• Bio-molecular Structure/Function (I cont’d)
  — NUCLEIC ACID
    • DNA sequence and structure
    • Protein/nucleic acid interactions
  — CARBOHYDRATES
    • Sugars - mono and disaccharides
    • Polysaccharides
    • Glycerides and glycerol
  — FATS AND LIPIDS
    • Chemistry and nomenclature for fatty acids
    • Saturated and unsaturated fatty acids
    • Fluid mosaic model of membrane structure
BioChem 330 - Course Outline

• Metabolism and Bioenergetics (II)
  – ENZYME CATALYSIS:
    • kinetic constants $k_{cat}$, $K_m$
    • Catalytic strategies, the serine proteases

  – CATABOLISM (breakdown)
    • Carbohydrates
      – Glycolysis
      – Tricarboxylic Acid Cycle
      – Electron Transport
      – Chemiosmosis and ATPase

• Fatty acids and amino acids
Intro to Metabolism
  - ATP, the energy currency of the cell
  - sugar structure

Glycolysis Phase I
  - gly 1-5

Glycolysis Phase II
  - gly 6-10

Control in Glycolysis
Metabolism

• Catabolism
  – Degradation reactions
  – More complex to simpler molecules

• Anabolism
  – Biosynthesis
  – Less complex to more complex molecules

• Entropy:
  – Catabolism is favorable, and should have $\Delta G^o' < 0$
  – Anabolism is unfavorable, and should have $\Delta G^o' > 0$
Over 1000 different reactions are being catalyzed in an *e.coli* cell, we’ll focus on about two dozen of them which form the blue central line of carbohydrate metabolism.

http://www.genome.jp/kegg/
Why must a living organism metabolize nutrients such as fats, proteins, carbohydrates?

ANS: To generate ATP, the fuel that drives all cellular events

\[
\text{ATP}^-4 + \text{H}_2\text{O} \rightarrow \text{ADP}^-3 + \text{PO}_4^-3 + 2\text{H}^+ \quad \Delta G^o' = -30.5 \text{ kJ/mole}
\]

ATP hydrolysis liberates energy making it available for biological processes that require energy.
Phosphate Hydrolysis Potential Energy

- Molecules higher in the table can transfer a phosphate group to other molecules below them (so net $\Delta G^o' < 0$).
- A reaction far away from its equilibrium ratio of product/reactant and from the standard state can have additional free energy.
- In the case of ATP hydrolysis, cells can be many orders of magnitude away from equilibrium and are certainly NOT at the standard state. How does this affect the free energy available from hydrolysis?

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\Delta G^o'$ (kJ·mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphoenolpyruvate</td>
<td>$-61.9$</td>
</tr>
<tr>
<td>1,3-Bisphosphoglycerate</td>
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<td>$-43.1$</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>$-43.1$</td>
</tr>
<tr>
<td>PPi</td>
<td>$-33.5$</td>
</tr>
<tr>
<td>ATP ($\Rightarrow$ AMP + PPi)</td>
<td>$-32.2$</td>
</tr>
<tr>
<td>ATP ($\Rightarrow$ ADP + P$_i$)</td>
<td>$-30.5$</td>
</tr>
<tr>
<td>Glucose-1-phosphate</td>
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</tr>
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<td>Fructose-6-phosphate</td>
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</tr>
<tr>
<td>Glycerol-3-phosphate</td>
<td>$-9.2$</td>
</tr>
</tbody>
</table>

Which phosphate bonds in ATP and ADP are “high-energy” bonds?

- Breakdown of O-P phosphoanhydride bond at Pγ of ATP produces ADP and Pi and energy.
- Breakdown of O-P phosphoanhydride bond at Pβ of ADP produces AMP and Pi and energy.
- Breakdown of O-P phosphoanhydride bond at Pβ from ATP produced PPi and AMP and energy and then some.
Why is the phosphoanhydride bond a “high-energy” bond?

