Chapter 22
Gluconeogenesis, Glycogen Metabolism, and the Pentose Phosphate Pathway

Biochemistry
by
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Essential Question
1. What is the nature of gluconeogenesis, the pathway that synthesizes glucose from noncarbohydrate precursors
2. How is glycogen synthesized from glucose
3. How are electrons from glucose used in biosynthesis?

Outline of chapter 22
1. What Is Gluconeogenesis, and How Does It Operate?
2. How Is Gluconeogenesis Regulated?
3. How Are Glycogen and Starch Catabolized in Animals?
4. How Is Glycogen Synthesized?
5. How Is Glycogen Metabolism Controlled?
6. Can Glucose Provide Electrons for Biosynthesis?

22.1 – What Is Gluconeogenesis, and How Does It Operate?

Synthesis of "new glucose" from common metabolites
• Humans consume 160 g of glucose per day
• 75% of that is in the brain
• Body fluids contain only 20 g of glucose
• Glycogen stores yield 180-200 g of glucose
• So the body must be able to make its own glucose
**Substrates for Gluconeogenesis**

*Pyruvate, lactate, glycerol, amino acids and all TCA intermediates can be utilized*

- Fatty acids cannot! Why?
- Most fatty acids yield only acetyl-CoA
  - Except fatty acids with odd numbers of carbons
- Acetyl-CoA (through TCA cycle) cannot provide for net synthesis of sugars in animal, but plants do! Why?

**Gluconeogenesis**

- Occurs mainly in liver (90%) and kidneys (10%)
- Not the mere reversal of glycolysis for 2 reasons:
  - Energetics: must change to make gluconeogenesis favorable ($\Delta G$ of glycolysis = -74 kJ/mol)
  - Reciprocal regulation: Gluconeogenesis must turn one on and the other off, and vice versa

**Gluconeogenesis**

*Something Borrowed, Something New*

- Seven steps of glycolysis are retained:
  - Steps 2 and 4-9
- Three steps are replaced:
  - Steps 1, 3, and 10 (the regulated steps!)
- The new reactions provide for a spontaneous pathway ($\Delta G$ negative in the direction of sugar synthesis), and they provide new mechanisms of regulation
**Pyruvate Carboxylase**

**Pyruvate is converted to oxaloacetate**

- The reaction requires ATP and bicarbonate as substrates

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\begin{align*}
\text{Pyruvate} & \quad \text{+ HCO}_3^- \quad \text{ATP} \quad \rightarrow \quad \text{Oxaloacetate} \\
\text{Pyruvate} & \quad \text{Bicarbonate} \quad \text{ATP} \quad \rightarrow \quad \text{Oxaloacetate} + \text{ADP}
\end{align*}
\]

- Biotin as a coenzyme and Acetyl-CoA is an allosteric activator

**Biotin is covalently linked to the e-amino group of a lysine residue**

**The mechanism is typical of biotin**

- Bicarbonate must be activated for attack by the pyruvate carbanion. This activation is driven by ATP and involves formation of a carbonylphosphate intermediate—a mixed anhydride of carbonic and phosphoric acids. (Carbonylphosphate and carboxyphosphate are synonyms.)
**Pyruvate Carboxylase**

- Acyl-CoA is an allosteric activator
- The conversion is in mitochondrial matrix
- Regulation:
  - If levels of ATP and/or acetyl-CoA are low, pyruvate is converted to acetyl-CoA and enters TCA cycle
  - If levels of ATP and/or acetyl-CoA are high, pyruvate is converted to oxaloacetate and enters gluconeogenesis → glucose

**PEP Carboxykinase**

*Conversion of oxaloacetate to PEP*

- Lots of energy needed to drive this reaction
- Energy is provided in 2 ways:
  - Decarboxylation is a favorable reaction
  - GTP is hydrolyzed
- GTP used here is equivalent to an ATP

\[
\begin{align*}
\text{Oxaloacetate} & \quad + \quad \text{GTP} \quad \rightarrow \quad \text{PEP} \quad + \quad \text{GDP} \\
\end{align*}
\]

