanges for NAD⁺ present in the medium (kinetic evidence for this) or can reduce NAD⁺ present in the medium without dissociating from the enzyme.

   c) This free-energy is preserved by cleavage of the S-acyl enzyme by phosphorolysis i.e. by nucleophilic attack of the oxygen atom of inorganic phosphate. The sulfhydryl group is liberated and the acyl phosphate, 1,3-bisphosphoglycerate, is released.

2) The enzyme *phosphoglycerate kinase* now transfers the P attached to the carboxyl group to ADP. Thus the large amount of chemical free energy available from the oxidation of the aldehyde to the carboxyl has been preserved by the formation of 1 mole of ATP (and also 1 mole of NADH).

1,3-bisphosphoglycerate is a mixed-anhydride (analogous to an acyl halide). The phosphoryl is a good leaving group and readily transferred to ADP.

\[
\begin{align*}
\text{O} & \quad \text{O} \\
C-O-P & \quad C-OH \\
H-C-OH & \quad H-C-OH \\
\text{CH}_2-O-P & \quad \text{CH}_2-O-P \\
& \quad + \text{ADP} \quad + \text{ATP}
\end{align*}
\]

More accurately, the O⁻ on the β-P of ADP attacks as a nucleophile on the P of the carboxyl phosphate. The overall free energy for this sequence of events is quite favorable (ΔG°' = -3.0 kcal/mole). This overall free energy arises from the trade-off between two strongly energetic reactions. The oxidation of the -CHO to the -COOH is very exergonic (ΔG°' = -10.3 kcal/mole). The synthesis of the anhydride bond between the carboxyl carbon and the phosphate group is strongly endergonic (ΔG°' = +11.8 kcal/mole) and would not occur to any measurable extent if it were not coupled to the prior oxidation reaction. The overall ΔG°' is 1.5 kcal/mole for this part of the process. But the free energy expended in synthesizing the phosphoanhydride bond is subsequently recovered when 1,3-bisphosphoglycerate is utilized for ATP synthesis. In summary
Phosphoglycerate Mutase: (3C analog of phosphoglucomutase)

The next two reactions lead to the formation of the second mole of ATP obtained from each phosphoglyceraldehyde. 3-phosphoglycerate is a typical phosphate ester, the phosphate ester bond is not high-energy and cannot be activated. However as we shall soon see the adjacent carbon can be activated and so the next reaction is the conversion of 3-phosphoglyceric acid to 2-phosphoglyceric acid. This reaction is believed to be exactly analogous to the phosphoglucomutase reaction except that, in this case 2,3-bisphosphoglycerate (not G-1,6-bisP) is the relevant intermediate and there is a histidine residue (not serine) present at the active center. The histidine is initially phosphorylated, transfers a P to 3-phosphoglycerate to make 2,3-bisphosphoglycerate and then recaptures the P to yield 2-phosphoglycerate.

Enolase

The unactivated phosphate ester 2-phosphoglycerate is now dehydrated to an enol and trapped in that form as the phosphate ester. This is accomplished by a pair of glutamate acid residues (#'s 168, 211) which function together as a base to abstract the relatively non-acidic proton at C2, plus an active center Mg$^{2+}$ facilitating the process by capturing the hydroxyl at C3 (normally the OH is a poor leaving group). (See Biochemistry, Vol. 30, 2817). As long as this phosphate ester bond exists as the enol it cannot isomerize to the thermodynamically much more stable α-keto-acid tautomer; thus a high-energy enol phosphate is formed. The reaction is an internal redox step produced by the facile dehydration (dehydration followed by rearrangement (after the P has left)). The reaction proceeds by a carbanion intermediate.

Enolase has an absolute requirement for Mg$^{2+}$ which is part of the active center and this is the explanation for its potent inhibition by fluoride (history lecture). It is now known that fluoride reacts spontaneously with phosphate present in the medium to produce fluorophosphate; fluorophosphate has a high affinity for Mg and either removes it from solution thus depleting the Mg in the enzyme, or binds to the Mg at the active center, thus blocking reaction.

