Embden-Meyerhof-Parnas Pathway
a.k.a Glycolysis
Stryer’s 6th edition, chapter 16

and many more…
Hans & Eduard Buchner

- Yeast extract can ferment sugar to alcohol.
- Refutation of the vitalistic dogma (Pasteur).
- Things can happen outside of cells.
- Nobel prize medicine 1907.
Why glucose?

- Is formed in prebiotic conditions from formaldehyde.
- Glucose has a low tendency to glycosylate proteins since the cyclic form is favored.
Glucose phosphorylation

- Traps glucose in the cell
- Destabilizes glucose for future metabolism.

**Kinase**

The trick here is to add the phosphate to the substrate and not to water.

The reason for the difficulty is that water is far more abundant.

**Phosphoglucone isomerase: Aldose \( \rightleftharpoons \) Ketose**
Bis means two separate groups while di means two connected groups (e.g., ADP).

The name of the enzyme comes from the reverse reaction: aldol condensation.
The aldose to ketose conversion

- Without step II, the aldose cleavage would have resulted in two fragments:
  - 2 carbon unit
  - 4 carbon unit
- This would have required two separate pathways.
Rate enhancements of 1010.
- Diffusion limited
  - The reaction is unfavored, but is driven by subsequent steps.

Decomposition is favored 100 to 1.
Acyl phosphate: a mixed anhydride with high phosphoryl transfer potential
Coupling makes sure that this can work.

Hydride transfer

Substrate level phosphorylation
G3P dehydrogenase + PGK

- G3P an Aldehyde is oxidized to carboxylic acid.
- NAD⁺ is reduced to NADH.
- ATP is formed from Pi at the expense of carbon-oxidation energy.

The final 3 steps

- A rearrangement of (3-phosphoglycerate) 3PG to 2PG (2-phosphoglycerate).
- Dehydration of 2PG to an enol
Phosphoglycerate mutase

- The enzyme requires a catalytic amount of 2,3-BPG.
- This maintains an active site His in a phosphorylated form.

\[
\text{Enz-His-phosphate + 3PG} \rightleftharpoons \text{Enz-His-phosphate + 2,3-BPG}
\]

- Then the enzyme acts as a phosphatase:

\[
\text{Enz-His-phosphate + 2,3-BPG} \rightleftharpoons \text{Enz-His-phosphate + 2BPG}
\]

Enolase

- This step is critical in elevating the transfer energy of the enol phosphate:
  - A regular ester phosphate hydrolysis is $-13$ kJ mol$^{-1}$
  - An enol phosphate hydrolysis is $-62$ kJ mol$^{-1}$
- This enables the next step which is another substrate level phosphorylation.

The instability of phosphoenolpyruvate (PEP)

- The phosphate in (PEP) traps the molecule in the unstable enol form.
- Upon hydrolysis the enol can be converted to the much more stable ester.
The outcome

- Two ATP molecules invested
- Four ATP molecules gained
- Net is two ATPs per glucose and two NADHs:

\[
\text{Glucose} + 2\text{Pi} + 2\text{ADP} + 2\text{NAD}^+ \rightarrow 2\text{pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+ + 2\text{H}_2\text{O}
\]
What to do with the NADH?

- The redox balance hasn’t been maintained due to the formation of NADH (activity of GA3P dehydrogenase).
- Since NAD⁺ is limiting it must be regenerated (comes from niacin a vitamin).
- While glycolysis is constant, the fate of pyruvate isn’t.
Fermentations

• In the absence of oxygen the body can extract a small amount of energy out of glucose.
• In bursts of activity the ability to supply oxygen is limited.
• Some organisms cannot live in the presence of oxygen.

TABLE 16.3 Starting and ending points of various fermentations

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>lactate</td>
</tr>
<tr>
<td>Lactate</td>
<td>acetate</td>
</tr>
<tr>
<td>Glucose</td>
<td>ethanol</td>
</tr>
<tr>
<td>Ethanol</td>
<td>acetate</td>
</tr>
<tr>
<td>Arginine</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>Pyrimidines</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>Purines</td>
<td>formate</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>acetate</td>
</tr>
<tr>
<td>Threonine</td>
<td>propionate</td>
</tr>
<tr>
<td>Leucine</td>
<td>2-alkylacetate</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>propionate</td>
</tr>
</tbody>
</table>

Note: The products of some fermentations are the substrates for others.

Pyruvate metabolic pathway schematic.
The NAD⁺ binding pocket is diverse dehydrogenases is very similar pointing to evolutionary relationship

Usage of other sugars is via glycolysis as well
Net reaction:
Galactose + ATP → Glucose 1-phosphate + ADP + H^+
Lactase deficiencies

- During maturity lactase activity is down to 5-10% relative to youth.
- The undigested lactose is fermented by microorganisms in the gut to methane and $\text{H}_2$ with obvious side effects.
- The lactic acid causes water absorption with additional consequences.
Galactosemia

- Deficiency in transferase cause accumulation of galactose.
- This results is severe pathologies, such as clouding of the eye due to the osmotic behavior of galactitol.