**PEP Carboxykinase**

- The overall ΔG for the pyruvate carboxylase and PEP carboxykinase reactions under physiological conditions in the liver is -22.6 kJ/mol
- Once PEP is formed in this way, the other reactions act to eventually form fructose-1,6-bisphosphate
**Fructose-1,6-bisphosphatase**

**Hydrolysis of F-1,6-bisPase to F-6-P**
- Thermodynamically favorable - ΔG in liver is -8.6 kJ/mol
- Allosteric regulation:
  - citrate stimulates
  - fructose-2,6-bisphosphate inhibits
  - AMP inhibits (enhanced by F-2,6-bisP)

**Glucose-6-Phosphatase**

Conversion of Glucose-6-P to Glucose
- G-6-Pase is present in ER membrane of liver and kidney cells
- Muscle and brain do not do gluconeogenesis
- G-6-P is hydrolyzed as it passes into the ER
- ER vesicles filled with glucose diffuse to the plasma membrane, fuse with it and open, releasing glucose into the bloodstream.

**Lactate Recycling – Cori cycle**

*How your liver helps you during exercise...*
- Recall that vigorous exercise can lead to a buildup of pyruvate and NADH, due to oxygen shortage and the need for more glycolysis
- NADH can be reoxidized during the reduction of pyruvate to lactate
- Lactate is then returned to the liver, where it can be reoxidized to pyruvate by liver LDH
- Liver provides glucose to muscle for exercise and then reprocesses lactate into new glucose
22.2 – How Is Gluconeogenesis Regulated?

Reciprocal control with glycolysis

- When glycolysis is turned on, gluconeogenesis should be turned off, and vice versa.
- When energy status of cell is high, glycolysis should be off and pyruvate, etc., should be used for synthesis and storage of glucose.
- When energy status is low, glucose should be rapidly degraded to provide energy.
- The regulated steps of glycolysis are the very steps that are regulated in the reverse direction.

Allosteric and Substrate-Level Control

- Glucose-6-phosphatase
  - is under substrate-level control by G-6-P, not allosteric control.
- Pyruvate carboxylase
  - Activated by acetyl-CoA
  - The fate of pyruvate depends on acetyl-CoA; pyruvate kinase (-), pyruvate dehydrogenase (+), and pyruvate carboxylase (+).
- F-1,6-bisPase
  - is inhibited by AMP and Fructose-2,6-bisP
  - Activated by citrate - the reverse of glycolysis.
• Fructose-2,6-bisP
  – is an allosteric inhibitor of F-1,6-bisPase
  – is an allosteric activator of PFK
  – synergistic effect with AMP

• The cellular levels Fructose-2,6-bisP are controlled by phosphofructokinase-2 and fructose-2,6-bisPase which is bifunctional enzyme
  – F-6-P allosterically activates PFK-2 and inhibits F-2,6-BisPase
  – Phosphorylation by cAMP-dependent protein kinase inhibits PFK-2 and activates F-2,6-bisPase
22.3 – How Are Glycogen and Starch Catabolized in Animals?

Getting glucose from storage (or diet)
- α-Amylase is an endoglycosidase -- $\alpha(1\rightarrow4)$ cleavage
  - β-Amylase is an exoglycosidase (In plants)
- It cleaves dietary amylopectin or glycogen to maltose, maltotriose and other small oligosaccharides
- It is active on either side of a branch point, but activity is reduced near the branch points and stops four residues from any branch point
- limit dextrins

Metabolism of Tissue Glycogen

Digestive breakdown is unregulated - 100%!
- But tissue glycogen is an important energy reservoir - its breakdown is carefully controlled
- Glycogen consists of "granules" of high MW range from $6 \times 10^6$ ~ $1600 \times 10^6$
- Glycogen phosphorylase cleaves glucose from the nonreducing ends of glycogen molecules
- This is a phosphorolysis, not a hydrolysis
- Metabolic advantage: product is a glucose-1-P; a "sort-of" glycolysis substrate
22.4 – How Is Glycogen Synthesized?

