Chapter 10

Regulatory Strategy

Regulation of enzymatic activity:

1. Allosteric Control.
   Allosteric proteins have a regulatory site(s) and multiple functional sites
   Activity of proteins is regulated by small signaling molecules at regulatory site
   Allosteric proteins interact with a substrate cooperatively
   Enzymatic activity is regulated by a signaling molecule; transducers

2. Multiple Forms of Enzymes.
   Isoenzymes: homologous enzymes in a single organism that differ by K_M and V_{max} value and in regulatory properties. Often expressed in disstinct dissue and organell or at different stage of development.

1. Reversible Covalent Modification. Enzymatic activity is regulated by attachment of a small group to the enzyme; phosphorylation of Ser; regulation is catalyzed by kinases and phosphatases

2. Proteolytic Activation. Reversible conversion of an inactive enzyme to an active enzyme. Digestive enzymes and enzymes involved in bloot clotting.

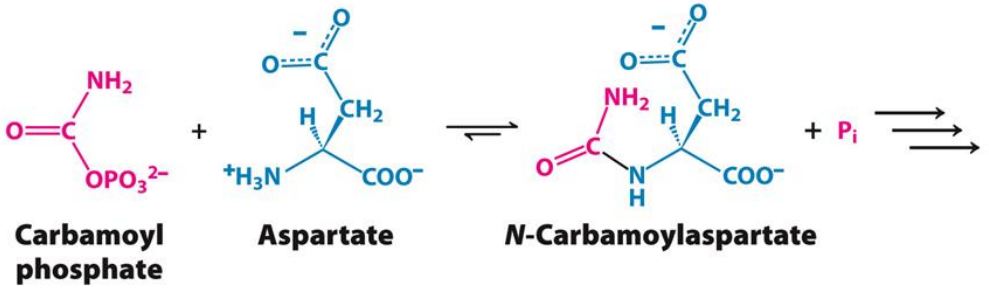
3. Controlling the Amount of Enzyme Present. Takes place at the level of transcription

Aspartate transcarbamoylase (ATcase)

Aspartate transcarbamoylase (ATCase) catalyzes the first step in pyrimidine synthesis.

ATCase is inhibited by the end product of the pathway, CTP, in an example of feedback inhibition. The inhibition by the product of the enzymatic pathway ensures that intermediates of the metabolic pathway are not produces when the end product of the enzymatic is abudant.

CTP exerts its effects by binding at a distinct regulatory or allosteric site on ATCase.



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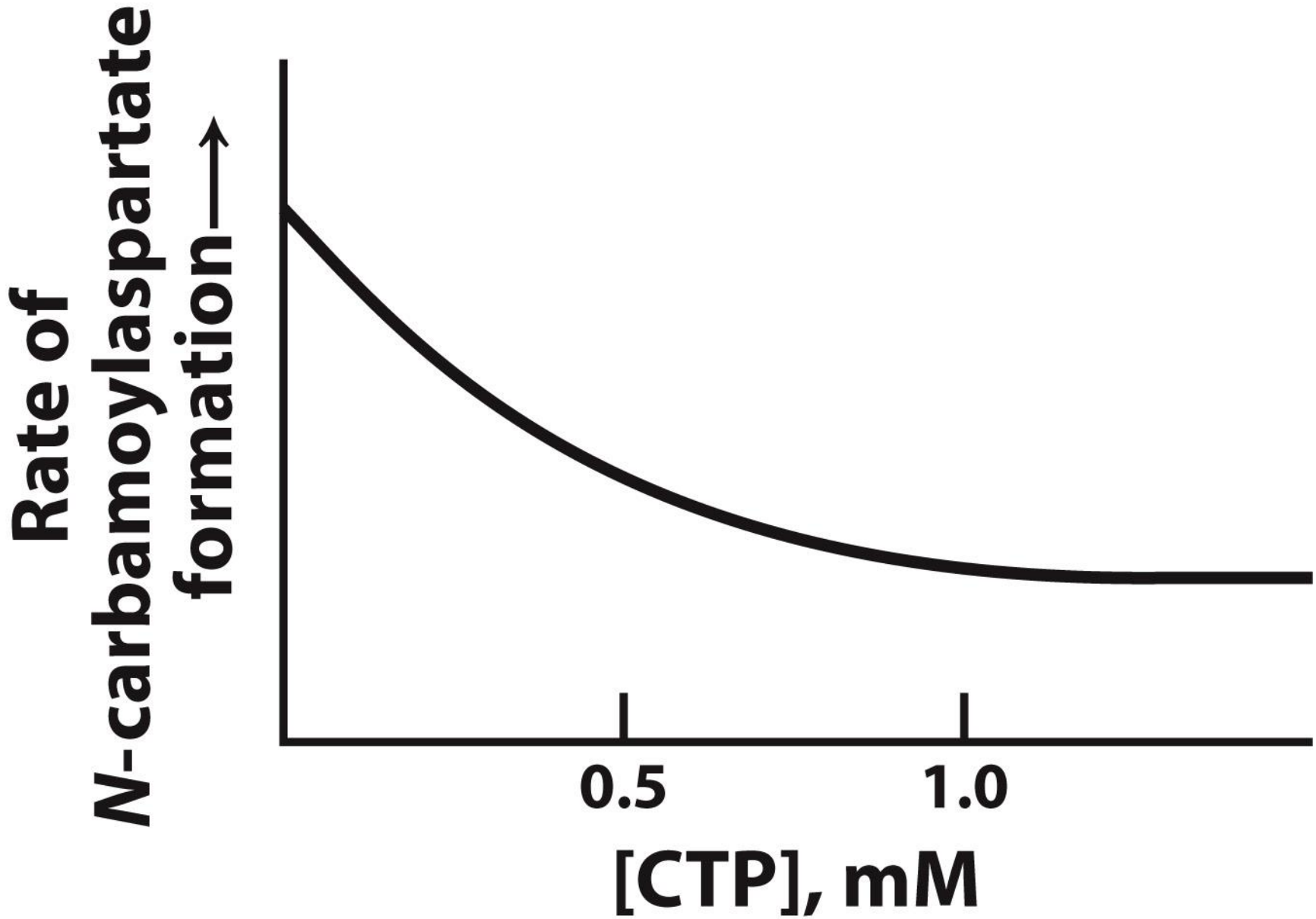

