

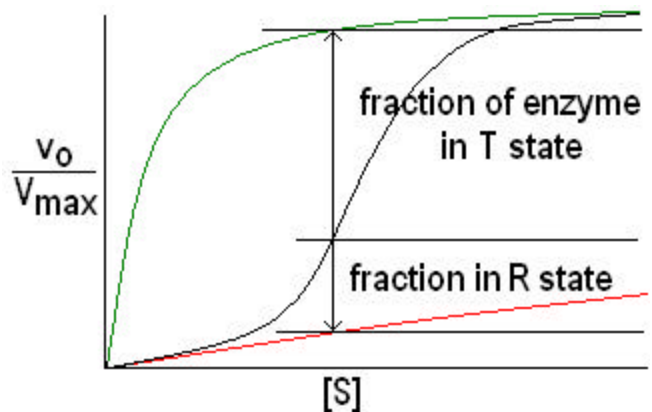
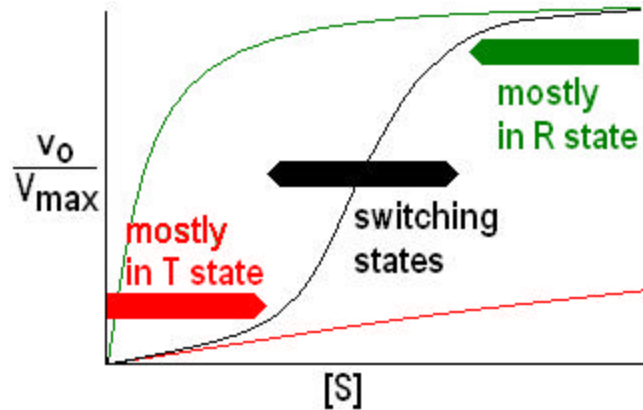
Chem*3560 Lecture 19: Review of regulation

What is meant by cooperative allosteric regulation?

Positive cooperativity - characteristic is the sigmoidal binding/activity curve

T-state has weaker affinity, meaning higher substrate concentration is needed to observe binding. T stands for **T**ense, a name derived because of state of tension that exists between Fe^{2+} , Helix F and neighbouring subunit in deoxyhemoglobin. R state has strong affinity, meaning lower $[\text{S}]$ is sufficient for binding. (**R** stands for **R**elaxed because Fe^{2+} is relaxed to its optimum position for O_2 binding in hemoglobin). The T/R terminology is now applied to all enzymes showing sigmoidal kinetics.

The steep rise in the sigmoidal curve occurs as the protein starts to switch states. Individual molecules of the enzyme do not go through intermediate stages. Rather, the intermediate stage represents a certain fraction of molecules in R state while the rest remain in T state.

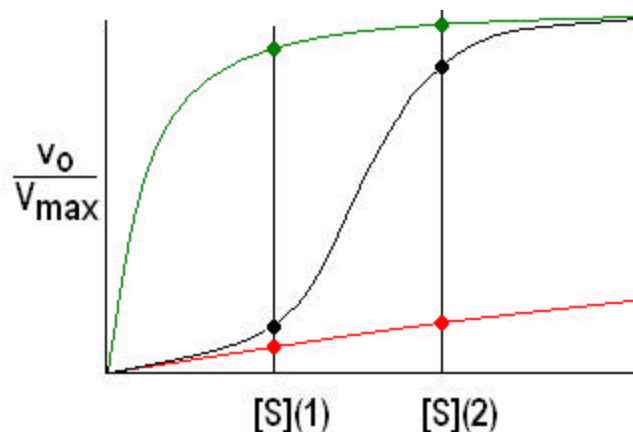


Why is positive cooperativity useful in biochemical regulation?

Narrower range of concentrations between point of strong binding and point of effective release

Multimeric proteins - affinity change is communicated to other subunits that are still unoccupied so that they benefit from higher affinity

Often associated with an "**all or none**" structural change, e.g. rotation of part of multimer relative to another part.

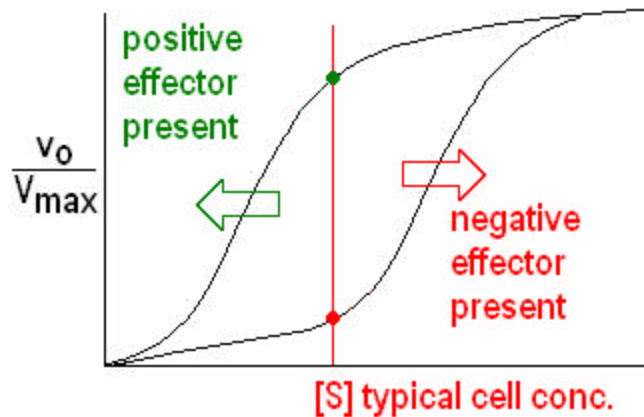


How is regulation exerted?

Other factors can shift the point at which the molecule switches from low affinity T-state to high affinity R-state.

Positive effectors favour R-state, shift curve to lower [S] (left).

Negative effectors favour T-state, shift curve to higher [S] (right).



Taking a typical cellular concentration of substrate [S], the effectors can achieve an effective **turn-on, turn-off** response.