- LOTS of energy is released due to
  - destabilization of reactants (electrostatic repulsion due to multiple negative charges)
  - stabilization of products (two resonance forms rather than 1 plus products are more easily hydrated b/c charge is more spread out)

Destabilized by electrostatic repulsion of negative charges at O⁻

:OH₂ attacks at Pγ or Pβ

better resonance stabilization
Energetics of making ATP

- \( \text{ADP} + \text{PO}_4^{-3} + \text{H}^+ = \text{ATP} + \text{H}_2\text{O} \quad \Delta G^\circ' = +30.5 \text{ kJ/mole} \)

It takes 30.5 kJ to make one mole of ATP under biological standard state conditions (1 M reactants and products and pH 7.0 295 K). At equilibrium..........

\[ K' = \exp\left(-\Delta G^\circ'/RT\right) \]

\[ = 5 \times 10^{-6} \text{ M}^{-1} \]

\[ = [\text{Prod}] / [\text{Reactants}] \]

\[ = [\text{ATP}] / [\text{ADP}][P_i] \]

\[ = 1/200,000 \]

But how far are cells away from equilibrium with regard to the synthesis of ATP?
Energetics of making ATP under cellular conditions

• Cells are far away from equilibrium and far away from standard state conditions. We have much more ATP than would be dictated by equilibrium; the ratio of ATP to ADP+P_i in some cells is as high as 200/1 rather than 1/200,000.

• This means that a cell can be far from equilibrium w.r. to this ratio, and now, through metabolism, we are going to make EVEN MORE ATP.

• Under these conditions, thermodynamics wants the system instead to hydrolyze the ATP and reach equilibrium, but instead, metabolic reactions will need to fight equilibrium; let’s calculate exactly how much more energy it will take to make this ATP:

\[ \text{ADP} + \text{PO}_4^{-3} + \text{H}^+ \nleftrightarrow \text{ATP} + \text{H}_2\text{O} \]

\[ \Delta G = ? \]
Energetics of making ATP under cellular conditions

• \( \text{ADP} + \text{PO}_4^{-3} + \text{H}^+ \not\rightarrow \text{ATP} + \text{H}_2\text{O} \) \( \Delta G = ? \)

To calculate this energy, remember from thermodynamics:

\[
\Delta G = \Delta G^o' + RT \ln Q^*
\]

(*At equilibrium, this equation simplifies to the more familiar eqn. \( \Delta G^o' = -RT \ln K \) because \( \Delta G = 0 \))

Where \( \Delta G \) is the free energy available to the system not at equilibrium, and \( Q \) is the mass action ratio:

\( Q = \frac{[\text{Products}]}{[\text{Reactants}]} \) when a cell is away from equilibrium....

so what is this value in an average cell?
Energetics of making ATP under cellular conditions

- \( \text{ADP} + \text{PO}_4^{-3} + \text{H}^+ \not\xrightarrow{} \text{ATP} + \text{H}_2\text{O} \) \( \Delta G = ? \)

In human erythrocytes for example:

\[ Q = \frac{[\text{ATP}]}{[\text{ADP}][\text{P}_i]} \]

\[ = \frac{2.25 \times 10^{-3}}{(0.25 \times 10^{-3} \times 1.65 \times 10^{-3})} = 5.45 \times 10^{3} \text{ M}^{-1} \]

\[ \Delta G = \Delta G^0' + RT \ln Q \]

\[ = 30.5 \text{ kJ/mole} + 21 \text{ kJ/mole} \]

\[ = +51.5 \text{ kJ/mole} \]
Overview Of Glycolysis

- **Stage I**
  - Uses 2 ATP’s per glucose
- **Stage II**
  - Generates 4 ATP’s
Glycolysis: a biochemical analogue of the movie “Roots”

\[
\text{Glucose} + 2\text{P}_i + 2\text{ADP} + 2\text{NAD}^+ = 2\text{pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+ + 2\text{H}_2\text{O}
\]

\[\Delta G^o = -85 \text{ kJ/mole}\]

(Note above NET reaction of glycolysis book keeps important biological molecules but is not charge balanced)

Phase I, Priming

Phase II, Cashing In
Phase I

**Glucose + 2 ATP = 2 GAP + 2 ADP**

**Priming the Pump in five steps**

* This phase of glycolysis requires the investment of two molecules of ATP.

* Gly3, PFK, is the committed step for glycolysis.

