Glycogen Metabolism

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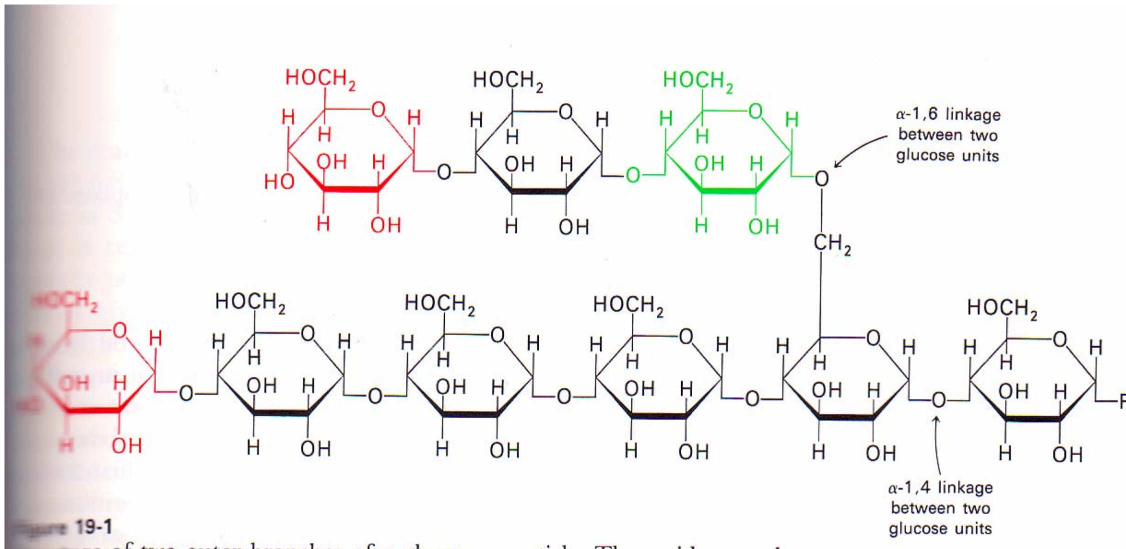
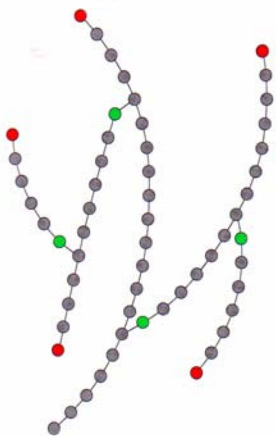
Bryant Miles

Glycogen is the storage polysaccharide of animals. It is present in all cells, but it is most prevalent in the liver and the muscles. Glycogen consists of glucose molecules linked together with $\alpha(1\rightarrow4)$linkages with $\alpha(1\rightarrow6)$ branch points occurring every 8 to 12 residues. The purpose of the high branched structure is to have many nonreducing ends so that glucose can be rapidly mobilized in times of metabolic needs.



The nonreducing ends are shown in red. The residue that starts a branch is shown in green.



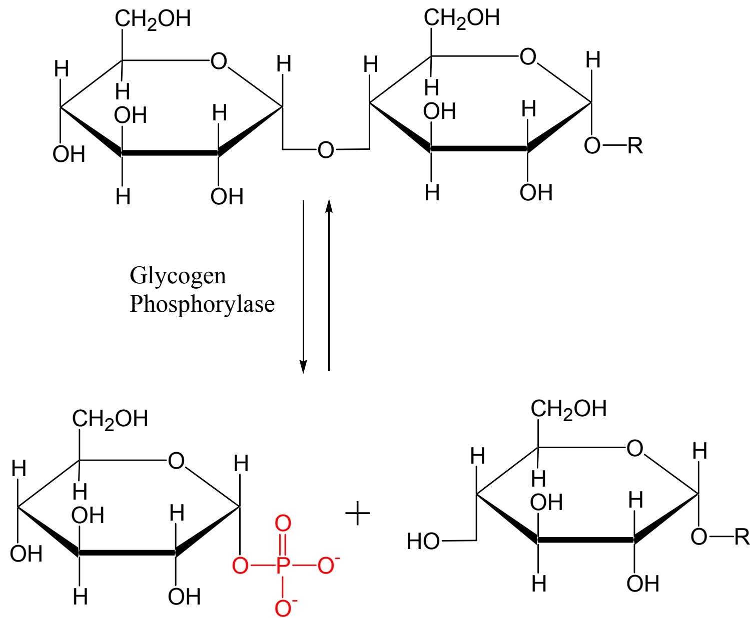
Glycogen metabolism is important for several reasons.