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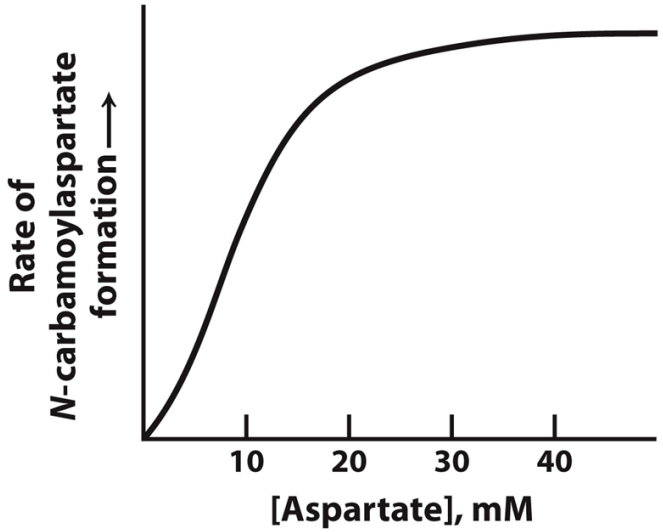
Allosterically regulated enzymes do not follow Michaelis –Menten kinetics

ATCase, like the majority of allosteric enzymes, displays sigmoidal kinetics.

Sigmoidal curves result from cooperation between subunits.



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The binding of the substrate to one binding site increases the probability of the substrate binding to the other biding site

ATCase structure

Native ATCase consists of six catalytic and six regulatory subunits (c_6r_6), organized as two catalytic trimers and three regulatory dimers.

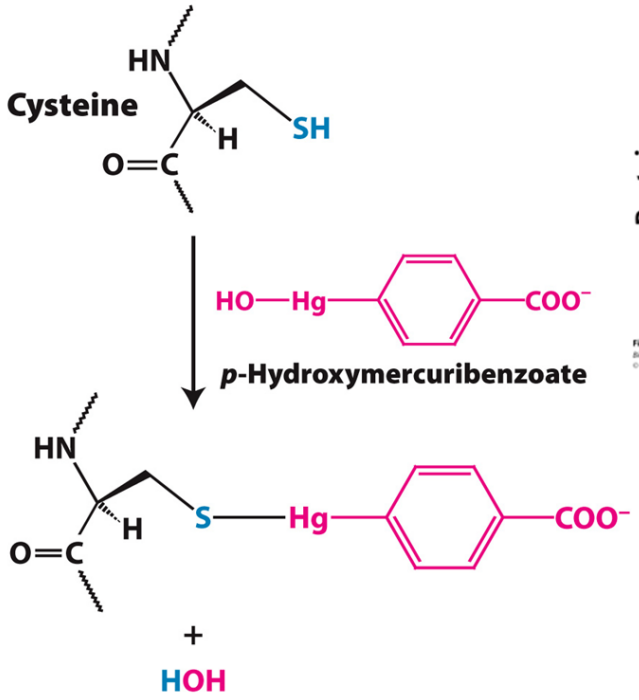
Treatment with *p*-hydroxymercuribenzoate dissociates the enzyme into a catalytic subunit consisting of three chains (c_3) and regulatory subunit of two chains (r_2) that can be separated by centrifugation.
Isolated subunits can be mixed to reconstitute the native enzyme.

Catalytic subunit:

- large; consist of three chains of 34 kDa
- enzymatic activity
- does not respond to CTP

Regulatory subunit:

- smaller; consist of two chains of 17 kDa
- no enzymatic activity
- binds CTP



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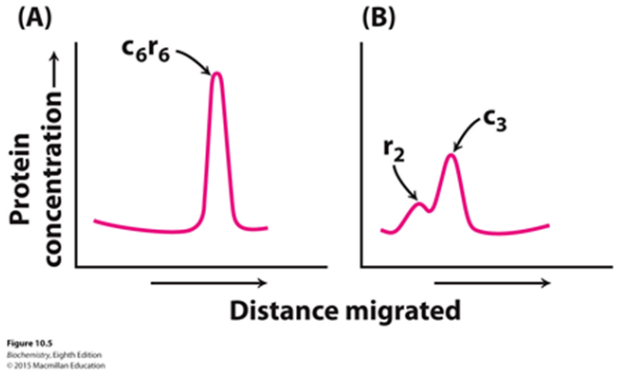

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Sedimentation velocity of the native protein (A) and the protein treated by p-hydroxymercuribenzoate

Structure of ATCase

The catalytic trimers are stacked upon one another, linked by the three regulatory dimers. Multiple contacts between the catalytic and regulatory subunits