* No oxidation and no involvement of molecular oxygen.
Gly 1 hexokinase: first ATP utilization

ATP + Glucose → Glucose-6-phosphate + ADP

* ATP is the second substrate, ADP the second product.

* Reaction type is phosphoryl shift: hexokinase catalyzes the transfer of the \( \text{g} \) phosphate group from ATP to glucose, C6 OH acting as a nucleophile attacks the P of terminal phosphate.

\[ \Delta G^\circ' = -16.7 \text{ kJ/mole} \]

What about the sugar cyclization?

What about the transition state?

What about metals?
Gly 1 hexokinase, glucokinase:

* Hexokinase shows multisubstrate binding, product inhibition, and induced fit.

* $K_m$ glucose is 0.1 mM, in erythrocytes $[\text{glucose}] = 5$ mM
  
  \[ K_m/S \text{ is } <<1, \text{ enzyme is saturated with glucose and operating at } V_{\text{max}} \]

* $K_m$ ATP is 4.0 mM in the absence of glucose --- weak binding
  
  \[ K_m \text{ ATP is } 0.1 \text{ mM in the presence of glucose --- tighter binding} \]

Glucose binding increases affinity of enzyme 40 fold for ATP

In liver, glucokinase is an isozyme of hexokinase, only binds glucose, only activates at higher glucose levels and doesn’t show product inhibition, precedes storage of glucose as glycogen, a process unique to liver and muscle cells.

All $K_m, S$ values throughout this handout are taken from Enzyme Structure and Mechanism by Alan Fersht, p256
Gly 1 hexokinase: Induced Fit, Hinge Motion upon Binding

Example of Induced Fit: Protein folds over substrates to exclude water, and catalyze reaction by proximity and orientation. Two lobes rotate by 12° resulting in relative movements of 8Å. Ligands that bind but do not induce conformational change are not substrates. Product dissociation is quite slow and rate limiting.
Substrates bind at domain interface

Hinge motion of protein domains: from Gerstein’s Lab at Yale
http://bioinfo.mbb.yale.edu/MolMovDB/
Steitz, Shoham, Bennett Jr.: Structural dynamics of yeast hexokinase during catalysis.

Image of hexokinase, PDB coordinates 2e2n.pdb vs 2e2q.pdb are morphed to represent the motion in the protein upon binding.
Gly 2 phosphoglucoisomerase

* Rxn type is isomerization: gly2 catalyzes the conversion of aldo sugar to keto sugar.

* ΔG’ about zero, reaction is almost at equilibrium

\[ \Delta G^{o'} = +1.7 \text{ kJ/mole} \]
Mechanism of Gly 2: Phosphoglucoisomerase
Mechanism of Gly 2: Phosphoglucone Isomerase

What would the free energy profile for this reaction look like?

Assume: \( K_m = 100 \text{ uM} \)
\( k_{\text{cat}} = \text{product dissociation} = 10^3 \text{s}^{-1} \)

Activation Barrier = \( \Delta G^t = [17.6 - 1.36 \log k_{\text{cat}}] \)

Binding Energy = \( \Delta G^o = -RT \ln(1/K_m) = -5 \text{ kcal} \)
Mechanism of Gly 2: Phosphoglucone Isomerase

What would the free energy profile for this reaction look like?

Assume: $K_m = 100 \text{ uM}$

$k_{\text{cat}} = \text{product dissociation} = 10^3 s^{-1}$

Free energy profile: $E + S \rightleftharpoons E_S c \rightleftharpoons E_S o \rightleftharpoons E I \rightleftharpoons E P_o \rightleftharpoons E P_c \rightarrow E + P$

$\Delta G^t = [17.6 - 1.36 \log k_{\text{cat}}]$  
$\Delta G^t = +12.5 \text{ kcal/mole}$  
$\Delta G^t = +50.0 \text{ kJ/mole}$

$\Delta G = \text{Binding Energy} = -RT\ln(1/K_m) = -5 \text{ kcal}$
Gly 2 Mechanism: Enolate formation

- Enol formation

- Enolate formation

Base

Acid
2.0 Å resolution structure of a dimer from rabbit phosphogluco-isomerase with 2 f6p, one bound in center of each monomer.
Committed (irreversible) step of glycolysis