- Glycogen stores in the liver are used to maintain a constant blood glucose concentration. Muscles also maintain glycogen stores as a reservoir of glucose for strenuous muscular activity.
- The synthesis and degradation of glycogen occur by different metabolic pathways allowing for reciprocal regulation.
- In addition, the enzymes of glycogen metabolism are under hormonal regulation.

The biochemical pioneers of glycogen metabolism were the Cori's, Carl and Gerty, a husband and wife team. They demonstrated the glycogen is broken down by phosphorolysis.

$\text{Glycogen}_n + \text{Pi}$ $\text{Glucose-1-phosphate} + \text{glycogen}_{n-1}$

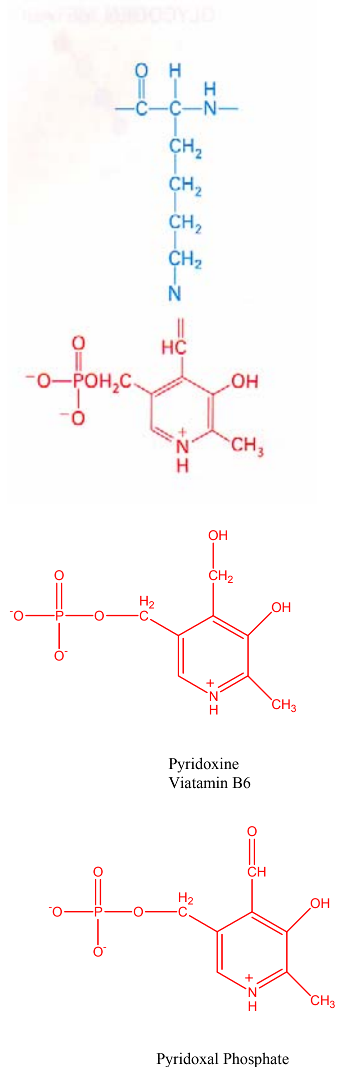
Glycogen phosphorylase catalyzes this reaction. This enzyme catalyzes the sequential phosphorolysis of glucose residues from a nonreducing end. The bond between the C1 carbon atom and the glycosidic oxygen atom is cleaved by inorganic phosphate in such a way that the stereochemistry at the C1 carbon is maintained.



This phosphorolytic cleavage is advantageous because the cell is saved the expense of phosphorylating glucose with ATP. In addition the phosphorylated glucose cannot diffuse out of the cell.

