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Chapter 19

Glycolysis

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Overview of Glycolysis

aka *The Embden-Meyerhoff Pathway*

- First pathway discovered
- Common to almost all living cells
- Occurs in cytoplasm of Eukaryotes
- Overall reaction:

Glucose + 2 NAD^+ + 2 ADP + 2 P_i

↓

2 Pyruvate + 2 NADH + 2 ATP

aka = "also known as"

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Glycolysis Overview, con't.

- Ten reactions overall
- Same in all cells, but relative rates and regulation varies among species and even between tissues (i.e. liver and muscle).
- Intermediates all bound to phosphate
  - Membrane impermeable
  - Important in energy conversion to ATP
- Occurs in two stages (or phases)

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Phases of Glycolysis

- Phase 1 – Preparatory Phase
  - Glucose converted to equilibrium mixture of triose phosphates
  - Investment of 2 ATP's required
- Phase 2 – Energy Yielding Phase
  - Triose phosphates converted to pyruvate
  - An oxidation step occurs
  - Yield of 4 ATP's, two for each triose phosphate

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Reactions of Glycolysis (Summary)

- Overall Pathway (Figure 19.1)
- List of Enzymes (Table 19.1a)
- Energetics of Reactions (Table 19.1b)
- Intermediate structures, first phase
  - (Figure 19.2)

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Phosphorylation of Glucose

- Energetics: "Driven" by ATP hydrolysis

	$\Delta G^{o\prime}$ kJ/mol
glucose + P_i ¾ glucose-6-P + H_2O	13.9
ATP + H_2O ¾ ADP + P_i	-30.5
glucose +ATP ¾ glucose-6-P +ADP	-16.7

- At Q of cell, ΔG can be much larger, -33.9 for erythrocytes, for example (Table 19.1b). The reaction is **removed from equilibrium**.
- (Review discussion of phosphate transfer energetics, Table 3.3, pp.67-69)
- Remember induced fit mechanism, Figure 15.1.

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Hexokinase and Glucokinase

- Phosphorylation "traps" glucose in the cell.
  - Figure 19.4
  - Transport systems vary. Some cells require insulin. Liver is freely permeable to glucose.
- Enzyme in most cells and organisms is hexokinase. Some isozymes exist. Muscle enzyme has Km = 0.1 mM.
- Liver has glucokinase, with Km = 10 mM.

Hexokinases have a broad specificity as the name implies, phosphorylating a variety of hexoses. They are also inhibited by the product, glucose-6-phosphate, presumably a regulatory function that prevents further phosphorylation if there is no demand for the product. Glucokinase is specific for glucose and is not inhibited by glucose-6-phosphate. The Km of glucokinase is near the normal blood concentration, so that the enzyme becomes more active when blood glucose increases, such as after a meal.

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Phosphoglucoisomerase

- aka **glucose phosphate isomerase** and **hexose phosphate isomerase**
- Isomerization of an **aldose** and a **ketose**.
  - (We will later see two more enzymes like this)
- Mechanism involves ring opening and formation of an intermediate enediol (Figure 19.6)
- Reaction is **near equilibrium**.

aka="also known as"

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Phosphofructokinase

- Forms fructose-1,6-bisphosphate
- Energetics similar to hexokinase
- $\Delta G^{o\prime}$ = -14.2 kJ/mol, ΔG slightly larger
  - Reaction is **removed from equilibrium**
- Regulatory, allosteric enzyme
  - ATP and citrate are inhibitors
  - AMP and Fructose-2,6-bisphosphate are activators
  - (Figures 19.8, 19.9, and 19.10)
- Major control point in **Pasteur effect** (inhibition of glycolysis by oxygen).

Regulation by ATP and AMP represents control by energy condition of the cell. When energy levels drop, ATP drops and AMP increases, signaling the need for more energy from glucose breakdown. If there is plenty of citrate as an alternative energy source, however, breakdown of glucose is inhibited. The level of fructose-2,6-bisphosphate is controlled by hormonal stimulation in a complex way we will discuss next term.

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Fructose Bisphosphate Aldolase

- aka **aldolase**
- Catalyzes reverse **aldol condensation**.
  - Figure 19.11
- Class I enzymes form Schiff base intermediates bound to enzyme
- Class II enzymes use metal ions to stabilize the enolate intermediate.
  - See Figure 19.13 for mechanism

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Aldolase, con't.

- One substrate, two products
- Large $\Delta G^{o\prime}$ (+23.9 kJ/mol) is misleading because dilution causes Q to drop rapidly.