$\Delta G^\circ = -14.2 \text{ kJ/mole}$

**highly regulated:** + regulators: Pi, AMP, - regulators: ATP, fatty acids, citrate, H+  also deinhibitors of ATP inhibition: F2-6BP (later)
(PFK1) Glycolysis: "Ribbon diagram of Phospho-Fructokinase, Gly-3. PFK catalyzes the conversion of F6P to F(1,6)BP. Here, the substrate F6P is shown bound at the center. A Mg(II) ion and ADP product molecule are adjacent to F6P. At the other end of the molecule, ADP is bound at a regulatory site."
\[ \Delta G^\circ = +23.8 \text{ kJ/mole} \]
The backward reaction (condensation) is spontaneous.
Important Themes in Gly 4

1. Schiff Base Linkage:
   - Active site Lysine
   - Covalently binds the substrate through a Schiff base linkage.
   - Activates substrate for bond cleavage by acting as an electron sink for negative charge that would otherwise build up on C3
2. Base/Acid Chemistry

- Tyrosinate at active site initiates C-C bond cleavage by removing a proton from alcohol on C4
- Aldehyde forms on C4, breaking C3-C4 bond
- P$_1$-GAP, leaves
- Tyrosine can donate proton back to C3
- Hydrolysis of bound enamine generated DHAP-P$_2$. (O$^{18}$ water, incorporated into DHAP)
Gly 4: Aldols, and Enols and Enamines
Oh, My!

- Enamine formation

Lysine

SHIFF BASE FORMATION

- General Aldol Condensation (Reverse Reaction)
Gly 4 Aldolase Substrate binding

<table>
<thead>
<tr>
<th>Muscle cells</th>
<th>$K_m$</th>
<th>$S$</th>
<th>$K_m/S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[FBP]</td>
<td>100 uM</td>
<td>32 uM</td>
<td>3.1</td>
</tr>
<tr>
<td>[G3P]</td>
<td>1000 uM</td>
<td>3 uM</td>
<td>333</td>
</tr>
<tr>
<td>[DHAP]</td>
<td>2000 uM</td>
<td>50 uM</td>
<td>40</td>
</tr>
</tbody>
</table>

* FBP binds best, $K_m/S$ shows about $1/2 \ V_{max}$
* DHAP, G3P 10X weaker binding, $K_m/S$ shows not at all maximized for this direction.
* Muscle cell has very little of G3P, this pulls the reaction forward.
Title Fructose 1,6-Bisphosphate Aldolase From Rabbit Muscle
Classification Lyase Compound
Mol_Id: 1; Molecule: Aldolase; Chain: A, B, C, D; Ec: 4.1.2.13;
Biological_Unit: Tetramer
Exp. Method X-ray Diffraction

Active site with Lys41 shown in blue, substrate analogue spacefilled and cpk colored
Gly 4 Aldolase 1ADO

Active site Tyr 301 shown spacefilled in green
Gly 5 Triose Phosphate Isomerase TIM

\[ \Delta G^o' \text{ ketone to aldehyde} = +7.5 \text{ kJ/mole} \quad \text{prod/react} = 1/20 \]
Gly 5 TIM Mechanism

- Enol formation requires both protonation and deprotonation
- Two groups involved in proton shuttles: Glu-165 and His-95.
Gly 5 TIM Kinetics for DHAP

- $k_{\text{cat}} = 2.0 \times 10^5 \text{ s}^{-1}$ ($\Delta G^{\text{tt}} = 10.4 \text{ kcal/mole}$)
  - modest catalysis
- $K_m = 8.7 \times 10^{-4} \text{ M}$
  - enzyme binds just well enough (0.87 mM)
- $k_{\text{cat}}/K_m = 2.4 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$
  - virtually the highest possible for bimolecular process of $E + DHAP = E + GAP$, *TIM is the perfect enzyme*

- Close to saturation?
  - $[S] = [\text{DHAP}] = 50 \text{ uM}$
  - $K_m = 870 \text{ uM}$
  - $K_m/S = 17/1$ enzyme not working at $V_{\text{max}}$, can go faster if necessary
Gly 5 TIM structure