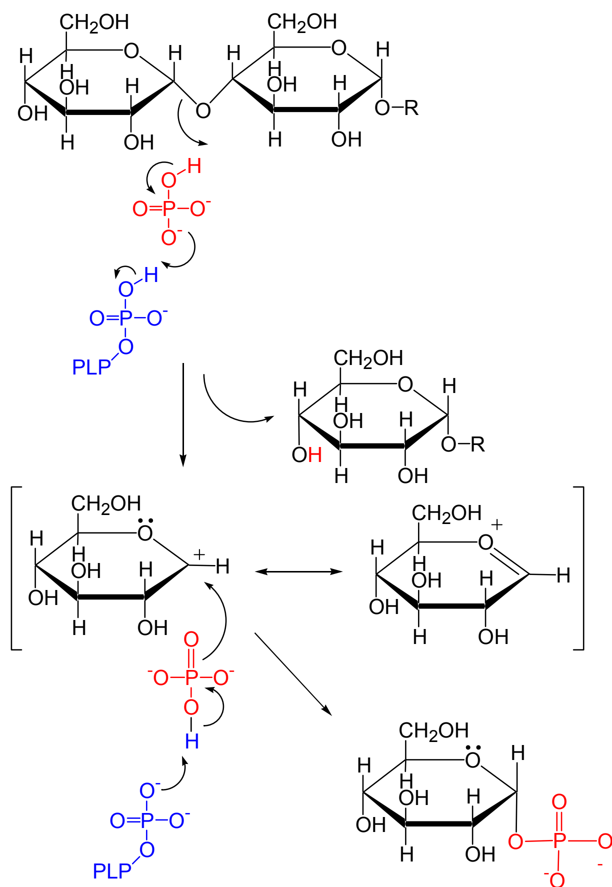
The retention of configuration at the stereocenter is an important clue into the mechanism of this enzyme. Sn2 substitution reactions proceed with inversion of configuration at the stereocenter. Retention implies a Sn1 like substitution mechanism. Sn1 mechanism begins with the dissociation of the leaving group to form a carbocation.

Glycogen phosphorylase requires a pyridoxl-5'-phosphate cofactor. This cofactor is covalently bound to a lysine residue via a Schiff base. Crystal structures show that the inorganic phosphate molecule lies between the pyridoxal-5'-phosphate and the glycogen substrate. It appears that the 5'-phosphate of pyridoxal phosphate functions as a general acid/base during catalysis. The inorganic phosphate donates its hydrogen to the glycogen n-1 residue to form a resonance stabilized oxonium ion. The oxonium ion is attacted by the phosphate to form a-glucose-1-phosphate. One important aspect of this enzyme is that water is completely excluded from the active site.