At standard states of 1 M, $Q = \frac{[\text{DHAP}][\text{G-3-P}]}{[\text{F-1,6-BP}]} = 1$

But dilute 1000 fold: $Q = \frac{[10^{-3}][10^{-3}]}{[10^{-3}]} = 10^{-3}$

and $RT\ln Q = (8.314 \frac{J}{mol-K})(10^{-3}\frac{kJ}{J})(310\ K)\ln(10^{-3}) = -17.8 \frac{kJ}{mol}$

so reaction is near equilibrium.

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Triose Phosphate Isomerase

- Equilibrates DHAP and G-3-P
  - (Figure 19.14)
- **Aldose** and **Ketose** interconversion
- Enediol intermediate (Figure 19.15)
- Reaction operates near equilibrium
- Ketose favored at equilibrium, but aldose is used for next step, so the effect is to convert hexoses into 2 G-3-P's.
- Two ATP's have been "invested" in Phase I to make the overall conversion spontaneous.

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Phase II of Glycolysis

- Reactions of Phase II (Figure 19.16)
- For each hexose entering glycolysis, two trioses go through the pathway.
- Each triose yields 2 ATP and 1 NADH
  - Total of 4 ATP and 2 NADH for two trioses
  - Overall glycolysis yield of 2 ATP and 2 NADH
- Large free energy of a redox reaction is "captured" by a coupling reaction.
- Two "high energy phosphate" intermediates are formed as donors in ATP synthesis

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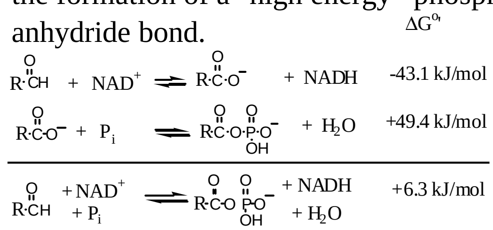
Glyceraldehyde-3-Phosphate Dehydrogenase

- Overall reaction (Figure 19.17)
- Energy of a redox reaction is "coupled" to the formation of a "high energy" phosphate anhydride bond.



Glyceraldehyde-3-phosphate dehydrogenase is also sometimes known as **triose phosphate dehydrogenase.**

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G-3-P Dehydrogenase, con't.

- Mechanism involves covalent catalysis, with formation of an enzyme-bound intermediate. (Figure 19.18)
- Reaction operates near equilibrium
- Regulation by availability of NAD^+
- Arsenate (AsO_4^{3-}) can replace phosphate, but the anhydride is unstable and readily hydrolyzes to form 3-phosphoglycerate.

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Phosphoglycerate Kinase

- Overall reaction (Figure 19.20)
- Free energy of hydrolysis of 1,3-BPG (-43.1 kJ/mol) is conserved as ATP by direct phosphate transfer to ADP.
- Arsenate would cause this energy conservation step to be lost.
- The reaction operates near equilibrium.
  - (Note it is actually named in reverse)
- Regulation by availability of ADP

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Phosphoglycerate Mutase

- An **isomerase** (Figure 19.23)
- Enzyme is phosphorylated as an intermediate step
- Two different mechanisms for different enzyme sources
  - 2,3-BPG is an intermediate in yeast and muscle enzyme. (Figure 19.24)
  - Glyceric acid is an intermediate in wheat germ enzyme. (Figure 19.25)
- Remember 2,3-BPG role in oxygen binding to hemoglobin. It is made from 1,3-BPG (Figure 19.21)

The mutase forming 2,3-BPG is actually the sum of two bimolecular reactions, with 3-phosphoglycerate as an intermediate (See Figure 19.22).

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Enolase

- The reaction (Figure 19.26)
- Reaction is near equilibrium
- But it generates a “high energy” phosphate compound, phosphoenolpyruvate (PEP), which has a $\Delta G^{o\prime}$ of hydrolysis of –62.2 kJ/mol (Recall Table 3.3)
- Inhibited by fluoride ion.

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Pyruvate Kinase

- Catalyzes transfer of phosphate from PEP to ADP. (See Figure 19.27)
- High negative free energy change comes from enol to keto tautomerism .
  - (Figure 19.28)
- Reaction is **removed from equilibrium.**
  - (Overall $\Delta G^{o\prime}$ of –31.7 kJ/mol means this reaction is **completely irreversible**).

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Regulation of Pyruvate Kinase

- Third site of regulation in glycolysis
  - AMP, F-1,6-BP, allosteric activators
  - ATP, acetyl-CoA, alanine, allosteric inhibitors
- Liver enzyme also regulated by covalent modification.
  - Hormone stimulated phosphorylation inactivates the enzyme (preserving PEP for gluconeogenesis).