Pyruvate Kinase

As before the energy capture step is followed by an enzymatic step whereby the activated phosphoryl group in PEP is transferred downhill to form ATP with the oxygen anion of ADP acting as a nucleophile during the phosphoryl transfer (attacking the P of PEP):

The enzyme pyruvate kinase exhibits an unusually complex response to cations: Cs$^+$, K$^+$, NH$^+$_4 and Rb$^+$ all activate the enzyme whereas Li$^+$ and Na$^+$ both inhibit. $\Delta G^o$ for the reaction is -7.5 kcal/mole; thus the reaction is overwhelmingly in favor of ATP synthesis and for all practical purposes this step in glycolysis is considered to be irreversible. This irreversibility is also apparent in the kinetic parameters. The enzyme has a turnover number (TN) in the forward reaction of 6000 sec$^{-1}$ while that in the reverse reaction is 12 sec$^{-1}$.

Fermentation vs. Glycolysis. The Fate of Pyruvate

It is at this point that the metabolic processes of Fermentation and Muscle Glycolysis diverge. The three products of glycolysis to this point are 2 pyruvate, 2 NADH and 2 ATP. As the cell continues to function it will utilize the ATP and regenerate ADP and Pi. However a mechanism must exist for recycling the NADH, otherwise the depletion of NAD$^+$ would block further reaction. The strategy that is adopted for this purpose is different in yeast and in muscle and is the most striking difference between these two systems, although in both cases the NADH is recycled by the further metabolism of pyruvate.
1) **Muscle**: The NADH is oxidized by reducing the pyruvate to lactate by lactate dehydrogenase (LDH): 

\[
\text{CH}_3\text{COCOOH} + \text{NADH} + \text{H}^+ \Leftrightarrow \text{CH}_3\text{CHOHCOOH} + \text{NAD}^+
\]

The reaction sequence is typical for NAD\(^+\)-linked enzymes (see NAD\(^+\) lecture) although the enzyme has some unusual features.

As detailed by Dr. Olson (lecture #11, Bios 301) LDH is a protein of 140,000 kDa containing 4 subunits. The enzyme occurs in animal tissues as 5 different ISOZYMES (multiple molecular forms) which are readily separable by electrophoresis. The isozyymes arise through different combinations of two different subunits. These subunits are called A (previously designated M, for Muscle) and B (previously designated H for Heart) and differ by virtue of variations in amino acid sequence between residues 298-315 out of a total chain length of 330. (The A subunits have a more negative charge than the B subunits and so move more rapidly towards the anode (+) of the electrophoresis apparatus.) LDH from skeletal muscle is a mixture which is dominated by the tetramer A\(_4\) (M\(_4\)); liver LDH is also M\(_4\). In heart the dominant species is B\(_4\) [A\(_4\) is dominant in tissues that have high glycolytic activity (white, fast twitch) whereas B\(_4\) predominates in tissue with high aerobic activity (pink, slow twitch)]. The B subunits are inhibited by excess pyruvate and only favor rapid formation of lactate at low concentrations of pyruvate; in contrast the A isozymes are much less sensitive to inhibition by high levels of pyruvate and can catalyze the formation of lactate even when pyruvate is high and the cell has a need for energy under anaerobic conditions; see Zubay p.321. In other tissues there are more complex distributions and comparable amounts of all 5 alternative combinations A\(_4\), A\(_3\)B\(_1\), A\(_2\)B\(_2\), A\(_1\)B\(_3\), B\(_4\) are present.

Isozyme analysis is frequently used in medical diagnosis! The LDH isozyme, B\(_4\), which predominates in heart tissue, greatly increases in the blood plasma after a heart attack due to the destruction of some of the heart tissue and consequent release of this cytosolic enzyme into the blood. In liver damage e.g. infectious hepatitis, the blood will contain the isozymes characteristic of the liver with a dominance of the A isozymes A\(_4\) and A\(_3\)B\(_1\).