![Galactose and Galactitol structures]

Glycolysis regulation

- Glycolysis not only supplies energy but also metabolites, hence it must be tightly regulated.
- The regulation occurs in the three irreversible steps in the pathway:
  - Phosphofructokinase
  - Hexokinase
  - Pyruvate kinase

Phosphofructokinase

- Activated by AMP (Le Chatelier).
- Inhibited by ATP (Le Chatelier).
- The ATP/AMP ratio reflects the energy state of the cell.
- AMP is a better indicator than ADP due to the activity of adenylate kinase:
  - $ADP + ADP \rightleftharpoons AMP + ATP$
- It is also inhibited by low pH that reflects lactic acid accumulation due to anaerobic respiration.
Hexokinase

- Hexokinase is inhibited by its product glucose 6-phosphate.
- When PFK is inactive fructose 6-phosphate is accumulated.
- In turn fructose 6-phosphate is converted into glucose 6-phosphate and inhibits Hexokinase.
- This a communication between 2 enzymes.
**PFK versus Hexokinase**

• PFK is the most important regulatory point.
• The reason is that glucose 6-phosphate can be used to synthesize glycogen as well.
• So the activity of PFK represents the 1st committed step in the catabolic pathway of glycolysis.

**Pyruvate Kinase (PK)**

• Inhibited by ATP allosterically.
• Alanine inhibits the enzyme as well to signal that there is enough building blocks.
• Fructose 1,6-bisphosphate allosterically activates PK to make sure that it “keeps up” with the rest of the pathway.
The liver

- The liver is different from the rest of the body since it controls the blood glucose levels.
- It can store glucose as glycogen and release glucose into the blood stream when [glucose] falls.
- So it has additional interesting pathways and regulatory strategies.

PFK in the liver

- Not inhibited by lowering the pH since lactate doesn’t accumulate in the liver (it is converted back to glucose).
- PFK is inhibited by citrate a key intermediate in the Krebs cycle thru enhancing the inhibitory effects of ATP.
- High levels of citrate mean that there is plenty of metabolites.
PFK and glucose in the liver

- An increase in [glucose] results in an increase in [fructose 6P].
- This in turn results in an increase in [fructose-2,6-BP].
- F-2,6-BP increases the affinity of PFK to F-6P and diminishes the effect of ATP.
- Thus glycolysis is accelerated when glucose is abundant.
- This is called feed-forward stimulation.

Remember that ATP is both a substrate and an allosteric inhibitor!

Liver Hexokinase

- Most enzymes are highly tuned towards their substrates, having a very low $K_m$.
- Since often $[S] > K_m$, a change in substrate concentration does not change the reaction rate appreciably.
- Thus, controlling a metabolic flux is not normally achieved by varying substrate concentrations.
- A notable exception is glucokinase (Hexokinase IV) in the liver which has a very high $K_m$ which is roughly comparable to blood glucose concentration.
- This enzyme was thought to be the glucose sensor in the body.
Pyruvate kinase in the liver

- There are several PK isozymes.
- L-PK isozyme behaves like M-PK except for the fact that it is inhibited by phosphorylation which is a result of glucagon stimulation (see later on).
- This prevents the liver from consuming glucose when concentration of glucose is low and it is needed in the brain.

Remember the blood [glucose] is 4-8 mM

<table>
<thead>
<tr>
<th>Name</th>
<th>Tissue Location</th>
<th>K_M</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT1</td>
<td>All mammalian tissues</td>
<td>1 mM</td>
<td>Basal glucose uptake</td>
</tr>
<tr>
<td>GLUT2</td>
<td>Liver and pancreatic β cells</td>
<td>15-20 mM</td>
<td>In the pancreas, play a role in the regulation of insulin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>In the liver: removes excess glucose from the blood</td>
</tr>
<tr>
<td>GLUT3</td>
<td>All mammalian tissues</td>
<td>1 mM</td>
<td>Basal glucose uptake</td>
</tr>
<tr>
<td>GLUT4</td>
<td>Muscle and fat cells</td>
<td>5 mM</td>
<td>Amount in muscle plasma membranes</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLUT5</td>
<td>Small intestine</td>
<td>---</td>
<td>Increases with endurance training</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Primarily a fructose transporter</td>
</tr>
</tbody>
</table>
Glycolysis and cancer

- Tumors develop faster than the ability of blood vessels to support them.
- Hence they are in the state of hypoxia – oxygen deprivation.
- So they rely on glycolysis for energy.
- In hypoxia the transcription factor HIF-1 induces many enzymes in glycolysis.