Examples of effector action

Hemoglobin

- $[H^+]$ is higher in peripheral tissue during active metabolism (**pH 7.2** versus **7.4** in lung)
- higher $[H^+]$ increases tendency that **His HC3** is protonated
- **His HC3 links to Asp FG1 as an ion pair, which transmits tension in T-state**
- this makes O_2 release easier in peripheral tissues.

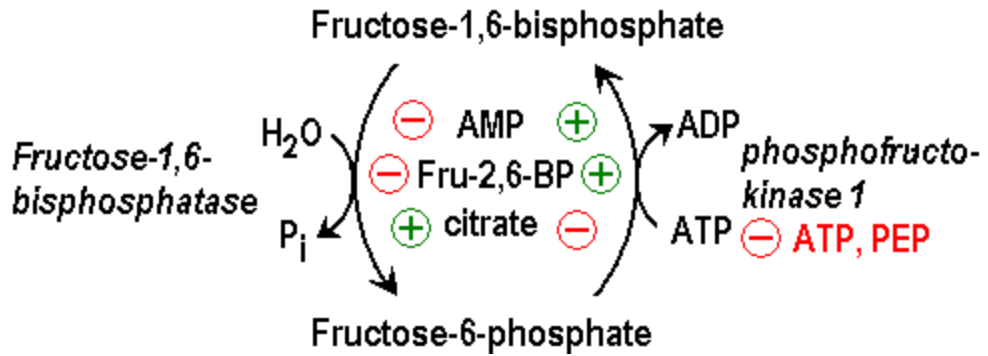
Phosphofructokinase 1

- AMP, Fru-2,6-bisP stabilize extra turn in "contact" helix
- this places **Arg 162** near the **negative Fru-6-P substrate**; this **favours** substrate binding and gives rise to R-state binding behaviour.
- ATP, citrate, PEP promote partial unwinding of "contact" helix
- this puts **Glu 161** in position to repel **negative Fru-6-P substrate**, leads to T-state behaviour.

Homotropic effect (effect of substrate itself) in PFK1 is the converse.

- as substrate binds, it attracts Arg 162 and repels Glu 161
- partial substrate occupancy increases likelihood of switch to R-state
- when switch occurs, the remaining vacant sites in the tetramer bind with high affinity

How do effectors relate to function?



- AMP is an indicator of need to make ATP
- Fru-2,6-bisP relays signal from hormones } classic example of **reciprocal regulation**
- citrate indicates TCA cycle has enough substrate
- PEP indicates glycolytic products are accumulating (setting up for gluconeogenesis?)
- ATP indicates adequate energy status

Allosteric regulation is not always cooperative

Allosteric means "other shape"

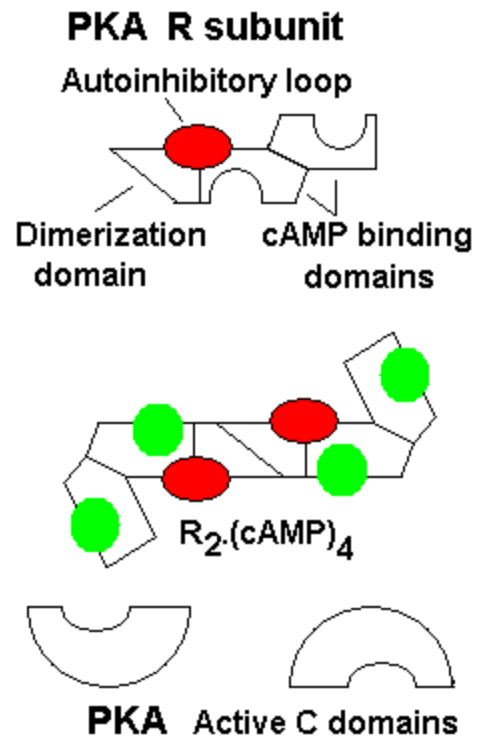
Binding of regulator exerts **induced fit** effect

- enzyme adjusts shape as it binds, and can reconfigure the catalytic site.

Non-cooperative allosteric effects often show as change in V_{max} rather than K'

Example

- **cyclic AMP** binds to **R-subunit of protein kinase A**
- shape change in R-subunit hides the autoinhibitory loop (a substrate-like peptide)
 - active C subunit is released
 - change in V_{max} of Protein kinase A is observed



Regulation by phosphorylation

Ser, Thr, Tyr (and His in bacteria) are potential targets for modification. Many different kinds of protein kinase exist, can be highly specific for substrate

e.g. pyruvate dehydrogenase kinase causes specific phosphorylation that inactivates pyruvate dehydrogenase when Acetyl-CoA builds up.

What determines how a protein kinase recognizes a target sequence?

Protein kinase A controls pathways related to ATP production and glucose distribution.

Its target sequences follow the pattern Arg-Arg-x-Ser-y (x = any, y = non polar)

Enzymes like protein kinase A select Ser for phosphorylation 1) based on accessibility, and 2) based on correspondence to a particular pattern of amino acids. A "consensus sequence" represents the pattern of amino acids most frequently observed around a particular target.