2) **In YEAST** the NADH is recycled as the result of a two-step mechanism. (Yeast appears not to have LDH though fungi and bacteria do). First of all, the pyruvate is decarboxylated to yield acetaldehyde and CO\(_2\). This reaction is catalyzed by the enzyme pyruvate decarboxylase; this enzyme utilizes a prosthetic group called thiamin pyrophosphate. The discussion of this prosthetic group and the mechanism of this reaction will be postponed for the time being.

Finally the acetaldehyde produced above is reduced to ethanol with the concomitant oxidation of NADH by alcohol dehydrogenase (ADH).

\[
\text{Acetaldehyde} + \text{NADH} + \text{H}^+ \Leftrightarrow \text{Ethanol} + \text{NAD}^+
\]

This dehydrogenase is also a tetramer but in this case the subunits are identical. Each subunit contains two atoms of Zn, one of which is removed from the active center but the other is present at the substrate binding site and serves to enhance the polarization of the C=O function [discussed in detail in section on NAD\(^+\)].

**Energetic Balance**

**1. Muscle**: The overall reaction is

\[
\text{glucose} \Rightarrow 2 \text{ lactate} + 2\text{H}^+ \\
\Delta G^' = 2(-124 - 9.6) - (-218) \\
= -267 + 218 \\
= -49 \text{ kcal/mole}
\]

Note that because the reaction is conducted at pH = 7 we are not at a standard-state for protons (pH = 0) and hence must include the free energy change associated with dilution of the proton (7 x -1.37 = - 9.6 kcal/mole).

VI-8
2 ATP are consumed in converting glucose ⇒ FDP

1 ATP is synthesized in oxidizing glyceraldehyde-3-P to phosphoglyceric acid. This step is occurs twice ⇒ 2 ATP.

Likewise the reaction of PEP to pyruvate occurs twice so that another 2 ATP are generated. The net yield of ATP is 2 and the energy recovered from the overall sequence is 2(-7.6) = -15.2 kcal/mole. The efficiency is thus calculated to be -15.2/(-49) ≈ 30%.

If we had started from glycogen then only 1 ATP would have been expended per mole; the efficiency would then be 3(-7.3)/49 =45%.

2. Yeast:

For glucose ⇒ 2 ethanol + CO₂

the free energy of reaction is -56 kcal/mole. The ATP balance is the same as for muscle, a net yield of 2 ATP so that the efficiency is 2(7.3)/56 = 27%.

Note however that the complete combustion of glucose to CO₂ and H₂O yields 686 kcal of free energy. Thus approximately 600 kcal are still present in the products of glycolysis.

Catabolism of Carbohydrates

Even though we have presented glycolysis as if glucose were the defined starting point, it is perhaps better to think of it as the conceptual starting point because it is very possible to have diets that have little-or-no free glucose, even though glucose is the most abundant monosaccharide. For example one could imagine that the sole source of carbohydrate in the diet is starch, either animal starch (glycogen) or plant starch. Then a necessary preamble to glycolysis is the conversion of the starch to glucose. Both of these storage carbohydrates are polysaccharides with α(1⇒4) linear segments; in addition glycogen and amylopectin contains α(1⇒6) branch points.

Amylose is degraded by the enzyme amylase, an endo-glycosidase which catalyzes random hydrolytic acts at points far from chain ends, thus reducing the polysaccharide in size to maltose and maltotriose. These are then reduced to free glucose by the enzyme maltase. (Amylopectin-the major component of plant starch resembles glycogen in structure; with amylopectin amylase yields a limit dextrin as a third product (see glycogen below); this is converted to glucose by the action of a dextrinase).

