Glycogen Metabolism

Glycogen Breakdown

Glycogen Synthesis

Control of Glycogen Metabolism

Glycogen Storage Diseases
Glycogen

**Glycogen** - animal storage glucan

100- to 400-Å-diameter cytosolic granules

up to 120,000 glucose units

α(1 → 6) branches every 8 to 12 residues

muscle has 1-2% (max) by weight

liver has 10% (max) by weight

~12 hour supply

Although metabolism of fat provides more energy:

1. Muscle mobilize glycogen faster than fat

2. Fatty acids of fat cannot be metabolized anaerobically

3. Animals cannot convert fatty acid to glucose (glycerol can be converted to glucose)
Glycogen Breakdown

Three enzymes:

- glycogen phosphorylase
- glycogen debranching enzyme
- phosphoglucomutase

Glycogen phosphorylase (phosphorylase) - phosphorolysis of glucose residues at least 5 units from branch point

\[
\text{Glycogen + P}_i \rightleftharpoons \text{glycogen} + \text{glucose-1-phosphate}
\]

(n residues) (n-1 residues)

homodimer of 842-residues (92-kD) subunits

allosteric regulation - inhibitors (ATP, glucose-6-phosphate, glucose) and activator (AMP), T ⇔ R

covalent modification (phosphorylation) - modification/demodification

- phosphorylase \(\alpha\) (active, Ser\(\text{OP}_2\)\(^{2-}\))
- phosphorylase \(\beta\) (less active, Ser)

narrow 30-Å crevice binds glycogen, accommodates 4 to 5 residues

Pyridoxal-5-phosphate (vit B\(_6\) derivative) cofactor - located near active site, general acid-base catalyst

Rapid equilibrium Random Bi Bi kinetics
Glycogen Breakdown

Glycogen debranching enzyme - possesses two activities

\[ \alpha(1 \rightarrow 4) \text{ transglycosylase} \ (\text{glycosyl transferase}) \ 90\% \]

glycogen \( \rightarrow \) glucose-1-phosphate

departs trisaccharide unit from "limit branch" to nonreducing end of another branch

\[ \alpha(1 \rightarrow 6) \text{ glucosidase} \ 10\% \ 	ext{glycogen} \rightarrow \text{glucose} \]

Debranching activity < phosphorylase activity
Glycogen Breakdown

**Phosphoglucomutase** - a phosphoenzyme (Ser)

reaction similar to that of phosphoglycerate mutase

formation of **glucose-1,6-bisphosphate** (required for full activity)

**phosphoglucokinase** - provides product

glucose-1-phosphate + ATP $\rightarrow$ glucose-1,6-bisphosphate
Glycogen Breakdown

Thermodynamic considerations

phosphorylase reaction:

\[ \Delta G^\circ = +3.1 \text{ kJ mol}^{-1} \]

\[ \Delta G = 0 \text{ when } [\text{P}_i/\text{glucose-1-phosphate}] = 3.5 \]

under physiological conditions

\[ [\text{P}_i/\text{glucose-1-phosphate}] \sim 30 \text{ to } 100 \]

\[ \Delta G^\circ = -5 \text{ to } -8 \text{ kJ mol}^{-1} \]

Glycogen breakdown is exergonic (favorable)

Glycogen synthesis must occur by a separate pathway
Glycogen Synthesis

Three enzymes:

**UDP-glucose pyrophosphorylase**
**glycogen synthase**
**glycogen branching enzyme**

**UDP-glucose pyrophosphorylase** - phosphoanhydride exchange

Glucose-1-phosphate + UTP ⇌ UDP-glucose + PP$_i$

$\Delta G^o = 0 \text{ kJ mol}^{-1}$

$\text{H}_2\text{O} + \text{PP}_i \rightarrow 2\text{P}_i$

$\Delta G^o = -33.5 \text{ kJ mol}^{-1}$

Common biosynthetic strategy generates:

glucose-1-phosphate + UTP → UDP-glucose + 2P$_i$

$\Delta G^o = -33.5 \text{ kJ mol}^{-1}$
Glycogen Synthesis

**Glycogen synthase** - adds glycosyl unit from UDP-glucose to form $\alpha(1 \rightarrow 4)$ glycosidic bonds

**Glycogen Primer:**

**glycogenin** - protein to which glucose is added to Tyr residue by **tyrosine glucosyltransferase**

autocatalytically extends chain up to 7 glucose residues by UDP-glucose

glycosyl oxonium ion intermediate (similar to phosphorylase and lysozyme mechanisms)

