**Glycolysis - Enzyme mechanisms**

4. Regulating a metabolic pathway

**Control of flux**
Metabolic flux = amount of metabolites going through a pathway per unit time

Flux through glycolysis varies by >100-fold in muscle depending on the need for ATP

**Maintaining homeostasis**
Levels of the glycolytic intermediates hardly change despite large changes in flux

What determines flux and homeostasis? THERMODYNAMICS

<table>
<thead>
<tr>
<th>enzyme</th>
<th>$\Delta G^\circ$ kcal/mol</th>
<th>$\Delta G$ kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>hexokinase</td>
<td>-4.0</td>
<td>-8.0</td>
</tr>
<tr>
<td>glucose 6-phosphate isomerase</td>
<td>+0.4</td>
<td>near equilibrium$^1$</td>
</tr>
<tr>
<td>phosphofructokinase</td>
<td>-3.4</td>
<td>-5.3</td>
</tr>
<tr>
<td>aldolase</td>
<td>+5.7</td>
<td>near equilibrium</td>
</tr>
<tr>
<td>triose phosphate isomerase</td>
<td>+1.8</td>
<td>near equilibrium</td>
</tr>
<tr>
<td>glyceraldehyde 3-phosphate dehydrogenase</td>
<td>+1.5</td>
<td>near equilibrium</td>
</tr>
<tr>
<td>phosphoglycerate kinase</td>
<td>-4.5</td>
<td>near equilibrium</td>
</tr>
<tr>
<td>phosphoglycerate mutase</td>
<td>+1.1</td>
<td>near equilibrium</td>
</tr>
<tr>
<td>enolase</td>
<td>+0.4</td>
<td>near equilibrium</td>
</tr>
<tr>
<td>pyruvate kinase</td>
<td>-7.5</td>
<td>-4.0</td>
</tr>
</tbody>
</table>

$^1$near equilibrium means that $\Delta G$ is about zero.
Glycolysis - Enzyme mechanisms

4. Regulating a metabolic pathway
Pictorial analogy: water represents flux of metabolites, amount of water in flask represents amount of a particular intermediate, pipes between flasks are enzymes, vertical drop represents decrease in free energy

\[ \Delta G^\circ = \text{height difference between flask bottoms} \]
\[ \Delta G = \text{height difference between water levels} \]
Glycolysis - Enzyme mechanisms

4. Regulating a metabolic pathway

Control of flux
What limits flux through glycolysis?

A. Supply of accessible glucose
Analogy: If less water is input than the system can carry, the amount of input will dictate the amount of flux

B. Capacity of glycolytic enzymes to process glc
Analogy: Nonequilibrium rxns are shown as skinny pipes that can limit flow through the system

How regulate activity of nonequilibrium enzymes?
• Allosteric effectors
• Modifications (phosphorylation)
• Altering amount of protein
Glycolysis - Enzyme mechanisms

4. Regulating a metabolic pathway
Maintaining homeostasis

A. Requires coordinately regulating all nonequilibrium rxns
Analogy: narrowing just one skinny pipe causes water to back up in the system, narrowing all three does not

B. Intermediates must remain at a constant level
Glycolytic intermediates also involved in “feeding” other pathways, altering levels interferes with control of other pathways
Glycolysis - Enzyme mechanisms

4. Regulating a metabolic pathway
   Maintaining homeostasis
   Glycolytic intermediates are fed in from other pathways
Glycolysis - Enzyme mechanisms

4. Regulating a metabolic pathway
Maintaining homeostasis
C. Accumulating certain metabolites can have severe consequences

Example: galactosemia
Symptoms: vomiting, diarrhea, jaundice, mental retardation...
Caused by: accumulation of toxic substances.
this enzyme absent

lactose → glucose + galactose

ATP → ADP

galactose 1-phosphate

×

glucose 6-phosphate

glycolysis

Galactitol accumulates in the lens, water enters, cataracts develop

galactose

galactitol

Treatment: don’t drink milk!
Many other examples: phenylketonuria, gout, .... understanding metabolism leads to rational treatments
Glycolysis - Regulation

