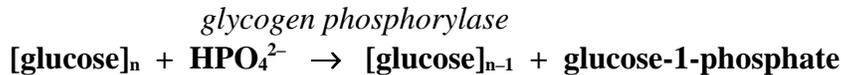


Chem*3560 Lecture 9: Glycogen phosphorylase

Glycogen synthesis and breakdown govern availability of glucose in animals

Glycogen is stored in the body in liver and in skeletal muscles. A normal 70 kg person has about 50-100 kJ worth of free glucose, and 5000 kJ worth of glycogen. **Glycogen phosphorylase** is the enzyme responsible for glycogen breakdown in liver and muscle. Glycogen phosphorylase uses inorganic HPO_4^{2-} to split glucose from the polysaccharide chains of glycogen (Lehninger p.283):

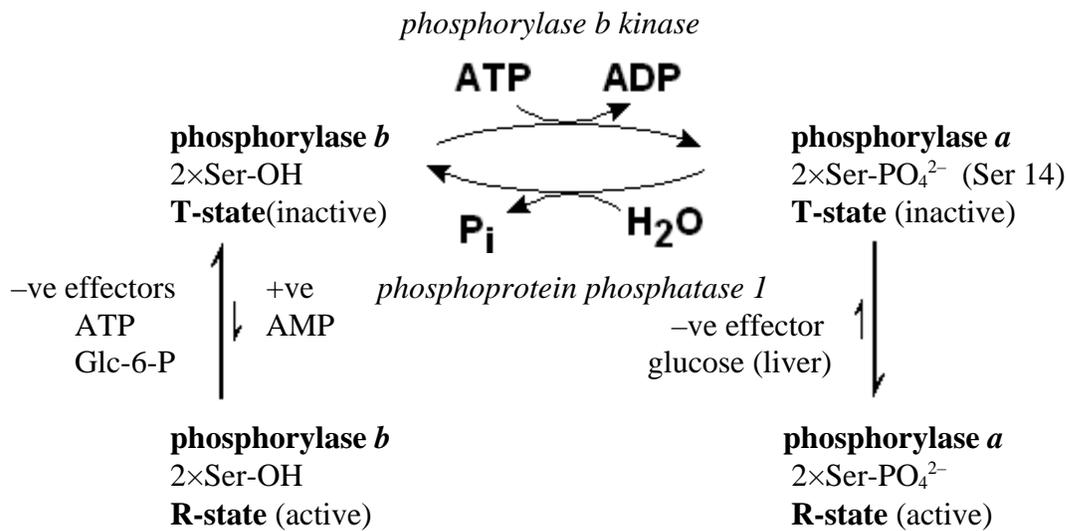


This makes glucose-1-phosphate available for glycolysis without using an ATP.

The term **phosphorylase** (a charter member of the group of Confusingly Named Enzymes) is not derived from the process of phosphorylation, but **phosphorolysis**, breaking a bond in glycogen by attack of HPO_4^{2-} , just as a **hydrolysis** is the breaking of bonds by attack of H_2O , catalyzed by enzymes classed as **hydrolases**. Phosphorylases other than glycogen phosphorylase also exist, e.g. polynucleotide phosphorylase.

Glycogen phosphorylase exists in two forms, **unphosphorylated phosphorylase b** and **phosphorylase a** with **two Ser- PO_4^{2-} groups**. Protein phosphate is added by **phosphorylase b kinase** in the PKA driven cascade already described. Phosphate is removed from serine in phosphorylase b kinase as well as from phosphorylase a by **phosphoprotein phosphatase 1**.