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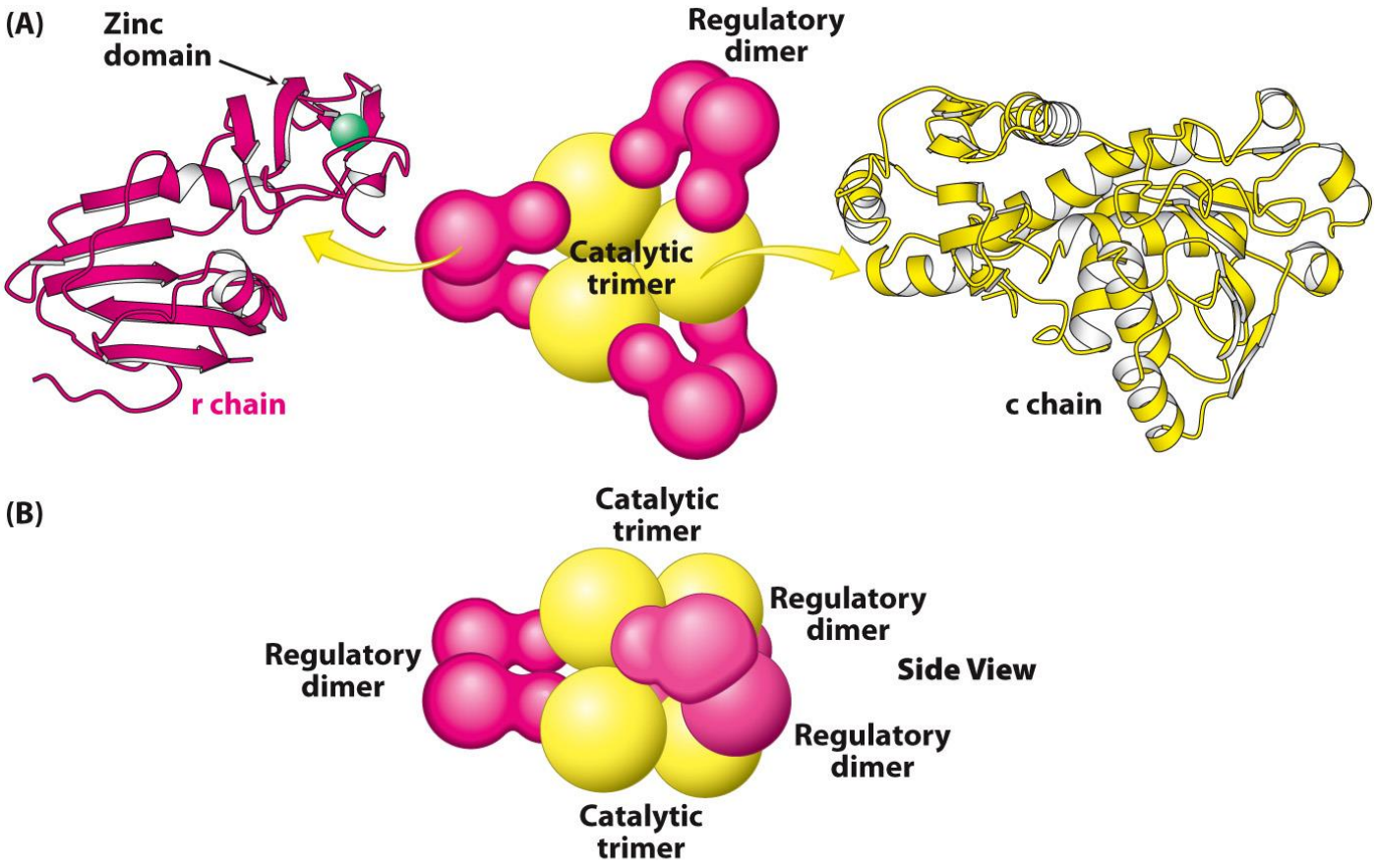

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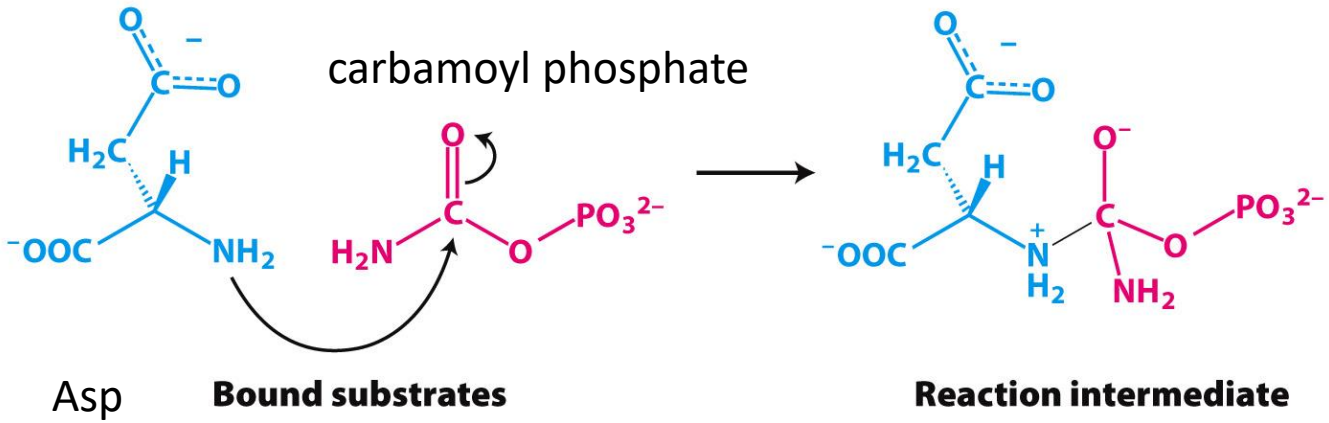
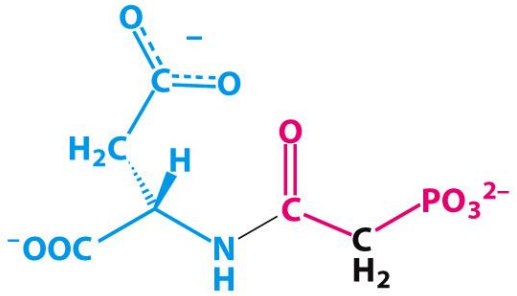
The active sites are located at the interface of the catalytic subunits.
The active site was identified by crystallization of ATCase in the presence of PALA

Binding of PALA causes structural changes that convert the compact, less active T state into the expanded, active R state





