

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 holoenzyme
- 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 re-arrangement 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

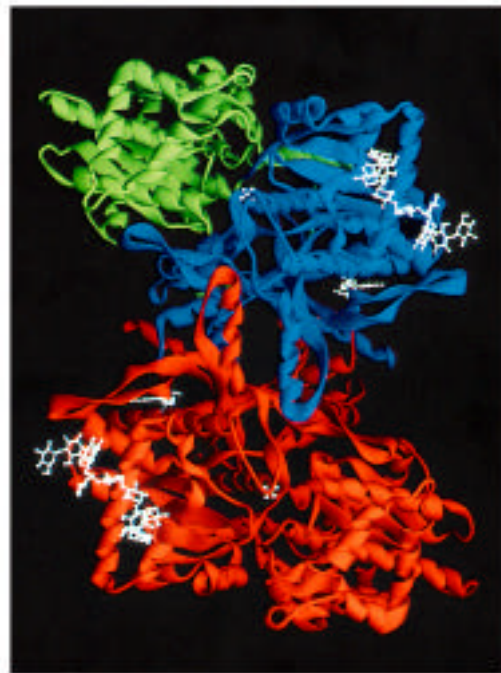
Phosphorylation of Ser 14 leads to a T to R conformation shift as the **negative charges of the phospho group interacts with positive charged Arg.**

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

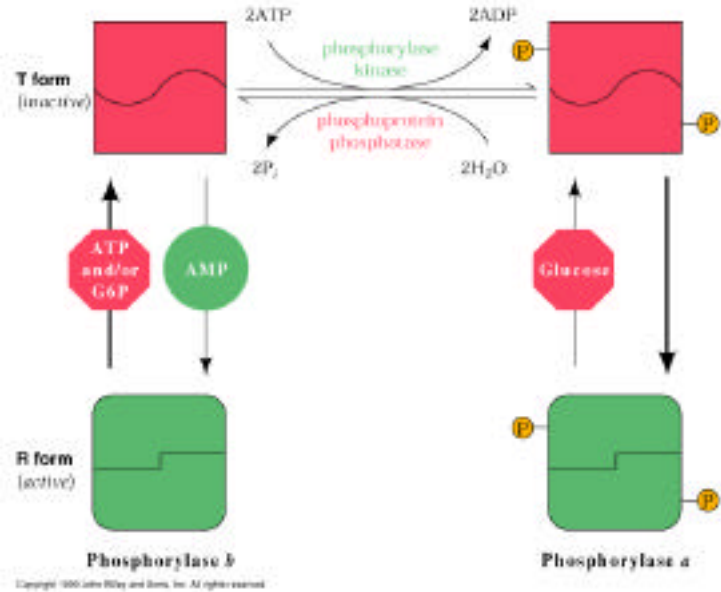
KNOW the structural mechanics of this enzyme

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



Courtesy of Wayne Spring, University of Texas Southwestern Medical Center

- 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

- phosphorylates 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 muscle. 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 -

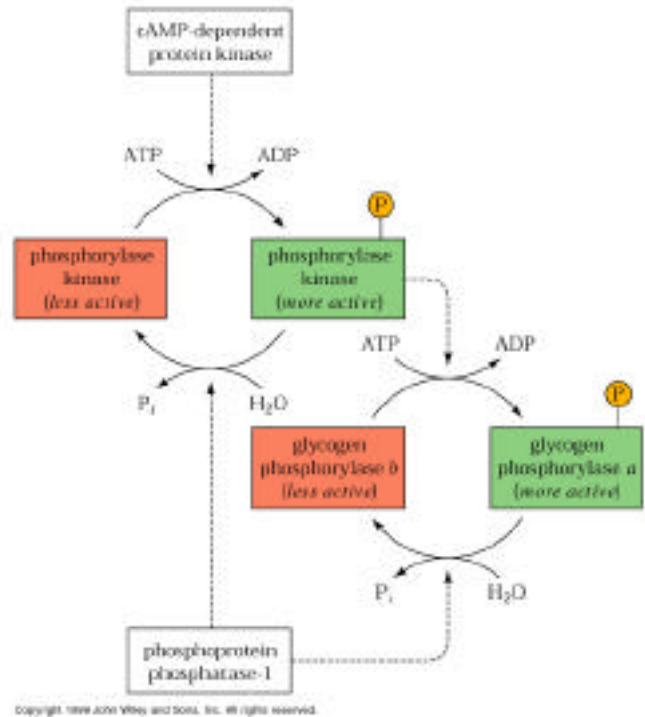
Protein Kinase A - regulates the activity of both phosphorylase and synthase.

