Glycogen metabolism

Glycogen review - 1,4 and 1,6 α-glycosidic links
~ every 10 sugars are branched - open helix with many non-reducing ends.
Effective storage of glucose

Glucose storage
Liver glycogen 4.0% 72 g
Muscle glycogen 0.7% 245 g
Blood Glucose 0.1% 10 g

Large amount of water associated with glycogen - 0.5% of total weight
Glycogen stored in granules in cytosol w/proteins for synthesis, degradation and control
There are very different means of control of glycogen metabolism between liver and muscle

The function of glycogen is to store metabolic glucose
- Efficient release of gluc from glycogen polymer requires many non-reducing ends for enzyme attack
- Must store largest amount of glycogen in smallest volume
- Chain length and branches support the "glucose bioavailability"
- G-glycogenin core with two branches, each continues to branch.
- Each layer (tier) of branches increase ends for enzymatic release of glucose

Glycogen biosynthetic and degradative cycle
Two different pathways - which do not share enzymes like glycolysis and gluconeogenesis
- glucose -> glycogen  glycogenesis - biosynthetic
- glycogen -> glucose 1-P  glycogenolysis - breakdown

Evidence for two paths
- Patients lacking phosphorylase can still synthesize glycogen
- hormonal regulation of both directions
**Glycogenolysis (glycogen breakdown)**

- **Glycogen Phosphorylase**
  - glycoegen (n) + Pi → glucose 1-p + glycogen (n-1)
  - Enzyme binds and cleaves glycogen into monomers at the end of the polymer (reducing ends of glycogen)
  - Dimmer interacting at the N-terminus.
  - Rate limiting - controlled step in glycogen breakdown
  - Glycogen phosphorylase - cleavage of 1,4 α glycosidic bond by Pi NOT H₂O
  - Energy of phosphorolysis vs. hydrolysis
    - low standard state free energy change - transfer potential
    - driven by Pi concentration
    - Hydrolysis would require additional step s/ cost of ATP
  - Think of the difference between adding a phosphate group with hydrolysis
    - phosphorylation locks glucose in cell (imp. for muscle)
    - Phosphorylase binds glycogen at storage site and the catalytic site is 4 to 5 glucose residues away from the catalytic site.
    - Phosphorylase removes 1 residue at a time from glycogen until 4 glucose residues away on either side of 1,6 branch point – sterically hindered by glycogen storage site
    - Cleaves without releasing at storage site
    - General acid/base catalysts
    - Inorganic phosphate attacks the terminal glucose residue passing through an oxonium ion intermediate.
    - Cofactor PLP pyridoxal 5’-phosphate
      - Covalently bound by shiff base
- Phosphate functional group of PLP acts as an acid/base catalyst
- Allows the exclusion of water - replaced by Pi

**Transferase/debranching enzyme**
Finishes what phosphorylase cannot
Two activities on same enzyme
- 1st - transferase - move 4 or 5 sugars from one chain to another
  - Exposes 1,6 branched sugar
- 2nd - debranching - specific cleavage (hydrolysis) of 1,6 branched glucose - giving free glucose

**Phosphoglucomutase**
glucose 1-P -> glucose 6-P
- mechanism involves phosphoryl shuttle from serine in active site to glucose hydroxyls
- no additional energy required
- equilibrium far to the right

**Glucose 6-phosphatase (G-6-Phos)**
glucose 6P -> glucose + Pi
- Found mostly in Liver and Kidney NOT Muscle or Brain
- Allows gluconeogenic tissue to supply and glucose for body while muscle and brain keep it for glycolysis

**Glycogenesis**

**UDP glucose transferase**
Glucose 1 P + UTP -> UDPglucose + PPi
- UTP acts as a high energy handle for many sugar polymers
- PPi hydrolysis - pulls reaction to right of reaction