The degradation of glycogen is more complex and requires the action of 3 enzyme activities. First the polymers are partially degraded to glucose-1-P by phosphorolysis of the α(1⇒4) bond. The enzyme that catalyses the phosphorolysis of glycogen is called phosphorylase a. It is an exo-glycosidase. The reaction is

\[(\text{glucose})_n + \text{Pi} \Rightarrow (\text{glucose})_{n-1} + \text{G-1-P}\]

Conceptually this reaction is the nucleophilic attack of the Pi upon the C₁ bond of the left-hand monomer unit. This reaction is carried out repeatedly from the outside of the polymer, the enzyme "nibbling" in along the α(1⇒4) chain from left to right. However this enzyme cannot cleave the α(1⇒6) bonds and once the enzyme gets within 3-4 residues of a branch point degradation stops. Further metabolism of this limit dextrin requires the action of a second enzyme which has two catalytic activities.
1) A glycosyl transferase activity moves the last three glucose residues as a unit and transfers them to the end of a second chain. Model building shows that the sugar tails of the limit dextrin are helical and the first glucose of the limit dextrin is physically adjacent to the glucose acceptor ($\Delta G^\circ$ for transfer may be zero).

2) The "naked" 1-6 branch point is now exposed and an $\alpha(1 \Rightarrow 6)$ glucosidase activity (the debranching enzyme) splits out the single residue at the branch point as glucose. Both activities (i.e. (1) and (2)) are part of the same polypeptide (160 kDa).

Phosphorylase can then continue with its exo-glycosidic action.

[Glycogen phosphorylase is under complex control. However discussion of this control will be postponed until the glycogen lectures after mid-term.]

Two other important carbohydrates in the diet are lactose and sucrose. Sucrose is our common kitchen sugar—it is synthesized only in green plants! The metabolism of sucrose begins with the action of the enzyme sucrase (invertase) which hydrolyzes sucrose to its component monosaccharides, glucose and fructose. Glucose enters glycolysis normally but fructose is metabolized in two different ways. In the heart fructose is first converted to F-6-P.

$$F + ATP \leftrightarrow F6P$$

This reaction like the analogous phosphorylation of glucose is catalyzed by hexokinase. The F-6-P is already on the glycolytic path. However in liver the reaction is

$$F + ATP \leftrightarrow F1P$$

catalyzed by fructokinase (liver does not contain hexokinase, rather it has glucokinase and fructokinase). The F-1-P is then cleaved by aldolase to yield DHAP and glyceraldehyde. The glyceraldehyde is then phosphorylated with ATP by glyceraldehyde kinase to yield G3P.

The disaccharide LACTOSE is a major constituent of milk. On hydrolysis by the enzyme, lactase, glucose and galactose are produced. Further metabolism of the galactose is quite complicated. First it is phosphorylated to Gal1P by the enzyme galactokinase:

$$\text{Gal} + \text{ATP} \Rightarrow \text{Gal1P} + \text{ADP}$$

Then the galactose-1-P is converted to glucose-1-P by an inversion of the orientation of the hydroxyl at C$_4$, an epimerisation reaction. This process occurs while the sugar is bound to a nucleotide, uridine diphosphate (UDP). This nucleotide occurs as its glucose complex (UDPG). It is an example of a nucleotide in which the
heterocyclic base is a pyrimidine and the glucose is attached to the pyrophosphate moiety via a phosphodiester link to C₁:

\[
\begin{array}{c}
\text{CH}_2\text{OH} \\
O \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{P} \\
\text{P} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{OH} \\
\text{OH}
\end{array}
\]

UDPG is made by the action of UTP pyrophosphorylase:

\[ \text{G}1\text{P} + \text{UTP} \Rightarrow \text{UDPG} + \text{PP} \]

The uridyl group of UDPG is transferred to galactose-1-phosphate in a transglycosylation reaction catalyzed by phosphogalactose uridyl transferase:

\[ \text{Gal}1\text{P} + \text{UDPglucose} \Rightarrow \text{UDPgalactose} + \text{G}1\text{P} \]

The G1P is then converted to G6P by phosphoglucomutase and enters the glycolytic sequence.

The next step is the epimerisation reaction

\[ \text{UDPgalactose} \leftrightarrow \text{UDPglucose} \]

\[ \text{UDP-glucose epimerase} \]

which regenerates the UDPglucose cofactor. This epimerisation actually proceeds by an oxidation-reduction reaction. The enzyme contains tightly bound NAD⁺ and the first step is the withdrawal of a H⁺ from C₄ to yield a C=O⁺ species. This is planar and addition of the H⁺ from the other side results in the epimerisation.