Regulatory proteins -

Phosphorylase kinase -

phosphorylates phosphorylase

- Dual controlled enzyme
- Exact regulation is still not totally clear, but there are four different subunits some in different amounts. α , β , and γ .
- 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



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Courtesy of Mike Carson, University of Alabama at Birmingham
 & Rex Krieger, Department of Chemistry, University of Tennessee at Birmingham

activate kinase (low active form) via nervous activity/muscle contraction/epinephrine

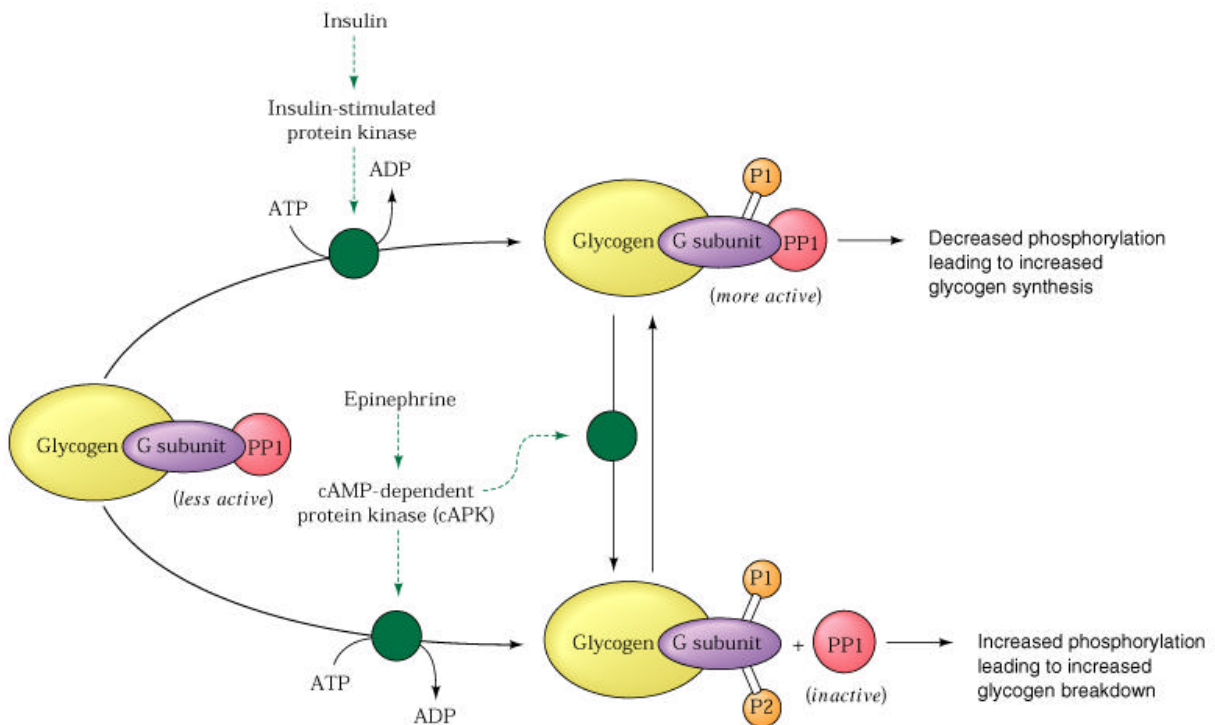
- Ca^{+2} activates by non-covalent interaction with a subunit of the phosphorylase kinase (calmodulin; CaM)
- CaM is a ubiquitous Ca^{+2} binding protein that interacts with many other proteins. Cytosolic Ca^{+2} levels are tightly controlled and only transiently increase.
- Most Ca^{+2} is stored in mitochondria and endoplasmic reticulum.
- Calmodulin is also a subunit of phosphorylase kinase ()
- 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 PP1 to glycogen complex
- Increases PP1/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.



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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 glycogene acts as normal. The reason for this is not known, but results in cardiomegaly and death occurs at an early age from heart failure.