**Glycogen synthase**
UDPglucose -> 1,4 glycogen + UDP
- Highly regulated enzyme of this path
- requires a preexisting primer of glycogen
  - Primer is glycogenin enzyme/protein
  - Glycogenin glycosylates itself
  - Glycogen “core”
- 1,4 glycogen addition until glycogen synthase loses contact with glycogenin core

**Branching enzyme**
1,4 glycogen -> 1,6 /1,4 glycogen
- once 11 or so glucose residues have been added, transfers 1,4 to another chain in a 1,6 linkage
- transfers about 7 sugar residues
Energy cost of Glycogen synthesis

\[
\begin{align*}
\text{glucose 6-phosphate} & \rightarrow \text{glucose 1 phosphate} \\
\text{glucose 1-phosphate} + \text{UTP} & \rightarrow \text{UDP-Glucose + PPI} \\
\text{PPI} + \text{H}_2\text{O} & \rightarrow 2 \text{Pi} \\
\text{UDP-glucose} + \text{glycogen}_n & \rightarrow \text{glycogen}_{n+1} + \text{UDP} \\
\text{ADP + ATP} & \rightarrow \text{UTP + ADP}
\end{align*}
\]

The cost of storage of glucose in the form of glycogen is very small as compared to the production of ATP from glucose

Glycogen Metabolism and Regulation

Three dimensional structure of glycogen phosphorylase
- 2 x 2 (heterotetramer)
- separate binding sites for each of the regulators, and glycogen particle site
- need to exclude water / active site (pyridoxal phosphate) interior of homoenzyme
- glycogen binding site distal (away) from the active site - permits several reactions before release of glycogen polymer
- concerted change to R active form through rotation of subunit and rearrangement of active site

Phosphorylase regulation
- Phosphorylase - 2 x 2 (heterotetramer)
  - phosphorylase a - phosphorylated
  - phosphorylase b - is not - phosphorylated
  R form active / T form inactive
  Shifting between R and T forms alters activity.
  phosphorylation state defines a or b but equilibrium between forms is also set by allosteric regulation

The active site in the T (b) form is hidden.
AMP (NOT cAMP) binding moves Ser 14 similar to that seen when the Ser is phosphorylated.

AMP leads to the opening of the active site without the requisite phosphorylation, thus the conversion from b to a form of phosphorylase. (a and b mean active and less active, it does not discuss the phosphorylation state)

ATP binds to the same site but does NOT cause the same shifts, rather it tends to stabilize the T form, and is thus an inhibitor.
Thinks of the logic of the energy state and how AMP and ATP relate to the results of glycogenolysis

Phosphorylation of Ser 14 leads to a T to R conformation shift as the negative charges of the phospho group interacts with positive charged Arg. This is similar to the changes in conformation found with AMP. Thus the very low energy state of the cell can overcome covalent modification of the enzymes activity

KNOW the structural mechanics of this enzyme

There are two levels of control of phosphorylase allosteric and covalent. Both are required for full activation

- Covalent - Phosphorylation by PKA
- Allosteric - Phosphorylase activator – AMP
- Allosteric - Phosphorylase inhibitor - ATP, G6P and glucose

Two proteins modulate activity by covalent modification – phosphorylation

1 phosphorylase kinase
   - phosphorlylates 1 Ser / subunit
   - activated by cAMP/PKA pathway (glucagon and epinephrine) and Calcium
2 protein phosphatase 1
   - general phosphatase under control of insulin

Differences between muscle and liver
Muscle phosphorylase
   - Inactive b form activated by low energy signal AMP (leads to increased glucose for muscular activity)
   - Glucose 6-P and ATP (high energy signals) reverse b form activation
Liver phosphorylase
   - Active a form is converted to the b form by glucose (not muscle form)
   - Therefore even with covalent modification when enough glucose is present in the liver cell, glycogenolysis stops, but not in the liver. You must remember that it is not easy to build up liver glucose levels due to G6Pase.