***N*-(Phosphonacetyl)- L-aspartate (PALA)**

Potent competitive inhibitor of ATCase

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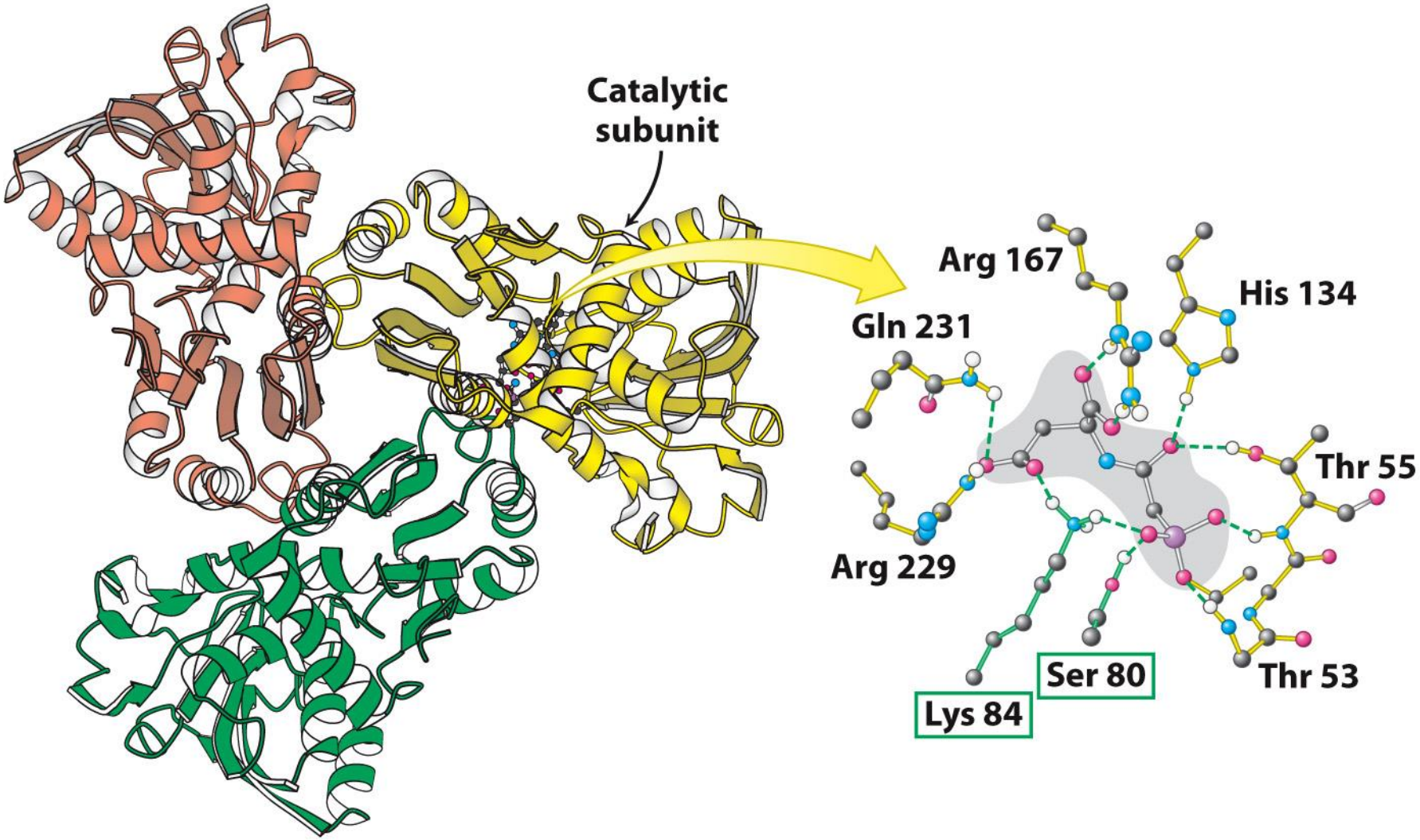

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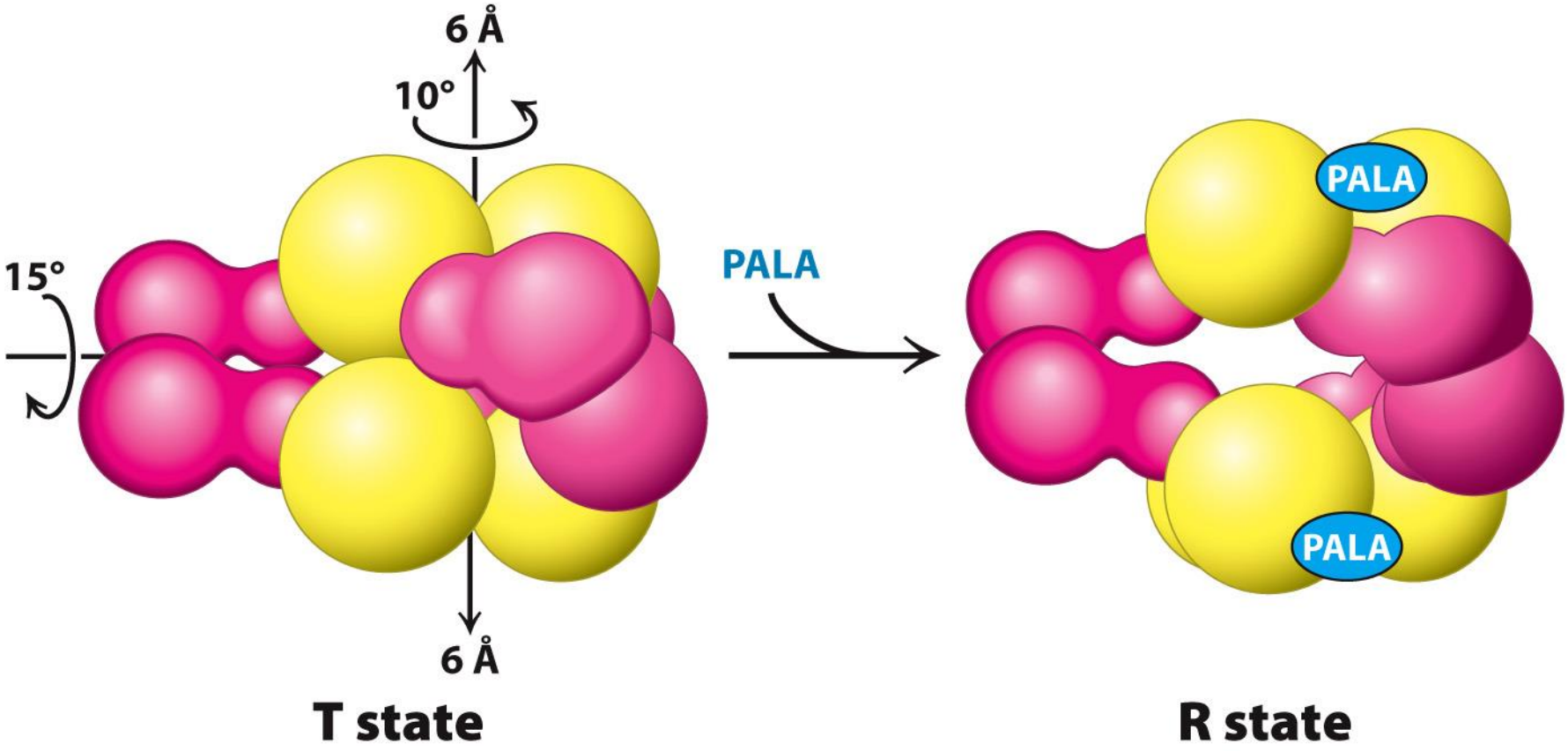
ATCase exists in two contormations



