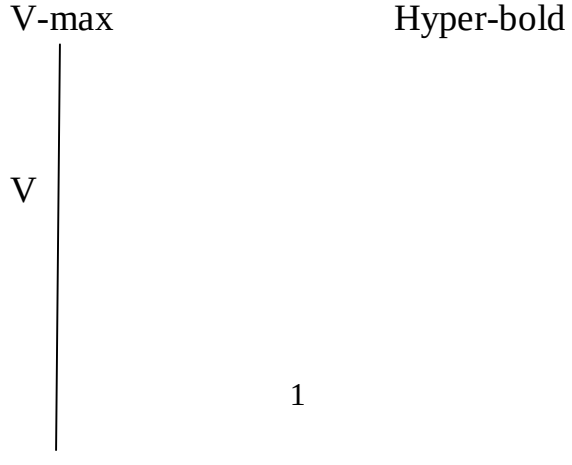
Properties of Allosteric Enzymes

(1) An allosteric enzyme possesses at least 2 spatially distinct binding sites on the protein molecules the active or the catalytic site and the regulator or the allosteric site. The metabolic regulator molecule binds at the allosteric site and produces a change in the conformational structure of the enzyme, so that the geometrical relationship of the amino acid residues in the catalytic site is modified. Consequently, the enzyme activity either increases (activation) or decreases (inhibition).

(2) Allosteric enzymes show 2 different types of control – heterotropic and homotropic depending on the nature of the modulating molecule. Heterotropci enzymes are stimulated or inhibited by an effector or modulator molecule other than their substrates, e.g. threonine deaminase the substrate is threamine and the modulator is iso-leucine. When the modulator promotes the binding of substrate to the allosteric enzyme, the modulator is said to be a +ve effector or allosteric activator whereas when the modulator diminishes the binding of substrate, it is called a –ve effector or allosteric inhibitor, +ve effectors increase the number of binding sites for substrate whereas – ve effectors decrease the number of binding sites for the substrate.

In homotropic enzymes, the substrate also functions as the modulator. Homotropic enzymes contain 2 or more binding sites for the substrate modulation depends on how many of the substrate sites are bound.

(3) Their Kinetics do not obey the Michaelis – Mention equation. A plot of V vs S for an allosteric enzyme yields sigmoid – or S-shaped curve rather than rectangular hyperbola.



Sigmoid

S

One possible explanation for the occurrence of these sigmoid Kinetics is that each molecule of enzyme possesses more than one catalytic site to which the substrate could be bound. The binding of the regulator molecule causes a conformational change in the protein so that the structure of the catalytic site is modified. This conformational change can be considered as information transfer between the regulator and the catalytic sites such that the binding of substrate at one site affects the binding of subsequent molecules of substrate at the remaining binding sites – a phenomenon referred to as Cooperativity. When the binding of the regulator results in more substrates being bound, then it is +ve cooperativity and the reverse as –ve cooperativity.

(4) Inhibition of a regulatory enzyme does not conform to any normal inhibition pattern and the inhibitor does not bear any obvious structural relationship to the substrate. The enzyme exhibits extreme specificity with regard to the regulator molecule.

(5) Allosteric enzymes have an oligomeric organization. They are composed of more than one polypeptide chain and have more than one S-binding site per enzyme molecule.

(6) Treatment of the allosteric enzyme with agents or conditions that exert a mild denaturing effect can result in loss of sensitivity to the effects of the regulatory molecule without changing the catalytic activity. This phenomenon is referred to as desensitization and this can be effected by high or low pH mercurials (such as mercuric chloride) urea or by gentle heating. Desensitisation causes dissociation of the ntive enzyme into its component sub-units and this prevents interaction between the regulator and catalytic sites.

Unlike most enzymes, many allosteric enzymes undergo reversible inactivation at O^oC.

(a) Covalently modulated enzymes.

This is a 2nd group of regulatory enzymes that are inter-converted between active and inactive forms by other enzymes by covalent modification of specific amino acid residues on the enzyme surface. Covalent modification may either reinforce or counteract the effects of allosteric regulators and hence may either intensify or tend to nullify allosteric regulatory effects. Regulation by covalent modulation is well documented in animals.

In mammalian systems, the 2 most common forms of covalent modification are Partial proteolysis and Phosphorylation and De-phosphorylation. B/C Cells lack the ability to reunite the 2 portions of a protein produced by hydrolysis of a peptide bond, proteolysis constitutes an irreversible modification. In contrast, phosphorylation is a reversible. Phosphorylation takes place on seryl, threomyl, or tyrosyl residues and it is catalysed by a group of enzymes known as protein Kinases. B/c PO_4 lation is versible, the hydrolytic removal of these phosphoryl groups is also possible and it is catalysed by enzymes called protein phosphatases.