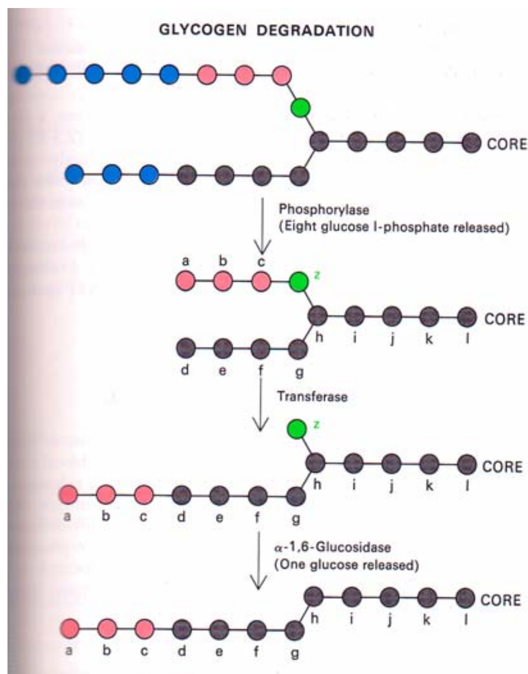


Pyridoxine
Viatamin B6

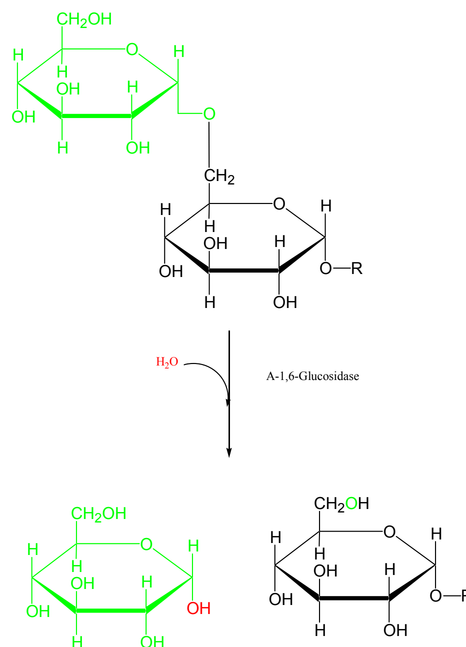
Pyridoxal Phosphate



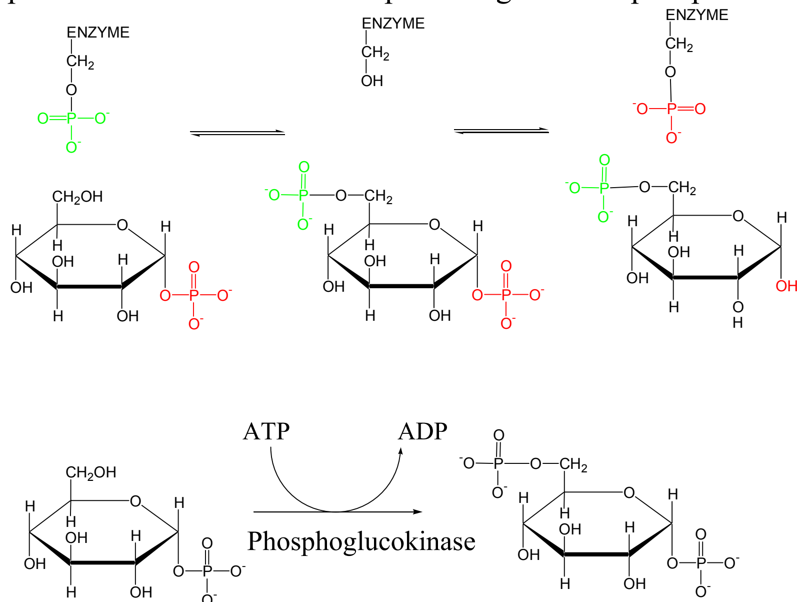
What happens at the branch points?



Glycogen phosphorylase degrades glycogen's α(1→4) glycosidic bonds sequentially until it gets four residues away from a α(1→6) branch point where its activity ceases. A another enzyme is required to remove the branches. This enzyme is called the debranching enzyme. The debranching enzyme has two active sites, each with its own unique activity. One active site, *the transferase*, catalyzes the transfer of blocks of 3 glycosyl residues from one outer branch to another. The other enzyme active site, *the α-1,6-glucosidase*, cleaves the α(1→6) glycosidic linkage.



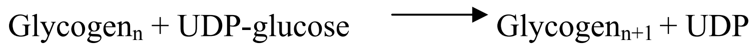
The glucose-1-phosphate formed by glycogen phosphorylase is isomerized into glucose-6-phosphate by phosphoglucoisomerase. The enzyme has an active site serine residue that must be phosphorylated for the enzyme to be active. The mechanism of the isomerization is very similar to that of phosphoglycerate mutase. The phosphoryl group attached to the serine is transferred to the C6 hydroxyl group to form a glucose-1-6-bisphosphate intermediate followed by the transfer of the phosphoryl group attached to the C1 position back to the serine to produce glucose-6-phosphate.



The glucose-1,6-bisphosphate formed can dissociate out of the active site before transferring the C1 phosphoryl group. When this happens, the active site serine must be rephosphorylated. There is an enzyme called phosphoglucokinase which phosphorylates glucose-1-phosphate to form glucose-1,6-bisphosphate. Which can bind to the dephosphorylated enzyme and transfer the C1-phosphoryl group to reactivated the enzyme and produce glucose-6-phosphate.

Glycogen Biosynthesis

Luis Leloir discovered the glycogen biosynthetic pathway.