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Compact, inactive form of the enzyme

Expanded state

The T state has a low affinity for substrate and has low catalytic activity.

The R state is the most active form.

The two states are in equilibrium, with the T state being favored.

The ratio of enzyme in the T state to that in the R state is the **allosteric coefficient (L)**

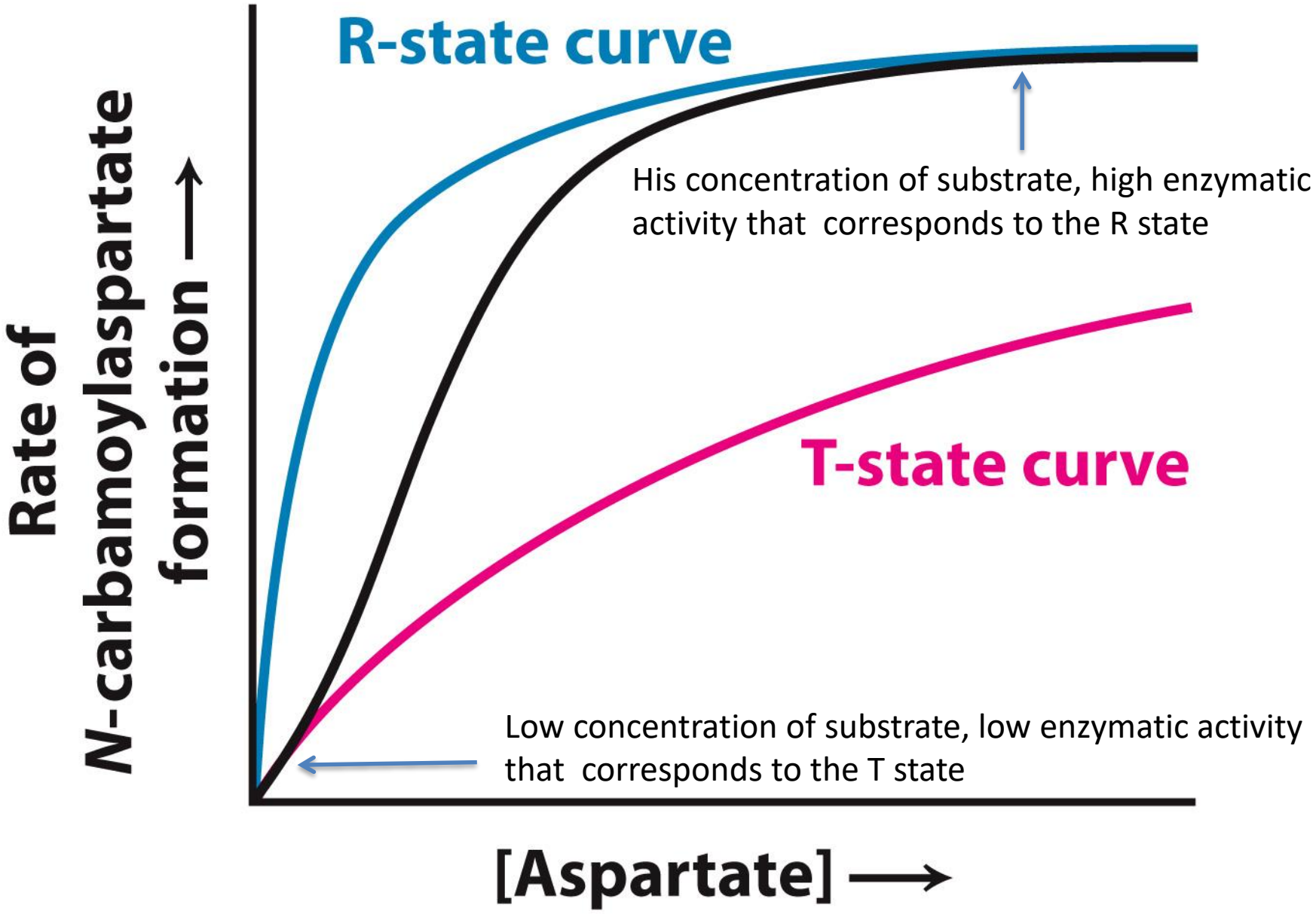
Binding of substrate disrupts the equilibrium in favor of the R state, a property called cooperativity.

$$\mathrm{R} \rightleftharpoons \mathrm{T} \qquad L = \frac{\mathrm{T}}{\mathrm{R}}$$

The effect of substrates on allosteric enzymes are called homotropic effects.

