Enzymes
Part III: regulation II

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Reversible covalent modification
This is a major mechanism for rapid and transient regulation of enzyme activity.

A most common mechanism is enzyme phosphorylation (the covalent addition of a phosphate group to one of its amino acid side chains).

The usual sites for phosphate addition to proteins are the serine, threonine and tyrosine R group hydroxyl residues.
Enzymes

ATP mostly is the phosphoryl donor in these reactions, which are catalyzed by protein **kinases**.

The removal of phosphoryl groups (dephosphorylation) by hydrolysis is catalyzed by protein **phosphatases**.

Note: dephosphorylation is not the reversal of phosphorylation.

The addition or removal of a phosphate group to an enzyme may activate or inactivate these enzymes.
Why is it effective?

- A phosphoryl group adds two negative charges to a modified protein allowing for the formation of new electrostatic interactions. Such structural changes can markedly alter substrate binding and catalytic activity.
- A phosphate group can form three or more hydrogen bonds allowing for specific interactions with hydrogen-bond donors.
- Phosphorylation and dephosphorylation can take place in less than a second or over a span of hours.
- Phosphorylation often causes highly amplified effects. A single activated kinase can phosphorylate hundreds of target proteins in a short interval. Further amplification can take place if the target protein is an enzyme.
A good example is glycogen Phosphorylase, which catalyzes removal of glucose molecules from glycogen. The Ser residue that is phosphorylated is remote from the active site of phosphorylase. The enzyme exists in two forms:

- A phosphorylated active form “a”
- A dephosphorylated inactive form “b”
Both phosphorylase $b$ and phosphorylase $a$ exist as equilibria between an active R state and a less-active T state.

Phosphorylase $b$ is usually inactive because the equilibrium favors the T state.

Phosphorylase $a$ is usually active because the equilibrium favors the R state.

The transition of phosphorylase $b$ between the T and the R state is controlled by the energy charge of the muscle cell.
Others

**Adenylylation (addition of adenylyl group).** AMP (from ATP) is transferred to a Tyr hydroxyl by a phosphodiester linkage. The addition of bulky AMP inhibits certain cytosolic enzymes.

**Uridylylation (addition of uridylyl group).**
ADP-ribosylation (addition of adenosine diphosphate ribosyl group). This inactivates key cellular enzymes.

Methylation (addition of a methyl group). Methylation on carboxylate side chains masks a negative charge and add hydrophobicity.

Acetylation (addition of an acetyl group donated by acetyl CoA). It masks positive charges when added to lysine residues.
Regulation via modulators
Small-molecule modulators can have dramatic effects on enzymes.

For example, cAMP, which is structurally modified AMP, can activate protein kinase A (PKA).
What do ATP and AMP do?

Muscle phosphorylase b is active only in the presence of high concentrations of AMP, which binds to a nucleotide-binding site and stabilizes the conformation of phosphorylase b in the R state.

ATP acts as a negative allosteric effector by competing with AMP and so favors the T state.

Glucose 6-phosphate also favors the T state of phosphorylase b, an example of feedback inhibition.
Protein kinase A (PKA), a serine/threonine protein kinase, phosphorylates several enzymes that regulate different metabolic pathways.

Glycogen phosphorylase kinase

PKA consists of two kinds of subunits: a regulatory (R) subunit, which has high affinity for cAMP, and a catalytic (C) subunit.

In the absence of cAMP, the regulatory and catalytic subunits form an inactive complex.
The binding of two molecules of cAMP to each of the regulatory subunits leads to the dissociation of R2C2 into an R2 subunit and two C subunits.

These free catalytic subunits are then enzymatically active.
Covalent and allosteric regulation of glycogen phosphorylase in muscle.

(a) The enzyme has two identical subunits, each of which can be phosphorylated by phosphorylase $b$ kinase at Ser$^{14}$ to give phosphorylase $a$, a reaction promoted by Ca$^{2+}$. Phosphorylase $a$ phosphatase, also called phosphoprotein phosphatase-1, removes these phosphate groups, inactivating the enzyme. Phosphorylase $b$ can also be activated by noncovalent binding of AMP at its allosteric sites. Conformational changes in the enzyme are indicated schematically. Liver glycogen phosphorylase undergoes similar $a$ and $b$ interconversions, but has different regulatory mechanisms.
Phosphorylation cascade

Epinephrine

Glucagon

Glycogen synthesis

Glycogen synthase a

Protein kinase A

Glycogen synthase b

ATP

ADP

Phosphorylase kinase

Phosphorylase kinase

Glycogen phosphorylase b

Glycogen phosphorylase a

ATP

ADP

Ca^{2+}

Glycogen degradation

cAMP

+ cAMP

Principles of Biochemistry, 4/e
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Regulation - Large regulatory molecules

G protein: a family of trans-membrane proteins causing changes inside the cell. They communicate signals from hormones, neurotransmitters, and other signaling factors.

- When they bind guanosine triphosphate (GTP), they are 'on', and, when they bind guanosine diphosphate (GDP), they are 'off'.

- The α subunit can be stimulatory or inhibitory.
Ca\textsubscript{2+}-calmodulin binds to several different proteins and regulates their function including glycogen phosphorylase kinase.

**Regulation of Glycogenolysis in Muscle**
- Epinephrine & Ca\textsuperscript{2+} \text{ON}
- Allosteric activator AMP \text{ON}

Muscle contraction
- Ca\textsuperscript{2+} release (via CaM)

Exercise
- AMP

Glycogen phosphorylase
- Inactive
- Active

Phosphorylase Kinase
- Inactive
- Active

Protein Kinase A
- Activates

Neurotransmitter signaling
- Epinephrine
- Activates

Flexible region between domains
When GTP is bound, the conformation of the G protein allows it to bind target proteins, which are then activated or inhibited.

The G protein hydrolyzes a phosphate from GTP to form GDP, which changes the G protein conformation and causes it to dissociate from the target protein.

GDP is exchanged for GTP, which reactivates the G protein.

The activity of many G proteins is regulated by
1. GAPs [GTPase-activating proteins]
2. GEFs [guanine nucleotide exchange factors]
3. GDIs [GDP dissociation inhibitors]