Note:

glycogen breakdown ($\Delta G^\circ = -5$ to $-8$ kJ mol$^{-1}$)

and

glycogen synthesis ($\Delta G^\circ = -13.4$ kJ mol$^{-1}$)

are thermodynamically favorable processes

The cost of controlling both is the hydrolysis of UTP (similar to ATP)!
Glycogen Synthesis

Glycogen branching enzyme (amylo-(1,4→1,6)-transglycosylase - transfer of ~7 glycosyl residue segments to form α(1 → 6) glycosidic bonds

Thermodynamic considerations

The overall free energy for debranching is:

<table>
<thead>
<tr>
<th></th>
<th>ΔG°'</th>
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<tbody>
<tr>
<td>Breaking α(1 → 4) bond</td>
<td>-15.5 kJ·mol⁻¹</td>
</tr>
<tr>
<td>Forming α(1 → 4) bond</td>
<td>+15.5 kJ·mol⁻¹</td>
</tr>
<tr>
<td>Hydrolyzing α(1 → 6) bond</td>
<td>-7.1 kJ·mol⁻¹</td>
</tr>
<tr>
<td>Total</td>
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The free energy change for branching is:

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<td>Total</td>
<td>-8.4 kJ·mol⁻¹</td>
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Control of Glycogen Metabolism

Glycogen phosphorylase and glycogen synthase:  
allosteric control  
substrate cycling  
covalent modification of activity  
(under hormonal control)

Direct allosteric control of glycogen phosphorylase and glycogen synthase

Precise flux control by having two opposing enzymes at a control step (far from equilibrium) in a pathway

**Glycogen phosphorylase**
activated by AMP
inhibited by ATP and glucose-6-phosphate

**Glycogen synthase**
activated by glucose-6-phosphate

High demand for ATP: glycogen breakdown
low [ATP], low [G6P], high [AMP]
glycogen phosphorylase **stimulated**
glycogen synthase **inhibited**

Low demand for ATP: glycogen synthesis
high [ATP], high [G6P], low [AMP]
glycogen phosphorylase **inhibited**
glycogen synthase **stimulated**
Control of Glycogen Metabolism

Covalent modification of enzymes by cyclic cascades

Features:

1. Respond to greater number of stimuli
2. Greater flexibility in control patterns
3. Amplification potential in response to effector concentrations

Small change in [allosteric effector] of a modifying enzyme → large change in [active, modified target enzyme]

Cyclic cascades nomenclature:

- \( a \) - more active target enzyme
- \( b \) - less active target enzyme
- \( m \) - modified enzyme form
- \( o \) - original (unmodified) enzyme form

Recall that the rate of reaction = \( k[E_{\text{active}}][S] \)

Using a mathematical model (beyond the scope of this course) we could show quantitatively how changes in [effectors] modulate \( E_{\text{active}} \)

so a cyclic cascade allows an effector signal to be amplified
Control of Glycogen Metabolism

Glycogen phosphorylase bicyclic cascade
Superimposed on the ATP (inhibitor) and AMP (activator)
allosteric control is covalent modification

Covalent modification enzymes:

cAMP-dependent protein kinase (cAPK) - phosphorylates (activates) phosphorylase kinase, requires cAMP, $R_2C_2$ tetramer
cAPK consensus sequence - Arg-Arg-X-Ser/Thr-Y
$X =$ small residue  $Y =$ hydrophobic residue

phosphorylase kinase - phosphorylates Ser14 of glycogen phosphorylase $b$
oligomeric $(\alpha\beta\gamma\delta)_4$, $\alpha\beta\delta$ inhibitory, $\gamma$ activates
$\delta =$ Calmodulin, $Ca^{2+}$ activates (muscle contraction)

phosphoprotein phosphatase-1 - dephosphorylates (deactivates) glycogen phosphorylase $a$ and phosphorylase kinase
muscle - active when bound to glycogen-binding G subunit
liver - controlled by binding to $m$-phosphorylase $a$

Level of phosphorylase activity is determined by fraction present as glycogen phosphorylase $a$
Control of Glycogen Metabolism

Glycogen synthase bicyclic cascade

Not as well understood

Two forms of enzyme:

\( m \)-glycogen synthase \( b \) (inactive)

allosterically controlled - inhibited by ATP, ADP, \( P_i \)
overcome by \([\text{glucose-6-phosphate}] > 10 \text{ mM}\) (rare)