ATCase adheres to the concerted mechanism for allosteric enzymes, which postulates that all active sites are in the same state, either T or R.

The sigmoidal kinetic curve of allosteric enzymes allows increased sensitivity to changes in substrate concentration, or the threshold effect.



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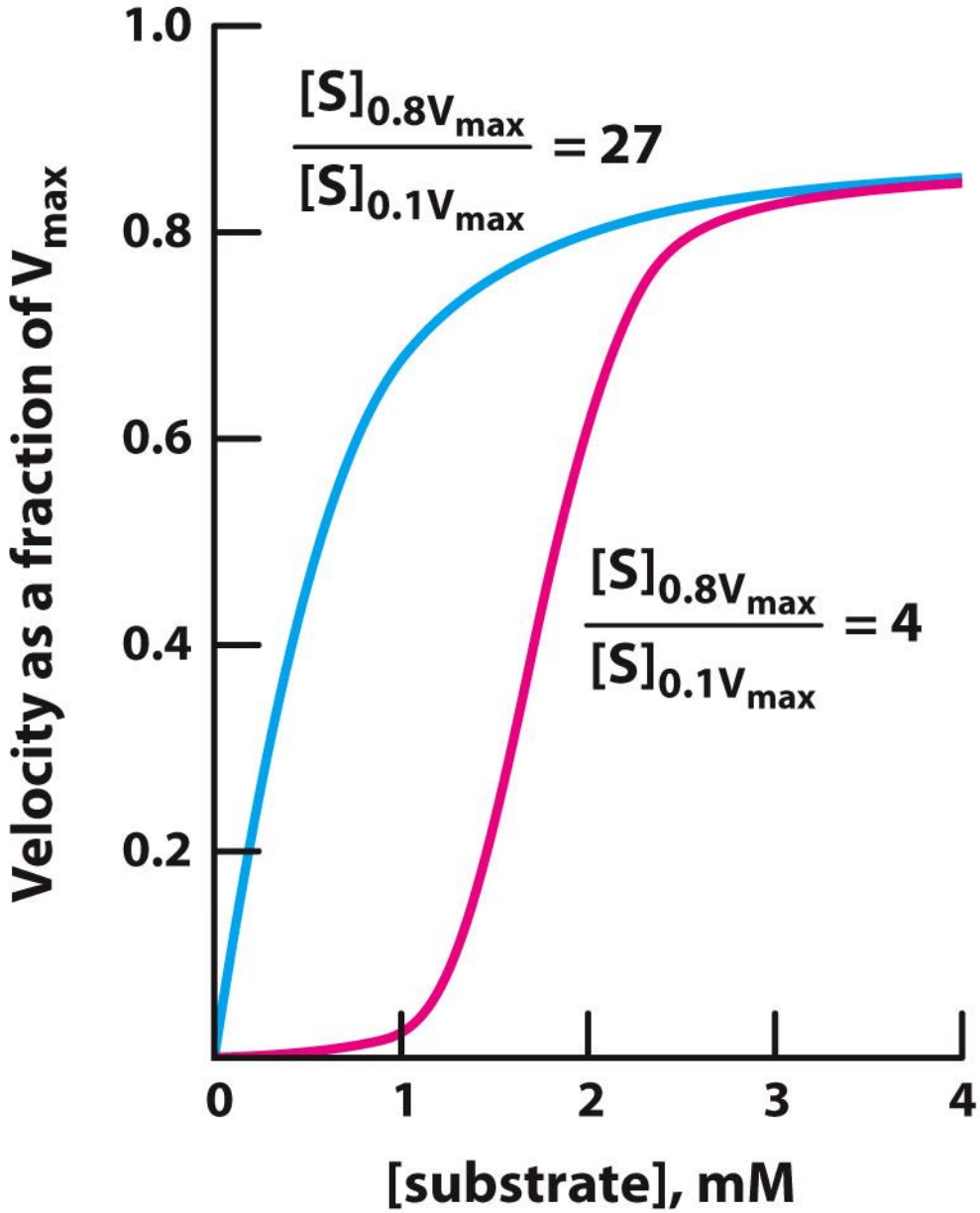

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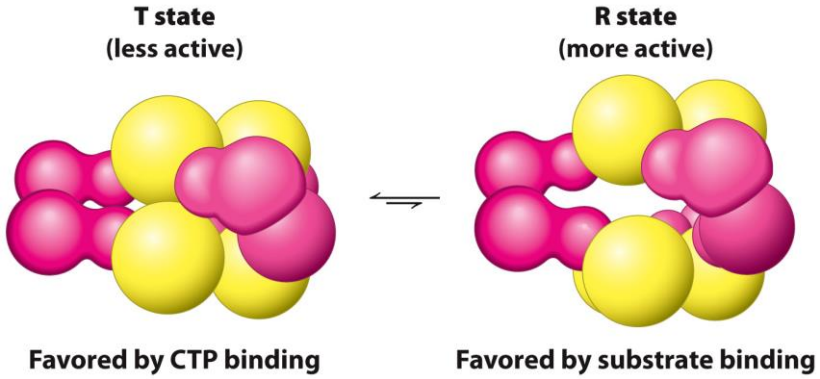
In case of allosteric enzymes; small increase in the substrate concentration has a substantial impact on the enzymatic activity

Allosteric Regulators modulate the T to R equilibrium

Binding of CTP to the regulatory site of ATCase alters the T-to-R equilibrium in favor of the T state, decreasing net enzyme activity.

ATP is a positive regulator of ATCase, altering the T-to-R equilibrium in favor of the R state, increasing net enzyme activity. ATP and CTP bind to the same regulatory site.