TABLE 16.5 Proteins in glucose metabolism encoded by genes regulated by hypoxia-inducible factor

<table>
<thead>
<tr>
<th>GLUT1</th>
<th>GLUT3</th>
<th>Hexokinase</th>
<th>Phosphofructokinase</th>
<th>Aldolase</th>
<th>Glyceroldehyde 3-phosphate dehydrogenase</th>
<th>Phosphoglycerate kinase</th>
<th>Enolase</th>
<th>Pyruvate kinase</th>
</tr>
</thead>
</table>

Gluconeogenesis

- Synthesis of glucose from non-carbohydrate sources.
- Glucose in the primary energy source of the brain and the only one for red blood cells.
- Daily glucose requirement of the body is 160gr, of which 120gr is for the brain.
- In the blood we have about 20gr and in glycogen another 190gr.
- So gluconeogenesis is needed at times of starvation.
- Most gluconeogenesis takes place in the liver and a small amount in the kidney.
Gluconeogenesis

• Conversion of metabolites to pyruvate and then to glucose.
• Other metabolites may enter at a later stages.
• The major inputs are:
  – lactate from glycolysis (lactate dehydrogenase)
  – Amino acid from protein breakdown during starvation.
  – Glycerol from lipid breakdown (see later).
• Note that fat cannot be used to make glucose in mammals.

Gluconeogenesis is not the reverse of glycolysis. (unique reactions are shown in red).
The reason is the the ΔG of Glycolysis is ~84 kJ/mol so it is essentially irreversible.
The “essentially” irreversible steps are in red again

<table>
<thead>
<tr>
<th>Glycolytic reaction step</th>
<th>$\Delta G^\circ$ (kJ/mol)</th>
<th>$\Delta S$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Glucose $\rightarrow$ GP $\rightarrow$ glucose 1-phosphate $\rightarrow$ 6-phosphate</td>
<td>$-10.7$</td>
<td>$-33.4$</td>
</tr>
<tr>
<td>2. Fructose 6-phosphate $\rightarrow$ fructose 1,6-bisphosphate</td>
<td>$-14.2$</td>
<td>$-22.2$</td>
</tr>
<tr>
<td>3. Fructose 1,6-bisphosphate + glucose-6-phosphate $\rightarrow$ glyceraldehyde 3-phosphate</td>
<td>$23.8$</td>
<td>$0 \times -1$</td>
</tr>
<tr>
<td>4. Glyceraldehyde 3-phosphate $\rightarrow$ glyceraldehyde 3-phosphate</td>
<td>$17.3$</td>
<td>$0 \times 4$</td>
</tr>
<tr>
<td>5. Fructose 1,6-bisphosphate $\rightarrow$ fructose 1,6-bisphosphate</td>
<td>$-8.5$</td>
<td>$-2 \times 2$</td>
</tr>
<tr>
<td>6. Fructose 1,6-bisphosphate + ATP $\rightarrow$ 1,2-bisphosphoglycerate + Pi</td>
<td>$-18.8$</td>
<td>$0 \times 2$</td>
</tr>
<tr>
<td>7. 3-Phosphoglycerate $\rightarrow$ 2-phosphoglycerate</td>
<td>$6.4$</td>
<td>$0 \times 0.8$</td>
</tr>
<tr>
<td>8. 3-Phosphoglycerate $\rightarrow$ 3-phosphoglycerate $\rightarrow$ 2-phosphoglycerate + Pi</td>
<td>$7.3$</td>
<td>$0 \times 3.5$</td>
</tr>
<tr>
<td>9. 3-Phosphoglycerate $\rightarrow$ 3-phosphoglycerate $\rightarrow$ G6P</td>
<td>$-31.4$</td>
<td>$-16.7$</td>
</tr>
</tbody>
</table>

Steps 1 and 3: “simple” hydrolysis by phosphatases

Fructose 1,6-bisphosphate + H$_2$O $\rightarrow$ fructose 6-phosphate + P$_i$

$\Delta G^\circ = -16.3$ kJ/mol

Glucose 6-phosphate + H$_2$O $\rightarrow$ glucose + P$_i$

$\Delta G^\circ = -13.8$ kJ/mol

Note that 5 proteins are required to Make glucose form G6P
Step 10: Forming PEP from pyruvate in 2 steps. The first step takes place in the mitochondria by pyruvate carboxylase, a biotin dependent enzyme.
We will cover the malate-aspartate shuttle later in the course.

The second step takes place in the cytoplasm by phosphoenolpyruvate carboxykinase. Note that the CO₂ is now taken off. Why was it added in the first place? The decarboxylation drives the reaction.

The cost of gluconeogenesis

- 6 molecules of ATP are needed to make glucose from pyruvate
- Versus only 2 that you get from glycolysis.
Reciprocal regulation

Glycolysis versus gluconeogenesis in the liver

- F 2,6-BP stimulates PFK and inhibits F 1,6 bisphosphatase.
- When [glucose] is low F 2,6-BP loses a phosphate to form F 6P which no longer binds to PFK.
- 2 enzymes control this reaction:
  - One that phosphorylates F 6P
  - One that hydrolyses F 2,6-BP

They are both on the same gene!
Phosphofructokinase 2 + fructose bisphosphatase 2
Phosphorylation of a single Ser when [glucose] is scarce
Activates the phosphatase and inhibits the kinase.
Stimulation via gene expression
Of insulin response elements and glucagon response elements

Substrate cycles for heat and regulation
Discussed previously
Evolution

- Glycolysis and gluconeogenesis deal with 6 and 3 carbon sugars.
- The enzymes that deal with trioses are very conserved and seem to have arisen very early in evolution.
- It can be used to generate riboses as well.
- So was the origin catabolic or anabolic?