ATP ADP

Mg^{2+}

Kinase
Phosphorylation

Enz-Ser-OH $Enz – Ser – O – PO_3^{2-}$

Phosphatase

Mg^{2+}

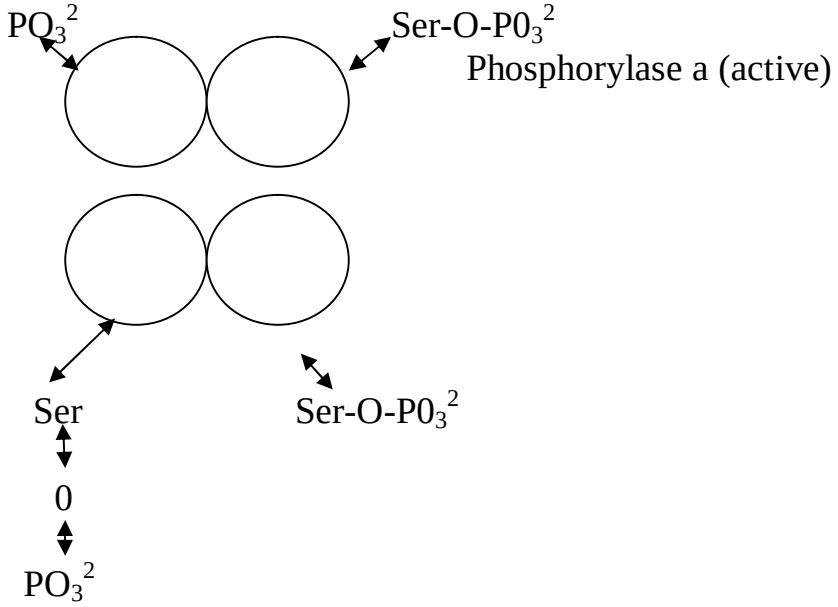
Pi Dephosphorylation H_20

The activities of protein Kinases and protein Phosphatases are themselves regulated; if not, their concerted action would be both thermodynamically and biologically unproductive.

A classical example of an enzyme regulated by covalent modification of its activity is glycogen phosphorylase of animal tissues which catalyses the breakdown of glyzogen.

$(Glucose)_n$ + Pi $(Glucose)_{n-1}$ + Glucose -1- $P0_4$
Glycogen Shortened glucogen molecule

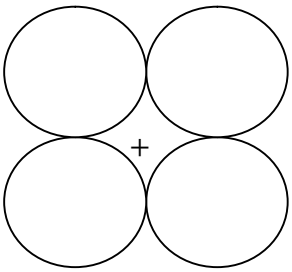
Glycogen phosphorylase occurs in 2 forms, phosphorylase a, the more active form and phosphory lase b, the less active form. Phosphorylase a is an oligomeric protein with 4 major sub-units. Each sub-unit:



$4H_20$ 4-ADP

Phosphorylase Phosphorylase
Phosphatase $4P_i$ Kinase

4ATP



Phosphorylase b (inactive)

Contains a serine residue that is phosphorylated at the OH groups; these PO_4 groups are required for maximum catalytic activity. The PO_4 groups in phosphorylase a can be hydrolytically removed by the enzyme phosphorulase phosphatase. Removal of the PO_4 groups causes phosphorylase a to dissociate into 2 half molecules, phosphorylase b, whicha re inactive. Reactivation of the inactive phosphorylase b to form active phosphorylase a can be brought about by the enzyme phosphorylae kinase, which catalyses the enzymatic PO_4 lation of the serine resolves at the expense of ATP. In this way, the activity of glycogen phosphorylase (glycogen breakdown is regulated by the action of 2 enzymes that shift the balance between its active and inactive forms.

The 2^{nd} string attribute of glycogen phosphorrylase and similar regulatory enzymes modulated by covalent modification is that they can greatly emplify a chemical signal. All enzymes can bring about amplification, i.e. one enzyme molecule can catalyse formation of thousands of product molecule from a given substrate in a given period of time. However, here an enzyme acts upon another enzyme as its substrate. One molecule of phsophorylase Kinase can convert thousands of molecules of phosphorylase

b into the active phosphorylae a, which in turn can catalyse the production of thousands of molecules of G-I-P molecules from glycogen. Phosphoglase Kinase and phosphorylase thus constitute an amplification cascade with 2 steps. Examples of mammalian enzymes whose activity is altered by covalent PO_4 lation-de-PO_4 lation.