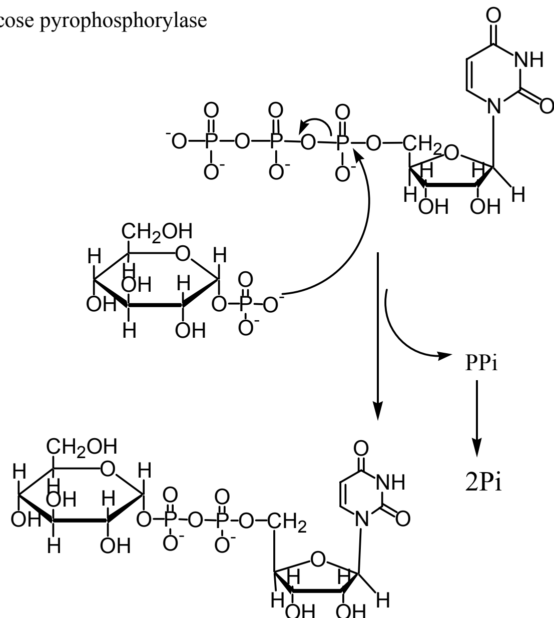
$\text{Glycogen}_n + \text{UDP-glucose} \longrightarrow \text{Glycogen}_{n+1} + \text{UDP}$

Compare the synthetic pathway to the degradative pathway:

$\text{Glycogen}_{n+1} + \text{Pi} \longrightarrow \text{Glycogen}_n + \text{Glucose-1-phosphate}$

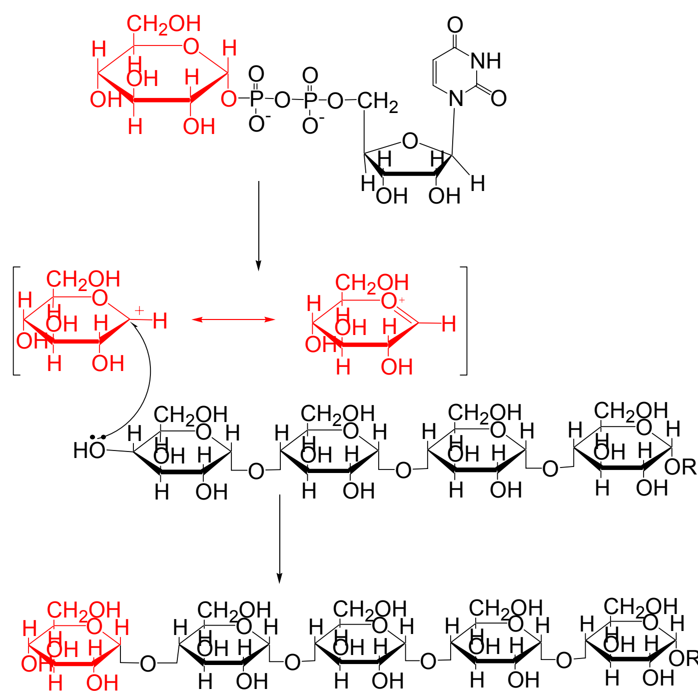
Clearly glycogen biosynthesis is not merely the reversal of the degradative pathway. The two pathways are distinct providing a mechanism for reciprocal control.

UDP-Glucose pyrophosphorylase



The first step of glycogen biosynthesis is the synthesis of UDP-glucose from glucose-1-phosphate and UTP. The enzyme that catalyzes this reactionis **UDP-glucose pyrophosphorylase**. Just as acyl-CoA carries activated acyl groups and ATP carries activated phosphoryl groups, UDP-glucose carries activated glucose molecules.

The phosphate oxygen of glucose-1-phosphate attacks the α-phosphoryl group of UTP to form UDP-glucose and pyrophosphate. The pyrophosphate formed is quickly hydrolyzed by the enzyme inorganic pyrophosphatase, which makes this step irreversible. This is one more example of a biosynthetic reaction driven by the hydrolysis of pyrophosphatase.



