Enzymes
Part III: regulation II

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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).
Protein kinase A (PKA), a serine/threonine protein kinase, phosphorylates several enzymes that regulate different metabolic pathways.

Example: glycogen phosphorylase kinase

When inactive, PKA consists of four subunits

- Two regulatory (R) subunits with high affinity for cAMP,
- Two catalytic (C) subunits
The binding of two molecules of cAMP to the regulatory subunits leads to the dissociation of R2C2 into an R2 subunit and two active C subunits.
Reversible covalent modification
Advantage

Rapid and transient.

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

Usually serine, threonine, and tyrosine.
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?

- Formation or removal of new electrostatic interactions and/or hydrogen bonds altering substrate binding and catalytic activity.
- It can happen in less than a second or over a span of hours.
- Phosphorylation often causes highly amplified effects.
Adenylylation (addition of adenylyl group). AMP is transferred to Tyr residues through phosphodiester linkage.

The addition of bulky AMP inhibits cytosolic enzymes.

Uridylylation (addition of uridylyl group).
ADP-ribosylation (addition of adenosine diphosphate ribosyl group) inactivates enzymes.
Methylation of carboxylate side chains masking negative charges.
Acetylation (from acetyl Co) to lysine residues masking positive charges.
Example: Glycogen phosphorylase

\[
\text{glycogen}_{(n \text{ residues})} + P_i \rightarrow \text{glycogen}_{(n-1 \text{ residues})} + \text{glucose 1-P}
\]

- GP catalyzes removal of glucose molecules from glycogen.
- The phosphorylated Ser residue is remote from the active site.
- The enzyme exists in two forms:
  - A phosphorylated active form “a”
  - A dephosphorylated inactive form “b”
The two forms of the enzyme

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 (ATP and AMP) of the muscle cell.
The diagram illustrates the conversion between the T form and R form of phosphorylase, which are inactive and active states, respectively. The T form can be activated through phosphorylation by phosphorylase kinase and dephosphorylated by phosphoprotein phosphatase.

- **T form (inactive)**: Requires ATP or G6P to activate.
- **R form (active)**: Can be dephosphorylated to revert to the inactive state.

The reactions involved are:
- ATP and G6P activate the T form to R form.
- AMP inactivates the R form to T form.
- Glucose activates the R form to T form.
- Water dephosphorylates the R form to T form.

The process involves the conversion of ATP to ADP and the conversion of P to water.
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.
Muscle glycogen phosphorylase b is activated by AMP and inhibited by ATP. Phosphorylase kinase converts phosphorylase b to phosphorylase a, which then phosphorylates glycogen to form glucose 1-phosphate. The reaction is driven by the hydrolysis of ATP to ADP and AMP.
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 + cAMP → Protein kinase A

Glycogen synthase a → ATP → ADP

Glycogen synthase b → ATP

Phosphorylase kinase + ATP → ADP

Phosphorylase kinase + Ca^{2+} → ATP

Glycogen phosphorylase b → ATP

Glycogen phosphorylase a

Glycogen degradation
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.
Monomeric G proteins

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]