Glucose units are activated for transfer by formation of sugar nucleotides

- What are other examples of "activation"?
  - acetyl-CoA: acetate
  - Biotin and THF: one-carbon group
  - ATP: phosphate

- Leloir showed that glycogen synthesis depends on sugar nucleotides
- UDP-glucose pyrophosphorylase catalyzes the formation of UDP-glucose
- ADP-glucose is for starch synthesis in plants

Glucose-1-P + UTP →
UDP-glucose + pyrophosphate.
**Glycogen Synthase**

*Forms α-(1→4) glycosidic bonds in glycogen*

- Glycogenin (a protein core) forms the core of a glycogen particle
- First glucose is linked to a tyrosine -OH on glycogenin
- Glycogen synthase transfers glucosyl units from UDP-glucose to C-4 hydroxyl at a nonreducing end of a glycogen strand.
- Oxonium ion intermediate (Fig. 22.19)

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**Glycogen branching occurs by transfer of terminal chain segments**

- Glycogen branches are formed by amylo-(1,4→1,6)-transglycosylase, also called branching enzyme
- α-(1→6) linkages, which occurs every 8-12 residues
- Transfer of 6- or 7-residue segment from the nonreducing end

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**22.5 – How Is Glycogen Metabolism Controlled?**

_A highly regulated process, involving reciprocal control of glycogen phosphorylase and glycogen synthase_

- Glycogen phosphorylase, allosterically activated by AMP and inhibited by ATP, glucose-6-P and caffeine
- Glycogen synthase, is stimulated by glucose-6-P
- Both enzymes are regulated by covalent modification - phosphorylation
**Phosphorylation of GP and GS**

*Covalent modification*
- In chapter 15 showed that protein kinase converted phosphorylase b (-OH) to phosphorylase a (-OP)
- Glycogen synthase also exists in two distinct forms
  - Active, dephosphorylated glycogen synthase I
  - Less active, phosphorylated glycogen synthase D (glucose-6-P dependent)
- Nine Ser residues on GS are phosphorylated

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**Enzyme Cascades and GP/GS**

*Hormonal regulation (insulin, Glucagon, epinephrine, and glucocorticoids)*
- Glucagon and epinephrine activate adenylyl cyclase
- cAMP activates kinases and phosphatases that control the phosphorylation of GP and GS
  - GTP-binding proteins (G proteins) mediate the communication between hormone receptor and adenylyl cyclase
- Dephosphorylation is carried out by phosphoprotein phosphatase-I (PP-I)

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**Hormonal Regulation**

*of Glycogen Synthesis and Degradation*
- Insulin is secreted from the pancreas (to liver) in response to an increase in blood glucose
- Insulin into the portal vein and traverses the liver
- Insulin stimulates glycogen synthesis and inhibits glycogen breakdown
- Other effects of insulin (Figure 22.22)
Hormonal Regulation

Glucagon and epinephrine

- Glucagon and epinephrine stimulate glycogen breakdown - opposite effect of insulin
- Glucagon (29 AA-res) is also secreted by pancreas and acts in liver and adipose tissue only
- Epinephrine (adrenaline) is released from adrenal glands and acts on liver and muscles
- A cascade is initiated that activates glycogen phosphorylase and inhibits glycogen synthase
**Hormonal Regulation**

- The phosphorylase cascade amplifies the signal
  - $10^{-10}$ to $10^{-8}$ M epinephrine
  - $10^{-6}$ M cAMP
  - Protein kinase
  - 30 molecules of phosphorylase b kinase
  - 800 molecules of phosphorylase a
  - Catalyzes the formation of many molecules of glucose-1-P
- The result of these actions is tightly coordinated stimulation of glycogen breakdown and inhibition of glycogen synthesis

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**The difference between Epinephrine and Glucagon**