Glycogen phosphorylase is a dimer of two identical subunits, with a slight difference in the liver and muscle versions. The enzyme is under allosteric regulation, with T and R states, as well as regulation by phosphorylation (Lehninger p.557). This makes the regulatory scheme **two dimensional**, with phosphorylation on the horizontal axis and allosteric regulation on the vertical axis:

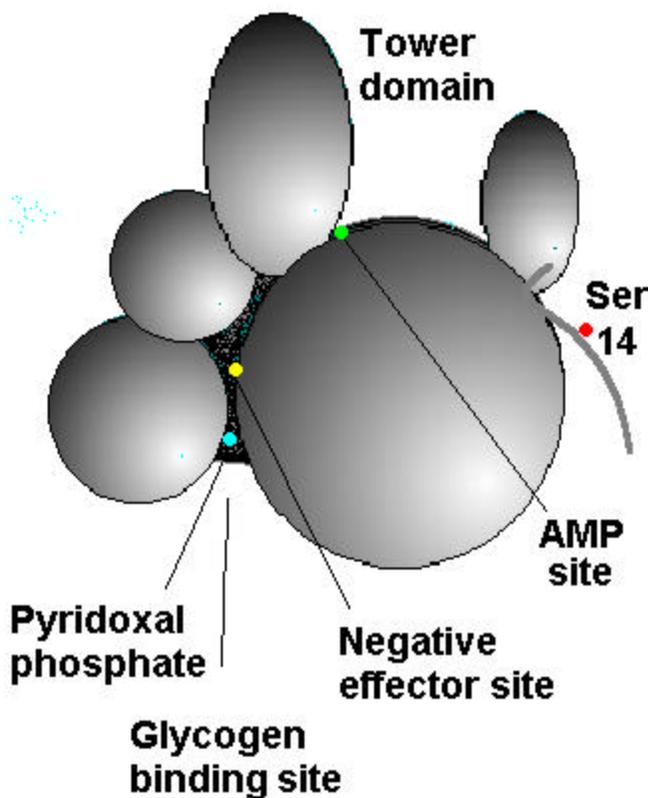


The key difference between phosphorylase *a* and phosphorylase *b* is that the *a* form (Ser-PO₄²⁻) is **biased in favour of active R state**, whereas the *b* form (Ser-OH) is biased in favour of T-state.

Phosphorylase *b* is sensitive to negative allosteric effectors ATP (high energy level means less need to breakdown glycogen) and Glucose-6-phosphate (when present, there's less need to continue glycogen breakdown). AMP is a positive effector for muscle glycogen phosphorylase, overriding the negative effect of ATP. AMP levels rise in active muscle, signalling need for more glucose release. Liver tends to conserve the glycogen reserve if energy is low (AMP high), and rely on other energy substrates such as amino acids.

The liver form of phosphorylase *a* is sensitive to glucose as a negative effector. This is to conserve glycogen if other glucose sources are currently available.

The phosphorylation control of glycogen phosphorylase is a response to **messages from outside the cell, signalled by hormone**. Allosteric control is a response to **conditions inside the cell** signalled by **molecules that would be present in different energy states of the cell**.