L is the equilibrium constant for the T-to-R equilibrium.
Allosteric effectors (heterotropic effectors) alter the value of L.
Heterotropic effect: effectors change the K_M value
Homotropic effect: substrate generate the sigmoidal curve
Both effector and substrate change L



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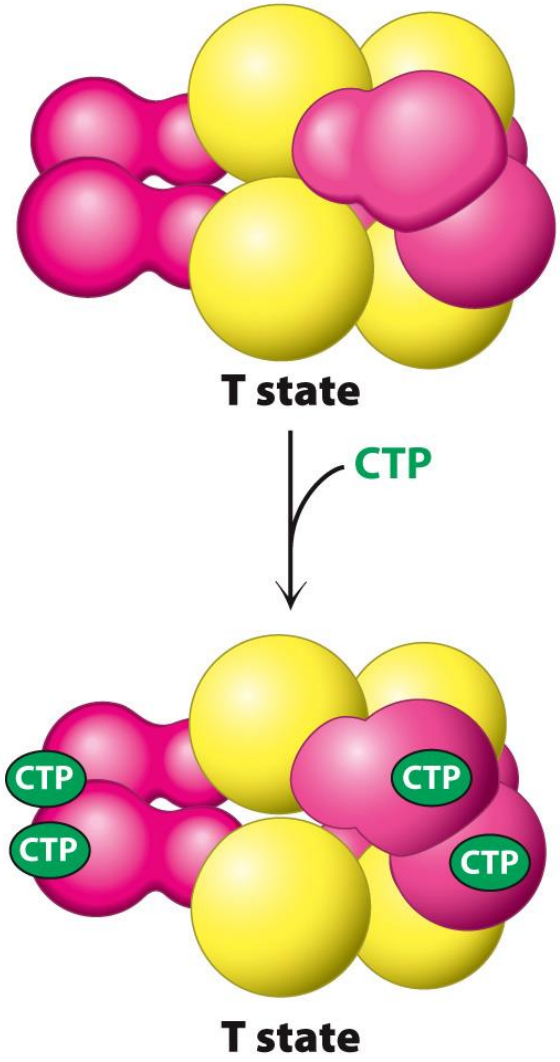

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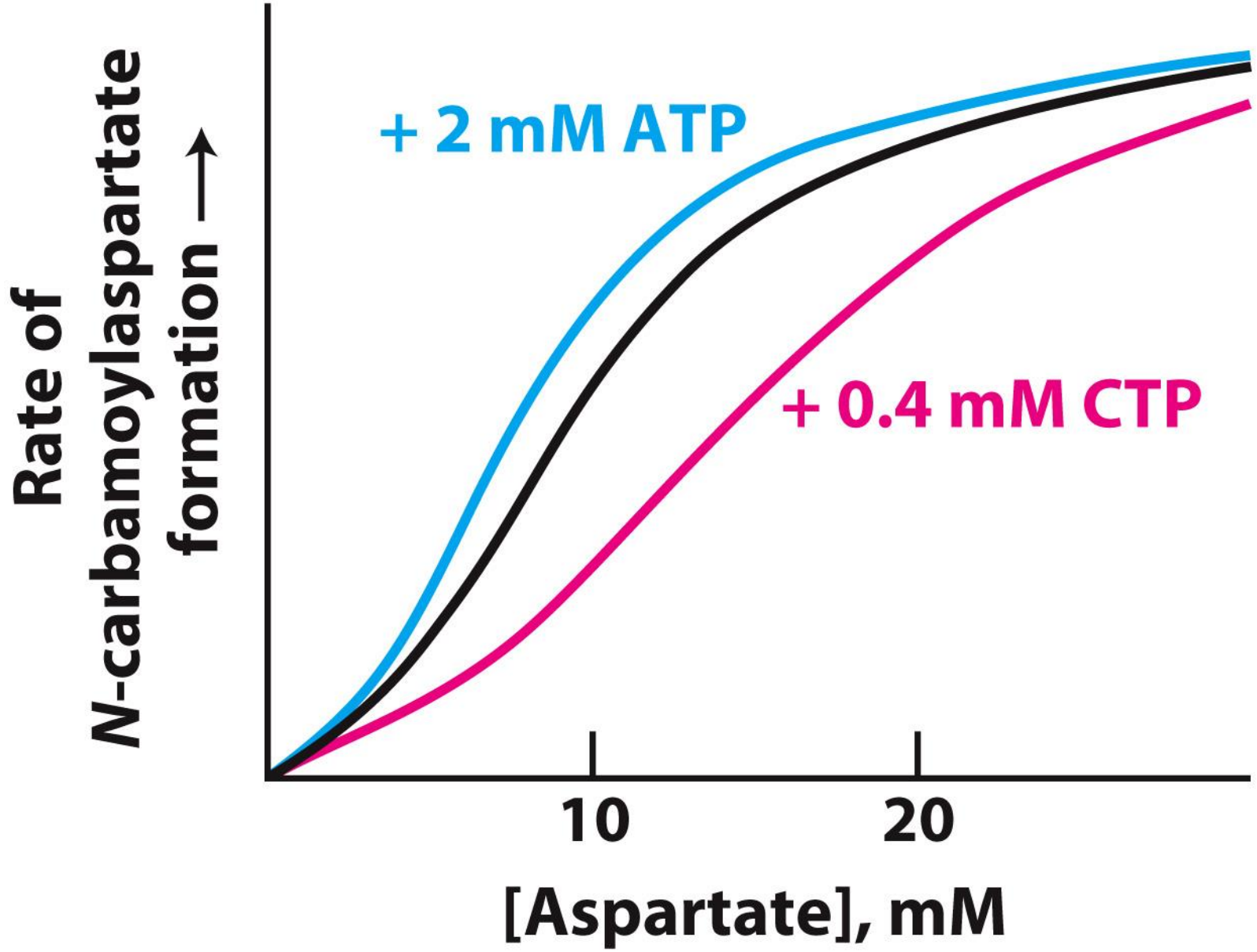

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Isoenzymes

Isoenzymes or isozymes are enzymes that differ by amino acid sequence

Isozymes catalyze the same reaction but display different regulatory properties.