Allosteric and covalent modification regulation of both muscle and liver leads to use of glycogen glucose for muscle and liver glucose for export
Glycogen synthase Regulation

Glycogen synthase -
- phosphorylated at C and N terminals increases net charge from -13 to -31.
- active (a) form is dephosphorylated
- inactive (b) form is phosphorylated
- phosphorylation controlled via cAMP by PKA

Glycogen Synthase Regulatory Proteins –
Protein Kinase A - regulates the activity of both phosphorylase and synthase.

Regulatory proteins -
Phosphorylase kinase - phosphorylates phosphorylase
- Duel controlled enzyme
- Exact regulation is still not totally clear, but there are four different subunits some in different amounts. α, β, γ and δ.
- Both the α and the β subunits are phosphorylated by PKA - this leads to a highly active phosphorylase kinase when Ca^{2+} is also present.
- The gamma subunit is similar to a protein kinase and acts as a pseudosubstrate (kind of like the regulatory subunits of PKA) for phosphorylase kinase -key glutamate
  - highly active form when phosphorylated by PKC
- Increased Ca^{2+} levels partially activate kinase (low active form) via nervous activity/muscle contraction/epinephrine
- Ca^{2+} activates by non-covalent interaction with a subunit of the phosphorylase kinase (calmodulin; CaM)
  - CaM is a ubiquitous Ca^{2+} binding protein that interacts with many other proteins. Cytosolic Ca^{2+} levels are tightly controlled and only transiently increase.
  - Most Ca^{2+} is stored in mitochondria and endoplasmic reticulum.
  - Calmodulin is also a subunit of phosphorylase kinase (δ)
  - Calmodulin binds Ca^{2+} in a central loop (EF
hand) that causes the central helix to alter its conformation.

- When Ca$$^{2+}$$ levels increase, calmodulin pulls the gamma subunit of phosphorylase away from the active site of phosphorylase kinase, allowing activation of the enzyme.

**Protein phosphatase 1 (PPI) multiple phosphorylation sites**

- There are several protein phosphatases - most are many times more active (think about what that means) than the protein kinases. Generally these are not specific PPases and are not highly regulated. Except for...
- PP1 increases glycogen synthesis and inhibits glycogen phosphorylase
- PP1 removes the phosphoryl groups from phosphorylase kinase (α and β subunit)
- PP1 also removes the phosphoryl group from glycogen synthase (think of this consequence)
- Regulation of PP1
- There are two subunits of PP1, the G protein (glycogen binding) and the catalytic domain.
- PP1, G protein and glycogen must be in a complex for phosphatase activity to occur.
- G protein binds glycogen and acts to recruit PPI to glycogen complex
- Increases PPI/G protein interactions result when the G protein is phosphorylated at 1 residue (controlled by insulin)
- PKA also phosphorylates the G protein at a different site and inhibits the G protein - PP1 from binding. The phosphatase is then inactive.
**Glycogen storage disorders**

clinical manifestations is fatty liver -> distended abdomen
many different kinds depending on mutated enzyme

Von Gierke’s Disease –
- clinical manifestations is fatty liver -> distended abdomen
- many different kinds depending on mutated enzyme
- Von Gierke’s Disease - G 6-Pase or transporters missing
- normal glycogen but high levels of trapped phospho-sugars
- surgery to liver and controlled feedings treat this disease. One of the patients was discovered in Fargo. Dr. Nordlie at UNDSM is the leader in the study of this disease.

McArdle’s disease - Found after cramps at onset of exercise
- ADP concentrations increase initially and decrease w/ more exercise
- phosphorylase kinase missing in muscle but liver present (isozymes) - what is happening? Muscle glycogen is NOT available. The muscles are probably damaged due to lack of ATP. With lower levels of activity, glucose (exported from liver) can enter and take the place of glycogenesis.

Pompe’s Disease - Missing glucosidase activity, usually found in lysosomes.
- Leads to large increases in glycogen found in lysosomes in nearly every tissue in the body. Once the glycogen particles are in the lysosome it can no longer function normally, although extralysosomal glycogen acts as normal. The reason for this is not known, but results in cardiomegaly and death occurs at an early age from heart failure.