	Activity	State
Enzyme	Low	High
Acetyl CoA carboxylase	EP	E
Glycogen synthase	EP	E
Pyruvate dehydrogenase	EP	E
HMG CoA reductase	EP	E
Glycogen phospyorylase	E	EP
Citrate lyase	E	EP
Pyospyorylase b Knase	E	EP
HMG CoA reductase Kinase	E	EP

Protein Kinase

These are converte enzymes that catalyse the ATP-dep PO_4 lation of serine, threonine or tyrosine OH groups in target proteins PO_4 lation introduces a bulky group bearing 2-ve charges, causing conformational changes that alter the target protein's function. Unlike a phosphoryl group, no amino acid side chain can provide 2 –ve charges. Protein Kinases differ in size, sub-unit structure and sub-cellular location. However, they share a common catalytic mechanism based on a conserved catalytic core/Kinase domain of 260 amino acid residues. Theya re classified as Ser/Thr and/or Tyr specific. They also differ in terms of the target proteins that they recognize and PO_4 late target selection depends on the presence of an amino acid sequence within the target protein that is recognized by the Kinase. For instance, cAMP-dependent protein Kinase phosphorylated proteins having Ser or Thr residues that occur in an Arg- (Arg or Lys) – (any amino acid) – (Ser or Thr) sequence segiments. Tyrosine Kinases are protein Knases that PO_4 lates Tyr-residues and occur only in multicellular organisms. They are components of signaling pathways involved in cell-cell communication.

Classification of Protein Kinases
Protein Kinase Class Activators

1. Ser/Thr Protein Kinases
   - A Cyclic nucleotide-dependent
     - cAMP-dependent — cAMP
     - cGMP-dependent — cGMP
   - B Ca^{2+} - calmodulin (CAM) dep. — Phosphorylation by P.K
     - Phyosphorylase Kinase — CA^{2+} - CaM
     - Myosin light-chainKinase (MLCK)
   - C Protein Kinase c(PKC) — Ca^{2+}, diacylglycerol
   - D Mitogen-activated protein Kinases — PO_4lation by MAPK Kinase
     - (MAP Kinase)
   - E G-protein-coupled receptors
     - β-Adrenergic receptor Kinase
     - (BARK) Rhodopsin Kinase

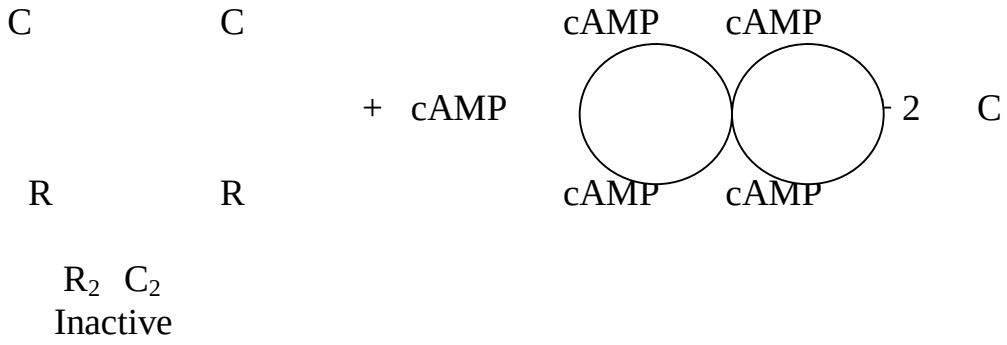
2 Ser/Thr/Tyr protein Kinases
- MAP Kinase Kinase — PO_4lation by Raf

3 Tyr protein Kinases
- A Cytosolic tyrosine Kinases
- B Receptor tyrosine Kinases (RTKs)
  - Plasma membrane receptors for hormones such as epidermal growth factor (ECF) or platelet-derived growth factor (PDGF)

Regulation of the Activity of Protein – Kinases and Protein Phosphatases

Targeting of protein Kinaes to particular consensus sequence elements within proteins creates a means to regulate these Kinases by a mechanism

referred to as Intrasteric control. Intrasteric control occurs when a regulatory submit has a pseudosubstrate sequence that mimics the target sequence but lacks OH-bearing side chain at the right place. For, e.g. the cAMP-binding regulatory sub-units of protein Kinase A possess the pseudo substrate sequence that binds to the active site of protein Kinase A catalytic sub-units, blocking their activity. This pseudo substrate sequence in protein Kinase A has an alanine residue where serine occurs in the target protein. Alanine is sterically similar to serine but lacks a phosphory latable OH group. When the regulatory subuntis of protein Kinase A bind cAMP, they undergo a conformational change and dissociate from the catalytic sub-units and the active site of protein Kinase A is free to bind and PO_4 late its target proteins. In other protein Kinases, the pseudosubstrate seaquence involved in intrasteric control and the Kinase domain are part of the same polypeptide chain. In these cases, binding of an allosteric effector (like cAMP) induces a conformational change in the protein that releases the pseudo-substrate sequence from the active site of the Kinase domain and the active site could then $P0_4$late its target. Thus, dissociation of the regulatory sub-units activates the catalytic subunits, whereas reassociation suppresses activity.



Regulation of protein phosphatases also involbves $P0_4$lation and de-PO_4lation phosphoprotein phosphatase inhibitor. (PP1-1) is a modulator protein that regulates the activity of phospho-protein phosphatase. When PPI-1 is PO_4lated on one of its serine residues, it binds to phosphor-protein phosphatase, inhibiting its phosphatase activity. The result is an increased PO_4lation of the itner convertible enzyme targeted by the protein Kinase/phosphoprotein phosphatase cycle.