3 non-equilibrium reactions that limit flux
All three must be coordinately regulated to control flux and maintain homeostasis
Glycolysis - Regulation

5. Regulating glycolysis
A. Regulation by cellular energy charge
B. Regulation by hormone
C. Regulation to coordinate glycolysis with other pathways
5. Regulating glycolysis

A. Regulation by cellular energy charge

Flux of glycolysis increases when \([\text{ATP}]\) is needed

ATP, ADP, and AMP are interconverted

\[
\text{ATP} + \text{AMP} \rightleftharpoons 2 \text{ADP}
\]

ATP and AMP allosterically regulate phosphofructokinase-1
High physiological \([\text{ATP}]\) ~ completely blocks PFK-1
Inhibition relieved by \([\text{AMP}]\)
\([\text{AMP}] / [\text{ATP}]\) controls PFK-1 activity

Energy charge affects the \(K_m\) of PFK-1 for its substrate (F6P)
5. Regulating glycolysis

A. Regulation by cellular energy charge

Energy charge affects the $K_m$ of PFK-1 for F6P

PFK-1 is an allosteric enzyme, allosteric effectors bind remote from active site and shift equilibrium between high and low affinity conformations.
Glycolysis - Regulation

5. Regulating glycolysis

A. Regulation by cellular energy charge
What about homeostasis?
Hexokinase and pyruvate kinase are regulated to match PFK-1

Hexokinase is **feedback inhibited** by G6P
Pyruvate kinase is **feed-forward activated** by F1,6-BP
Glycolysis - Regulation

5. Regulating glycolysis

A. Regulation by cellular energy charge

Hexokinase versus Glucokinase (isozymes)

HK - in most cells
- has a high affinity for glc ($K_m = 0.1 \text{ mM}$)
- allosterically inhibited by its product, G6P

GK - in liver
- affinity for glc ($K_m = 10 \text{ mM}$, blood [glc] = 5 mM)
- efficient glc transporters maintain [glc] in liver at 5 mM
- enzyme regulated by level of blood glc
- after meals blood glc high, excess blood glc moved to liver
- inhibited by F6P
Glycolysis - Regulation

5. Regulating glycolysis

A. Regulation by cellular energy charge
Why use PFK as the control point?

PFK is the first “committed step” - first nonequilibrium enzyme not also in other pathways
Glycolysis - Regulation

5. Regulating glycolysis

B. Regulation by hormone (glucagon)

Glucagon -

Glucagon inhibits PFK-2
PKF-2 is an allosteric activator of PFK-1 and it synthesizes F-2,6-BP
When glucagon is around, it binds its receptor on cell surface, activates a protein kinase that phosphorylates and inactivates PFK-2
5. Regulating glycolysis

C. Regulation to coordinate glycolysis with other pathways
Glycolysis - Regulation

Summary of Glycolysis Regulation

Insulin \textit{(muscle, fat)} \rightarrow (+) Glucose

Hexokinase $\xrightarrow{(-)}$

Glucose 6-phosphate

\textit{Glucagon} \textit{(liver)}

Fructose 6-phosphate

Phosphofructokinase-1

AMP $\rightarrow (+)$ \quad ATP $\xleftarrow{-}$ Citrate

Fructose 2,6-bisphosphate $\rightarrow (+)$

$\xrightarrow{-}$

Fructose 1,6-bisphosphate

$\xrightarrow{-}$

Phosphoenolpyruvate

Pyruvate kinase $\xrightarrow{(-)}$ ATP $\xleftarrow{-}$

This is a somewhat simplified view.
Glycolysis - Regulation

What about NAD+? Why don’t we run out of it?

Further metabolism:
NADH reduces O_2
regenerating NAD^+