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Regeneration of ADP and NAD^+

- For glycolysis to continue, there must be a supply of ADP and NAD^+.
  - ATP is utilized in many energy requiring processes in the cell. If the cell is not using energy, ADP will not be regenerated, glycolysis will stop.
  - NAD^+ must be regenerated by an oxidation reaction. If there is no possibility of reoxidation, glycolysis will stop.

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Regeneration of NAD^+

- Two possible ways to regenerate NAD^+
  - Aerobic regeneration
    - Reoxidation by mitochondrial electron transport chain by mechanisms shuttling electrons into the mitochondria (Chapter 21)
  - Anaerobic regeneration
    - Reduction of pyruvate to lactate in muscle by the enzyme **lactate dehydrogenase.**
    - Decarboxylation of pyruvate to acetaldehyde and reduction of acetaldehyde to ethanol in yeast by the enzyme **alcohol dehydrogenase.**

Recall our discussion about the isozymes of lactate dehydrogenase, where different tissues have enzymes with different kinetic properties.

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Lactic Acid Fermentation

- Anaerobic conditions in muscle
- Lactate dehydrogenase catalyzes reduction of pyruvate to lactate, regenerating NAD^+.
- (See Figure 19.30)
  - Remember the isozymes of LDH (page 467)
- Overall reaction becomes:

Glucose + 2 ADP + 2 $P_i \rightarrow$ 2 Lactate + 2 ATP

- Lactate is excreted into the blood and sent to the liver for further metabolism.

Excess accumulation of lactate leads to cramps and muscle fatigue, so anaerobic work cannot be carried on indefinitely.

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Alcoholic Fermentation

- Anaerobic conditions in yeast
- Alcohol dehydrogenase catalyzes reduction of acetaldehyde to ethanol, regenerating NAD^+.
  - (See Figure 19.30)
- Acetaldehyde is formed from pyruvate by decarboxylation.
- **Pyruvate decarboxylase** has thiamine pyrophosphate as a prosthetic group.
- Overall reaction becomes:

Glucose + 2 ADP + 2 $P_i \rightarrow$ 2 ethanol + 2 CO_2 + 2 ATP

We will explore the interaction of pyruvate with the thiamine pyrophosphate prosthetic group in the next chapter when we discuss the enzyme **pyruvate dehydrogenase**.

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Overall Energetics of Glycolysis

- Three steps are far from equilibrium.
  - Hexokinase
  - Phosphofructokinase
  - Pyruvate kinase
    - (See Figure 19.31)

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Fate of Glucose Carbon Atoms

- To interpret isotopic tracer experiments, it is important to understand what happens to each carbon atom of glucose.
- Practice labeling a carbon of glucose and tracing the label through the pathway.

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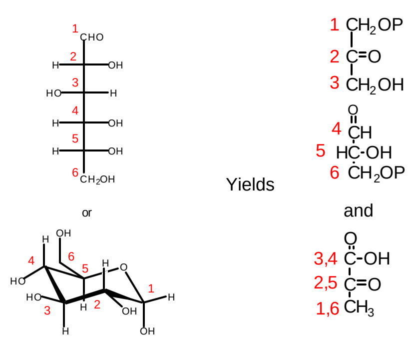
Fate of Glucose Carbon Atoms, con't.



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Other Sugars in Glycolysis

- Mannose is phosphorylated and isomerized to fructose-6-phosphate.
- Fructose is phosphorylated to **fructose-1-phosphate**, which is acted on by a special aldolase. (See Figure 19.32)
  - The regulatory enzyme **PFK** is bypassed.
- Galactose is slightly more complicated.

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Metabolism of Galactose

- Phosphorylation at C-1
- Transfer of UDP from UDP-Glc to form Glc-1-P and UDP-Gal
- Epimerization of UPD-Gal to UDP-Glc
  - See Figure 19.33
- Galactosemia is from a defect in the transferase.
  - Rarer forms of the disease involve defects in galactokinase or the epimerase.

In galactosemia, galactose cannot be metabolized, and ts accumulation causes cataracts, neurological disorders and liver problems. Prevention of the disease consists of removing galactose and lactose from the diet. In adults, another enzyme for activating galactose-1-phosphate with UTP alleviates the problem.

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Metabolism of Glycerol

- Glycerol is formed by hydrolysis of triglycerides.
- Glycerol kinase forms glycerol-3-phosphate
- Glycerol phosphate dehydrogenase converts it to dihydroxyacetone phosphate, a glycolytic intermediate.
  - (See Figure 19.36)