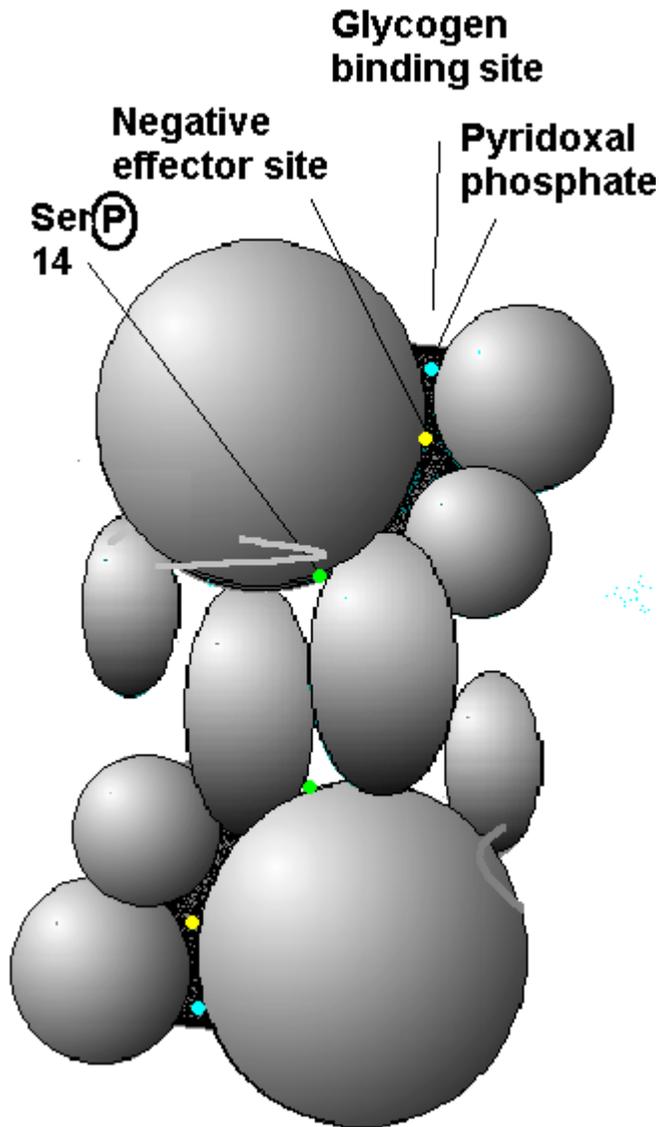
Structure of glycogen phosphorylase

Glycogen phosphorylase is a dimer of **two identical 94 kDa subunits**, arranged in several domains. One subunit of the unphosphorylated *b* form is shown, with the binding sites indicated.

A binding site for the negative effectors is located near the catalytic site, which contains **pyridoxal phosphate**. When ATP or glucose bind, a loop of amino acids (280-289 in the sequence) is displaced, resulting in the **low activity T state**. The position of this loop affects the conformation of the “tower” domain.

The positive effector, AMP, binds in a separate location near the base of the so-called “tower” domain and has the opposite effect on its conformation, promoting R state. When Glc-6-P binds here, it excludes AMP.

Ser 14, the phosphorylation site, is on an unstructured loop near the N-terminus.



The dimer forms by interlocking the “tower” domains, which communicate the allosteric state, T or R, between opposite subunits.

In phosphorylase *a*, Ser 14 is phosphorylated, and the N-terminal loop can rearrange itself so that the Ser-PO₄²⁻ can fit into the positive effector site, and play the same activating role as AMP.

Glycogen phosphorylase uses the coenzyme **pyridoxal phosphate** (PLP) as part of the catalytic mechanism. Pyridoxal phosphate is covalently bonded to Lys 679 by reaction of the lysine amino with the pyridoxal aldehyde group.

The phosphate group of PLP is used for catalysis. What is needed is a **weak acid** to act as a **general acid catalyst** in one step of the reaction, and for its anion to act as a **general base** in the second step.

Since phosphates have second pK_a = 6.5, they are equally good at donating or accepting back H⁺ ions at neutral pH, and can thus play the role of base better than low pK_a amino acid side chains like Glu or Asp.

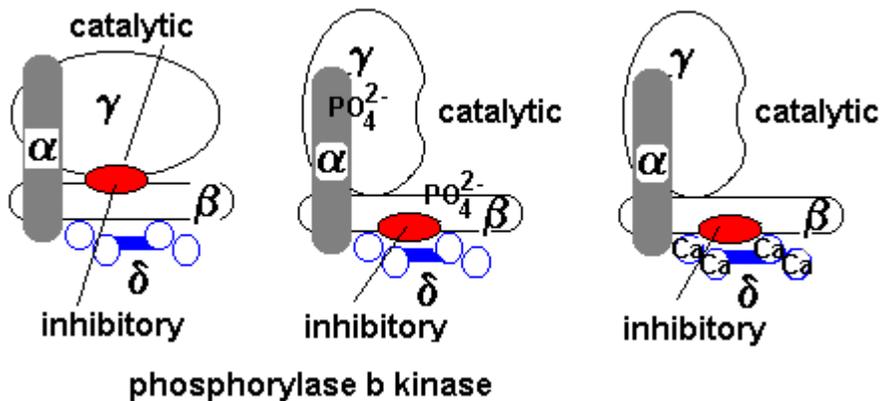
Regulation of phosphorylase b kinase

Phosphorylase b **kinase** (the enzyme that converts glycogen phosphorylase from inactive b to active a) is a tetramer of four **different** subunits **αβγδ**. Of these, the **γ-subunit** is very similar to PKA **C subunit**, and is the catalytic component of the kinase.