- **Enzyme Motion**
  - When the enzyme binds its substrate the loop closes over the active site, shielding the substrate from water. The loop appears to close as a rigid lid, stabilized by internal hydrogen bonds. Its motion involves movements of alpha carbons of up to 8 Å. The closure involves the filling of a cavity near the base of the helix to which the loop is connected and the formation of new hydrogen bonds and contacts.
  - Structures 2YPI, 3TIM, 6TIM
Gly 5 TIM Structure 2YPI

Crystallographic Analysis Of The Complex Between Triosephosphate Isomerase and 2-Phosphoglycolate At 2.5 Å

Classification Isomerase (Intramolecular Oxidoreductse) Compound Triose Phosphate Isomerase (TIM) (E.C. 5.3.1.1) Complex With 2-Phosphoglycolic Acid Exp. Method X-ray Diffraction
Gly 5 TIM Structure 2YPI

Glu 165 that acts as a base to abstract a proton is shown in blue at right.

His95 that participates in acid/base mechanism shown in yellow at left.
Phase II

2GAP + 2NAD$^+$ + 4 ADP + 4 HPO$_4^{-2}$

$\rightarrow$ 2 pyruvate + 2 NADH + 4 ATP + 6 H$_2$O

Cashing In, here we see a production of 2 NADH and when we subtract the 2 ATP invested from 4 ATP above, net 2 ATP

Note that reaction above isn’t charge or mass balanced, and protons aren’t accounted for directly, some reactions will generate.

6 waters produced, 2 come from substrate in gly 9 and 4 come from gly 7 and gly 10 (ADP$^{-4}$ + HPO$_4^{-2}$ = ATP$^{-6}$ + H$_2$O + H$^+$)
Gly 6: Glyceraldehyde-3-Phosphate Dehydrogenase:

• Note: overall oxidation
• Exergonic oxidation of aldehyde drives reaction
• Cofactor: NAD$^+$

\[ \Delta G^o' = +6.3 \text{ kJ/mole} \]
**Gly 6**: Mechanism

*C149 attacks GAP, H176 grabs proton*
*thiohemiacetal intermediate*
*hydride shift to NAD+ GAP oxidized*
*second acylthioester intermediate*
*NADH release is rate limiting*
*second NAD+ binds*
*P_i is 2nd substrate*
*1,3 BPG released*

This step is where the substrate is oxidized!!

Deprotonation of phosphate yields H+ in product

Base is H176
Gly 6: Saturation? Km and [S]

\[
\text{GAP} + \text{NAD}^+ + \text{Pi} = 1,3\text{ DPG} + \text{NADH}
\]

<table>
<thead>
<tr>
<th></th>
<th>Km</th>
<th>[S]</th>
<th>Km/[S]</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAP</td>
<td>70 uM</td>
<td>3 uM</td>
<td>23</td>
</tr>
<tr>
<td>NAD+</td>
<td>46 uM</td>
<td>600 uM</td>
<td>0.08</td>
</tr>
<tr>
<td>Pi</td>
<td>high</td>
<td>2,000 uM</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

- Km/[S] << 1 predicts that gly6 is saturated with NAD+ and not sensitive to changes in it, or controlled by its concentrations.
- GAP and Pi both have Km/[S] > 1, both are > 10 for very different reasons. GAP has good binding but a low concentration of substrate. Pi has horrible binding, but a high concentration of substrate. Since the binding won't change, we say that reaction is controlled by GAP because small changes in its concentration will dramatically alter the rate.
* Dimer structure from *e.coli*

* NAD\(^+\) binding in Rossman fold

* Pos. Cooperativity Hill 2.3
Gly 7: Phosphoglycerate Kinase
First ATP Generation

- Very exergonic, ATP generated
- Only monomeric enzyme on pathway
- Closely associated with Gly6

$$\Delta G^\circ = -18.5 \text{ kJ/mole}$$
Gly 7: Structure 16PK from *Trypnosoma brucei*

* ADP binding site looks like NADH binding site, here crystallized with NADH analogue
* ADP binds tightly, more like cofactor, Km/S = 0.05
* 1,3 DPG Km/S = 9
Gly 8: Phosphoglycerate Mutase

\[ \Delta G^o' = +4.4 \text{ kJ/mole} \]
**Gly 8: PGM Mechanism**

*Base (Lys?) interacts with carboxylic acid of 3PG for binding

* Active enzyme phosphorylated by 2,3-DPG

* His His duo at active site

* $K_m$ varies from 240 $\mu$M in brain to 5000 $\mu$M in muscle. (nature of base?)