\( o \)-glycogen synthase \( a \) (active)

deactivated by calmodulin-dependent protein kinase,
protein kinase C, glycogen synthase kinase-3
Control of Glycogen Metabolism  
(What the book does not illustrate)

Integration of glycogen metabolism control mechanisms

Maintenance of blood glucose levels - liver buffers  
\([\text{glucose}] \sim 5 \text{ mM}\)

The Cast

Hormones

Glucagon - polypeptide (liver)
Insulin - polypeptide (muscle, other tissues)
Epinephrine - adrenal

Second messengers

\(\text{Ca}^{2+}\)

Inositol-1,4,5-triphosphate (IP\(_3\)) - lipid-derived
Diacylglycerol (DAG) - lipid-derived

Phospholipase C - cleaves membrane lipid  
\((\text{phosphatidylinositol-4,5-bisphosphate, PIP}_2)\) to generate  
IP\(_3\) and DAG

Receptors

\(\beta\)-Adrenergic - binds adrenal hormones
\(\alpha\)-Adrenergic - binds adrenal hormones
Glucagon
Insulin
Control of Glycogen Metabolism

Glucagon Epinephrine Glucose

<table>
<thead>
<tr>
<th>Glucose transporter</th>
<th>cAMP</th>
<th>Ca^{2+}</th>
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<td>glycogen degradation</td>
<td>glycogen synthesis</td>
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Liver cell

Epinephrine

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Muscle cell

Insulin

<table>
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<tr>
<th>insulin receptor</th>
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<td>glycogen synthesis</td>
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Glucose transporter
Control of Glycogen Metabolism

Maintenance of blood glucose levels

**Hexokinase:**

Michaelis-Menten kinetics

*high glucose affinity* \( (K_m \sim 0.1 \text{ mM}) \)

*inhibited by glucose-6-phosphate*

**Glucokinase:**

monomeric

*Sigmoidal kinetics* (Hill constant of 1.5)

*lower glucose affinity* \( (K_{0.5} \sim 5 \text{ mM}) \)

not inhibited by physiological [glucose-6-phosphate]

*inhibited by glucokinase regulatory protein + fructose-6-phosphate*
Control of Glycogen Metabolism

Maintenance of blood glucose levels

Flux control by substrate cycle and covalent modification system

Phosphofructokinase-1 (PFK-1) - allosterically activated by fructose-2,6-bisphosphate

Fructose-1,6-bisphosphatase-1 (FBPase-1) - allosterically inhibited by fructose-2,6-bisphosphate

Phosphofructokinase-2 (PFK-2)/fructose-2,6-bisphosphatase-2 (FBPase-2):

Bifunctional homodimeric protein
phosphorylated (inactive)/dephosphorylated (active)

In liver - breakdown glycogen, release glucose into blood or take up glucose, synthesize glycogen

In heart - glycogen breakdown, increase glycolysis (different PFK-2/FBPase-2 gene)

In muscle - no phosphorylation site on enzyme, no cAMP-dependent phosphorylation control
Control of Glycogen Metabolism

Glucagon  Epinephrine  Insulin

Liver cell

fructose-6-phosphate

fructose-2,6-bisphosphate

fructose-1,6-bisphosphate

pyruvate

PFK-1

FBPase-1

PFK-2a

FBPase-2b

PFK-2b

FBPase-2a

Protein kinase A

cAMP

Phosphoprotein phosphatase

Liver cell
Control of Glycogen Metabolism

Glucagon  Epinephrine  Insulin

Liver cell

AMP  cAMP

Protein kinase A

fructose-6-phosphate  fructose-2,6-bisphosphate  fructose-1,6-bisphosphate  pyruvate

PFK-2 a  FBPase-2 b  PFK-1  PFK-2 b  FBPase-2 a

Phosphoprotein phosphatase

Liver cell
Control of Glycogen Metabolism

Glucagon  Epinephrine  Insulin

\[ \text{fructose-6-phosphate} \]

\[ \text{fructose-2,6-bisphosphate} \]

\[ \text{fructose-1,6-bisphosphate} \]

\[ \text{pyruvate} \]

\[ \text{PFK-1} \]

\[ \text{FBPase-1} \]

\[ \text{PFK-2} \]

\[ \text{FBPase-2} \]

\[ \text{Protein kinase A} \]

\[ \text{cAMP} \]

Heart tissue