Other protein kinases may have other amino acid patterns in their target, hence are selective for particular protein substrates.

A similar sequence (with Ser-PO₄²⁻) is recognized by phosphoprotein phosphatase 1

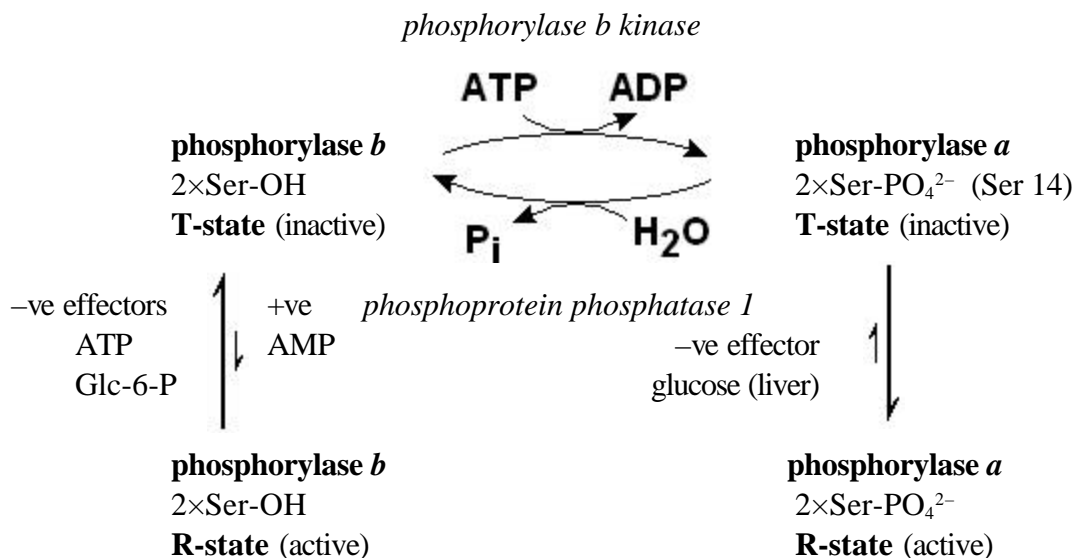
How does phosphorylation of amino acids affect protein activity?

Covalently bonded phosphate introduces -2 charge in place of neutral Ser.

- this can cause internal structural adjustments in protein
- this may **increase or decrease activity** of the target protein
- **phosphorylase b kinase, Liver FBPase 2, muscle PFK2** are all activated
- **glycogen synthase, pyruvate kinase, liver PFK 2, muscle FBPase 2** are inactivated

Phosphate's charge can mimic presence of effectors with similar charge

- in **phosphorylase a**, Ser14-PO₄²⁻ phosphate repositions itself near the AMP binding site
- this means that phosphorylase **a** prefers R state as if AMP were present.



What is an autoinhibitory loop/pseudosubstrate loop?

Enzymes that act on other proteins bind and recognize specific target sequences, e.g. protein kinase A, trypsin, thrombin, cyclin-CDKs, caspase 9, etc. **Inhibitory loops or pseudosubstrate loops** are segments of proteins that act as a **decoy** for these kinds of enzyme, by matching their target sequence and binding to the catalytic site to **block** activity. They are **autoinhibitory** if the decoy component is part of the same protein complex, e.g. protein kinase A R subunit, or the β -chain of phosphorylase b kinase.

Inhibitory loops or pseudosubstrate loops block activity by a variety of means:

- In the R subunit of protein kinase, the autoinhibitory loop matches the consensus sequence but **lacks an actual Ser to phosphorylate**.
- In pepsinogen, the loop binds to catalytic amino acids through a side chain instead of peptide backbone.
- In IAPs (inhibitor of apoptosis) the target sequence is backwards so the decoy sequence binds into the catalytic site of caspase the wrong way around.
- Secretory pancreatic trypsin inhibitor and in antithrombin III bind their "victims" very tightly, so block movement needed for catalysis, and products do not leave the active site..
- In phosphoprotein phosphatase 1, the inhibitor binds tightly so it is the preferred substrate; but it is simply intended to keep the phosphatase busy for a period of time.

How does the Fructose-2,6-bisphosphate bifunctional enzyme work?

The single protein contains two separate catalytic sites. The N-terminal half contains phosphofructokinase 2 (PFK2), and the C-terminal half contains fructose-2,6-bisphosphatase (FBPase2). Isozymes from liver and muscle contain both these activities but are regulated in opposite ways by phosphorylation.

Liver enzyme contains a protein kinase A target sequence at Ser 32; when phosphorylated, it masks the PFK2 site and exposes the FBPase 2 site. Cyclic AMP and Protein kinase A promote glycogen breakdown and **gluconeogenesis** in liver, so that glucose can be released into the blood.

Muscle enzyme contains protein kinase A targets at Ser 406 and Thr 475; when phosphorylated, these mask the FBPase2 site and expose the PFK2 site. Cyclic AMP and protein kinase A promote glycogen breakdown and **glycolysis** in muscle, so that ATP can be made to support muscle contraction.