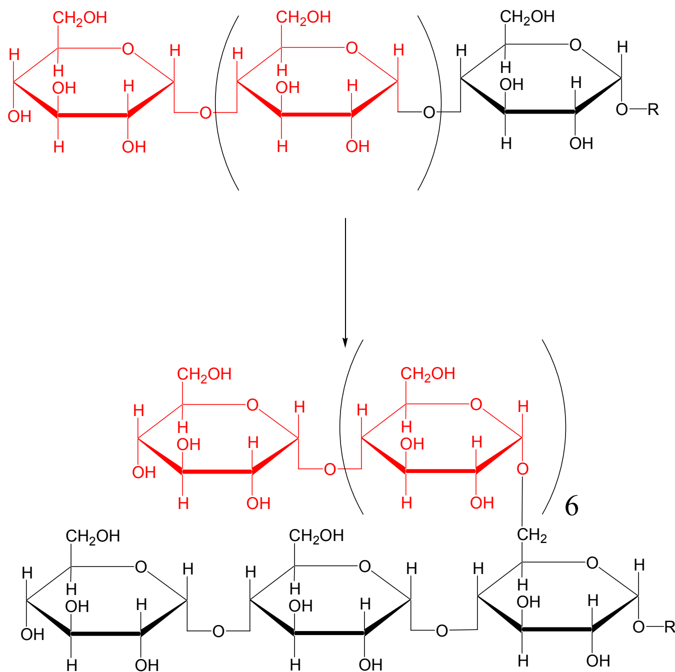
New glucosyl residues are added to the nonreducing ends of glycogen by the enzyme **glycogen synthase** which can add glucose residues to polysaccharide chain of four or more residues.

Thus glycogen synthesis requires a primer. The primer is a protein called **glycogenin** which contains a oligosaccharide of α-1,4-glucose residues attached to a phenolic oxygen of a tyrosine residue.

The first step of glycogen synthesis begins with the enzyme **tyrosine glucosyltransferase** which attaches a glucose molecule to the TYR-195-OH of glycogenin. Glycogenin then autocatalyitically adds up to seven glucose residues to form a glycogen primer. Each end of a glycogen molecule is attached to a molecule of glycogenin.

The Branching Enzyme.

Glycogen synthetase can synthesize α(1→4) linkages. Another enzyme is required to form the α(1→6) linkages that make the branches. Branching is important because it greatly increases the solubility of glycogen and increases the number of nonreducing ends increasing both the rate of glycogen biosynthesis and degradation.



The enzyme that synthesizes these branch points is called the **branching enzyme**.
The branching enzymes takes a block of seven or so residues of a nonreducing end and transfers these seven residues to an interior site and creates an α(1→6) linkage. The chain that donates the seven residues must be at least 11 residues long, and the new branch point must be at least four residues away from preexisting branch points.

The efficiency of storing glucose as glycogen.

The reactions of glycogen biosynthesis are shown below.

	Reaction	Enzyme
1.	Glucose-6-phosphate → Glucose-1-phosphate	Phosphoglucomutase
2.	Glucose-1-phosphate + UTP → UDP-Glucose + PPi	UDP-glucose pyrophosphorylase
3.	PPi + H_2O → 2Pi	Inorganic pyrophosphatase
4.	UDP-Glucose + $Glycogen_n$ → UDP + $Glycogen_{n+1}$	Glycogen synthase
5.	UDP + ATP → UTP + ADP	Nucleotide diphosphokinase

Sum: Glucose-6-phosphate + ATP + $Glycogen_n$ + H_2O → $Glycogen_{n+1}$ + ADP + 2Pi

90% of glycogen phosphorylytically cleaved into glucose-1-phosphate which is isomerized into glucose-6-phosphate.

10% are the branched residues which are hydrolyzed into glucose which can be phosphorlated into glucose-6-phosphate.

The complete oxidation of glucose-6-phosphate via glycolysis, the citric acid cycle and oxidative phosphorylation yields 38 molecules of ATP. The overall efficiency of storage is 97%.