- Both are glycogenolytic but for different reasons
- Epinephrine is the fight or flight hormone
  - Rapid breakdown of glycogen
  - Inhibition of glycogen synthesis
  - Stimulation of glycolysis
  - Production of energy
- Glucagon is for long-term maintenance of steady-state levels of glucose in the blood
  - Activates glycogen breakdown
  - Activates liver gluconeogenesis
- Glucagon do not activate the phosphorylase cascade in muscle
**Cortisol and glucocorticoid**

- Cortisol is a typical glucocorticoid
- In muscle (catabolic)
  - promotes protein breakdown
  - decreases protein synthesis
- In liver
  - stimulates gluconeogenesis
  - increases glycogen synthesis
  - amino acid catabolism

**22.6 – Can Glucose Provide Electrons for Biosynthesis?**

**Pentose Phosphate Pathway**

**Hexose monophosphate shunt**

**Phosphogluconate pathway**

- Provides NADPH for biosynthesis
- Produces ribose-5-P for nucleotide synthesis
- Several metabolites of the pentose phosphate pathway can also be shuttled into glycolysis

**Pentose phosphate pathway**

- Begins with glucose-6-P, a six-carbon, and produces 3-, 4-, 5-, 6, and 7-carbon sugars, some of which may enter the glycolytic pathway
- Two oxidative processes followed by five non-oxidative steps
- Operates mostly in cytoplasm of liver and adipose cells, but absent in muscle
- NADPH is used in cytosol for reductive reaction-- fatty acid synthesis
**Oxidative Steps**

- **Glucose-6-P Dehydrogenase**
  - Begins with the oxidation of glucose-6-P
  - The products are a cyclic ester (the lactone of phosphogluconic acid) and NADPH
  - Irreversible 1st step and highly regulated
  - Inhibited by NADPH and acyl-CoA

- **Gluconolactonase**
  - Gluconolactone hydrolyzed → 6-phospho-\(\delta\)-gluconate
  - Uncatalyzed reaction happens too
  - Gluconolactonase accelerates this reaction

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**Step 1**

The glucose-6-phosphate dehydrogenase reaction is the committed step in the pentose phosphate pathway.

**Step 2**

The gluconolactonase reaction.
Oxidative Steps

- 6-Phosphogluconate Dehydrogenase
  - An oxidative decarboxylation of 6-phosphogluconate
  - Yields ribulose-5-P and NADPH
  - Releases CO₂

The Nonoxidative Steps

Five steps, only 4 types of reaction...
- Phosphopentose isomerase
  - converts ketose to aldose
- Phosphopentose epimerase
  - epimerizes at C-3
- Transketolase (TPP-dependent)
  - transfer of two-carbon units
- Transaldolase (Schiff base mechanism)
  - transfers a three-carbon unit

Phosphopentose isomerase
- converts ketose to aldose
- Ribose-5-P is utilized in the biosynthesis of coenzymes, nucleotides, and nucleic acids
• Phosphopentose epimerase
  – An inversion at C-3

The phosphopentose epimerase reaction interconverts ribulose-5-P and xylulose-5-phosphate. The mechanism involves an enediol intermediate and occurs with inversion at C-3.

• Transketolase (TPP-dependent)
  – transfer of two-carbon units
  – The donor molecule is a ketose and the recipient is an aldose

The transketolase reaction of step 6 in the pentose phosphate pathway. The donor molecule is a ketose, and the acceptor is an aldose, with two-carbon units transferred. Despite the irony, these names persist for historical reasons.
• Transaldolase (Schiff base, imine)
  – transfers a three-carbon unit
  – Yields erythrose-4-P & Fructose-6-P

Variations on the Pentose Phosphate Pathway

1) Both ribose-5-P and NADPH are needed
2) More ribose-5-P than NADPH is needed
3) More NADPH than ribose-5-P is needed
4) NADPH and ATP are needed, but ribose-5-P is not
Large amounts of NADPH can be produced by the pentose phosphate pathway without significant net production of ribose-5-P. In this version of the pathway, ribose-5-P is recycled to produce glycolytic intermediates.

Both ATP and NADPH (as well as NADH) can be produced by this version of the pentose phosphate and glycolytic pathways.