* $[S]$ 3PG 40-60 $\mu$M

* $K_m/S = 6-80$, E not sat’d with S, room to speed up.
Gly 8: 3PGM structure from *Schizosaccharomyces pombe*

- Gly 8 has a relatively simple structure with a beta twist and helical unit, 3PG binds at interface.
- Two his 4 Å apart shown in blue wireframe
- His adjacent to C2 has Pi, His adjacent to C3 extracts Pi from C3 while OH on C2 extracts Pi from Pi-His
Is there next oxidation or reduction of substrate in Gly 9?

Answer No, because C2 becomes oxidized (0 to +1) while C3 becomes reduced (-1 to -2) so no net electron flow.

Dehydration to mobilize phosphate accompanied by shift from sp³ to sp² geometry on 2PG

Km 50 uM, S 5 uM, Km/S = 10

\[ \Delta G^{o'} = +7.5 \text{ kJ/mole} \]
Gly 9: 1E9I Structure from *E. coli*

* exists as tetramer, positive cooperativity

* Mg(II) must bind first, image shows 2 Mg(II) and Pi shown bound at active site.
**Gly 10: Pyruvate Kinase**

Second ATP Generation

**Is there next oxidation or reduction of substrate in Gly 10?**

Answer No, because C2 becomes further oxidized (+1 to +2) while C3 becomes further reduced (−2 to −3) so no net electron flow.

Transfer of P to ADP, is slow, conversion from enol to ketone is rapid.

\[ \Delta G^\circ = -31.4 \text{ kJ/mol} \]
Gly 10 1PKN from rabbit muscle

- Monomer rich in beta barrels with 3 domains, has both a $K^+$ and a $Mg^{+2}$ at active site.
- Exists as tetramer with positive coop 2.8 (only monomer shown here)
- Allosteric effectors
  - FBP (+)
  - ATP (-)
- R and T states
- Isozymes L and M
Fates of Glucose: Fermentation
LDH Mechanism: NADH redox

- Redox potential of NADH varies in different enzymes:
- Transfer of the proH_R or proH_S hydride to substrate depends on enzyme class
- Binding site selects conformation of the nicotinamide ring and only one stereoselected H is transferred (for reduction) or added (for oxidation).
- His 195 donates a proton to ketone, accepts a proton from alcohol
- Both His 195 and Arg 171 interact electrostatically to orient carboxylic acid of pyruvate in enzyme active site
Fermentation: Alcohol

$\text{Pyruvate}$ $\xrightarrow{\text{pyruvate decarboxylase}}$ $\text{Acetaldehyde}$ $\xrightarrow{\text{alcohol dehydrogenase}}$ $\text{Ethanol}$

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Pyruvate Decarboxylase Mechanism

Thiamine pyrophosphate, coenzyme
Control of Glycolysis

- Energy Coupling (review phosphorylation potential slide 5)
  - Gly 3? driven by ATP hydrolysis
    - uphill part is phosphorylation of sugar
  - Gly 7? driven by BPG hydrolysis
    - uphill part is ATP synthesis
  - Gly 10? driven by PEP hydrolysis
    - uphill part is ATP synthesis

Start Hexokinase PGI PFK Aldolase TIM G-3-P DH PGK PGM Enolase PK

Free Energy

Phosphofructokinase gly 3
Phosphoglyceratekinase gly 7
Pyruvate kinase gly10

Major control point
Not likely control since it is the last step in glycolysis
Free Energy of Hydrolysis

Table 13-2. Standard Free Energies of Phosphate Hydrolysis of Some Compounds of Biological Interest

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</tr>
<tr>
<td>Pₚ</td>
<td>−33.5</td>
</tr>
<tr>
<td>ATP (AMP + Pₚ)</td>
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<td>−13.8</td>
</tr>
<tr>
<td>Glycerol-3-phosphate</td>
<td>−9.2</td>
</tr>
</tbody>
</table>