The **β-subunit** contains an autoinhibitory loop that mimics the substrate sequence, and so binds to and **masks the catalytic γ-subunit**, similar to the mode of action of PKA **R subunit**. However, instead of binding cAMP to unmask the catalytic site, phosphorylase b kinase is modified by **PKA**. When key Ser-PO₄²⁻ phosphate groups are present in the α and β subunits, this frees up the γ-subunit for catalytic action.

Phosphorylase b kinase can also be activated by Ca^{2+}

What was called the δ -subunit of phosphorylase b kinase turns out to be the Ca^{2+} -binding protein, **calmodulin**, the Calcium-binding protein that **modulates** activity. Calmodulin is found as a Ca^{2+} dependent regulating factor in a number of other proteins, including several other kinds of protein kinase.



Calmodulin is a dumbbell shaped molecule, with helical domains at each end of a long straight helix. There are **four Ca^{2+} binding sites**, two at each end, which lie in loops that connect pairs of helices. The loops are rich in Glu and Asp, which serve as the Ca^{2+} ligands.

When the calmodulin in phosphorylase b kinase binds Ca^{2+} , it masks the autoinhibitory domain of the β -subunit, freeing up the catalytic γ -subunit, and providing a second mode of activation.

Muscle contraction is normally initiated by a rise Ca^{2+} , therefore the presence of high Ca^{2+} can also stimulate glycogen breakdown to provide substrate for glycolysis and ATP production.

Terminology that you need to keep straight

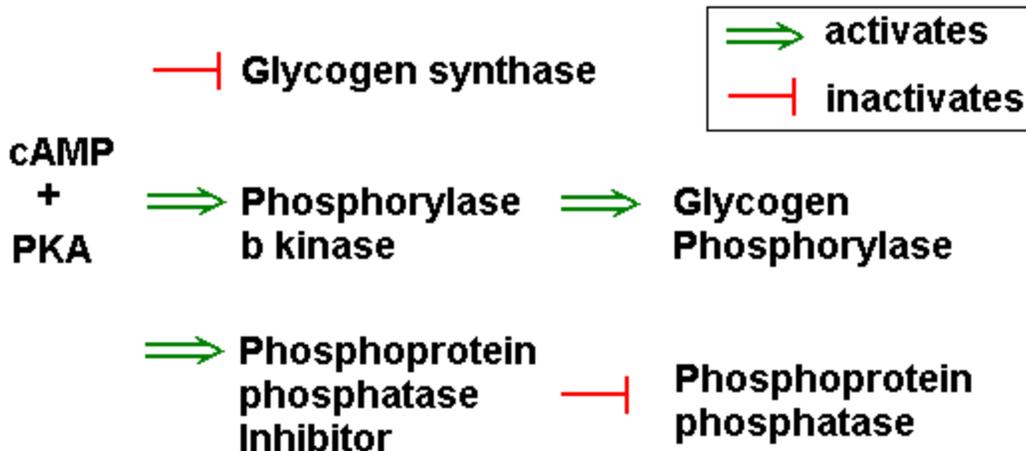
Phosphorylase: an enzyme that breaks a bond by attack of HPO_4^{2-} (phosphorolysis).

Kinase: an enzyme that transfers phosphate onto a substrate, usually from ATP as donor, a process often referred to as **phosphorylation**.

Phosphatase: an enzyme that removes phosphate by hydrolysis of the phosphate ester bond.

Regulation of phosphoprotein phosphatase 1

Phosphoprotein phosphatase 1 is responsible for removing the activating phosphate groups from Ser side chains in both phosphorylase b kinase and phosphorylase a. The activity of the phosphatase is affected by **phosphoprotein phosphatase 1 inhibitor**, a protein with a substrate-like sequence that occupies the catalytic site of the phosphatase.



The inhibitor binds to phosphatase only if the inhibitor is phosphorylated by PKA. The inhibitor's role is therefore to **keep the phosphoprotein phosphatase busy** while the other components of the glycogen phosphorylase cascade are activated. When the hormone signal disappears and PKA stops being active, the phosphatase will eventually remove the phosphate and deactivate its inhibitor, and will then be free to deal with Ser-PO₄²⁻ in the other components of the cascade.