20 mM in heart
PFK--Committed Step: Allosteric Control

Dimer of PFK shown

Substrate binding sites in center

Allosteric sites for ATP

R state, high affinity for F6P

T state, low affinity for F6P

No inhibitors (low [ATP])

1 mM ATP

1 mM ATP + 0.1 mM AMP

Phosphofructokinase activity vs. [Fructose-6-phosphate] mM

After data from Mansour, T.E. and Ahrefs, C.E., J. Biol. Chem. 243, 2523-2533 (1968). Copyright 1999 John Wiley and Sons, Inc. All rights reserved.

\[ \text{PFK}_T = \text{PFK}_R \]

Only T state conformation binds ATP at inhibitor site, high ATP, shift to T, low affinity for f6P

Voet, Voet, and Pratt Fundamentals of Biochemistry
Substrate Cycling

Different enzymes catalyze the forward and backward reactions

**Reciprocal Regulation:** Fructose 2,6 bisphosphate stimulates kinase and inhibits phosphatase.
Control of Glycolysis (1)

- Velocity $= V_{\text{max}} [S_t] = k_{\text{cat}} [E_t] [S_t]$
- $K_m + [S_t] = K_m + [S_t]$
- Typical enzyme concentrations, pM-μM
- How can Enzyme levels be controlled?
  - Sequestered storage, triggered release
  - Zymogens (inactive precursors)
    - quick inefficient
  - Transcriptional activation (small molecule metabolites or hormones bind to the genes)
    - slow, efficient
  - mRNA processing activation; (small molecules bind to untranslated nascent mRNA and affect translation) riboswitches
    - quick efficient
When is a ribozyme not just a ribozyme? When it’s a riboswitch http://www.yale.edu/breaker/riboswitch.htm
Control of gene expression by a natural metabolite-responsive ribozyme

Wade C. Winkler1, Ali Nahvi2, Adam Roth1, Jennifer A. Collins1 & Ronald R. Breaker1

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Most biological catalysts are made of protein; however, eight classes of natural ribozymes have been discovered that catalyse fundamental biochemical reactions. The central functions of ribozymes in modern organisms support the hypothesis that life passed through an ‘RNA world’ before the emergence of proteins and DNA. We have identified a new class of ribozyme that cleaves the messenger RNA of the glmS gene in Gram-positive bacteria. The ribozyme is activated by glucosamine-6-phosphate (GlcN6P), which is the metabolic product of the GlmS enzyme. Additional data indicate that the ribozyme serves as a metagenomic switch that repression of the glmS gene in response to rising GlcN6P concentrations. These findings demonstrate that ribozyme switches may have functioned as metabolite sensors in primitive organisms, and further suggest that mode retain some of these ancient genetic control systems.
Control of Glycolysis (2)

- Velocity = \( \frac{V_{\text{max}} [S_t]}{K_m + [S_t]} = \frac{k_{\text{cat}} [E_t] [S_t]}{K_m + [S_t]} \)
- Typical substrate concentrations, 10\(\mu\)M-10 mM
- How can substrate levels be controlled?
  - Sequestered storage (glycogen stores in muscle/liver), hormone triggered release (glucagon)
  - Conversion of related molecule (lactate to pyruvate)
  - Hunger signal to organism (hormone)
Control of Glycolysis (3)

- Velocity = \( V_{\text{max}} [S_t] = k_{\text{cat}} [E_t] [S_t] \)
- \( K_m + [S_t] \quad K_m + [S_t] \)
- Typical \( k_{\text{cat}} \), \( 10^2-10^6 \) s\(^{-1}\)
- How can \( k_{\text{cat}} \) be increased/decreased?
  - Allosteric effectors (example PFK-gly3)
  - Reversible covalent modification
    - Phosphorylation, adenylation, methylation, acetyllation, others (example pyruvate dehydrogenase)
Control of Glycolysis (3b)

- Velocity: $V_{\text{max}}[S_t] = k_{\text{cat}}[E_t][S_t]$
- $K_m + [S_t] = K_m + [S_t]$
- Typical $K_m$, 10-1000 uM
- How can $K_m$ be increased/decreased?
  - Self Inhibition