Isozymes may be expressed in a tissue-specific or developmentally specific pattern.

The appearance of certain isozymes in the blood is a sign of tissue damage.

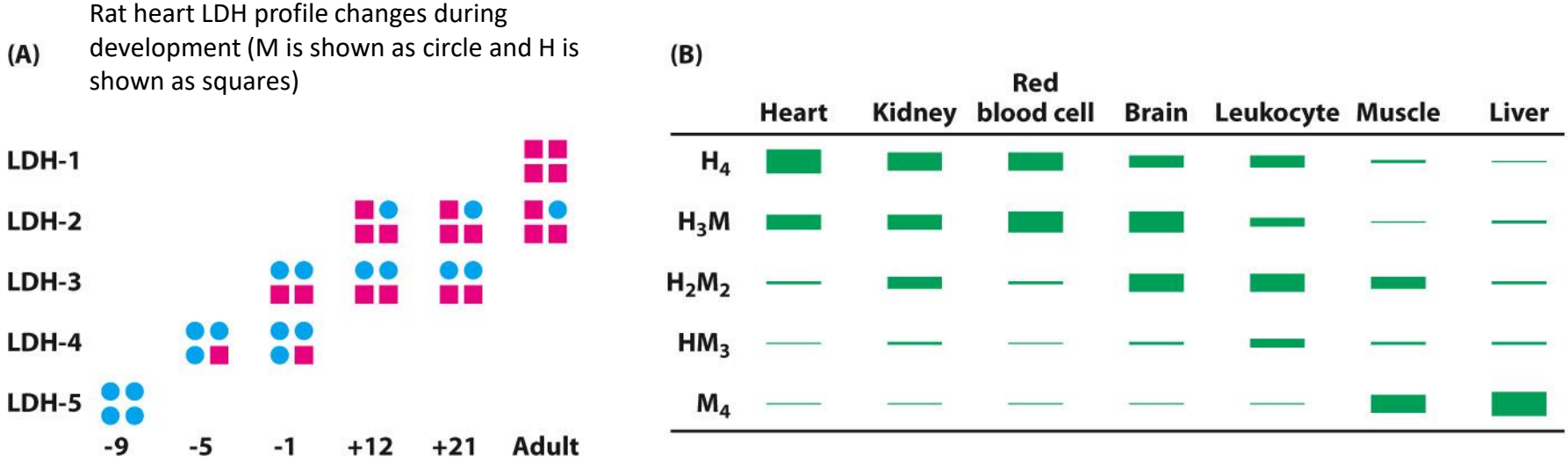
Encoded by different genes (through gene duplication and divergence)

Disttinct physical properties (electrophoretic mobility)

Lactate dehydrogenase

Involved in anaerobic glucose metabolism and synthesis
Each enzyme is heterotetrameric
In humans two isozymic polypeptide chains are expressed
H: expressed in heart muscle and M is expressed in skeletal muscle
H_4 has higher affinity for substrate and is inhibited by pyruvate than M_4 form

Rat heart LDH profile changes during development (M is shown as circle and H is shown as squares)



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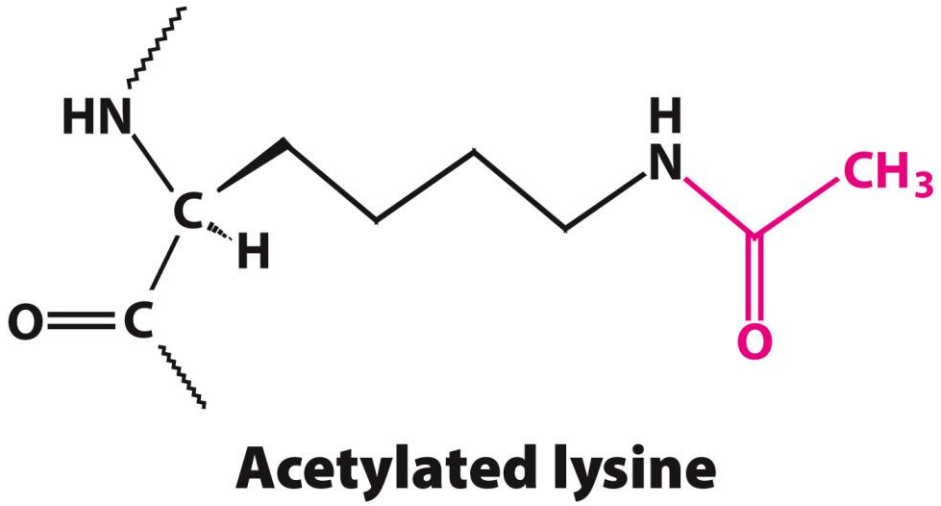
Covalent modification of enzymes

Enzymes can be modified by the covalent attachment of a molecule.

Phosphorylation and acetylation are common modifications.

Most covalent modifications are reversible.



Acetylated lysine

TABLE 10.1 Common covalent modifications of protein activity

Modification	Donor molecule	Example of modified protein	Protein function
Phosphorylation	ATP	Glycogen phosphorylase	Glucose homeostasis; energy transduction
Acetylation	Acetyl CoA	Histones	DNA packing; transcription
Myristoylation	Myristoyl CoA	Src	Signal transduction
ADP ribosylation	NAD^+	RNA polymerase	Transcription
Farnesylation	Farnesyl pyrophosphate	Ras	Signal transduction
γ-Carboxylation	HCO_3^-	Thrombin	Blood clotting
Sulfation	3′-Phosphoadenosine-5′-phosphosulfate	Fibrinogen	Blood-clot formation
Ubiquitination	Ubiquitin	Cyclin	Control of cell cycle

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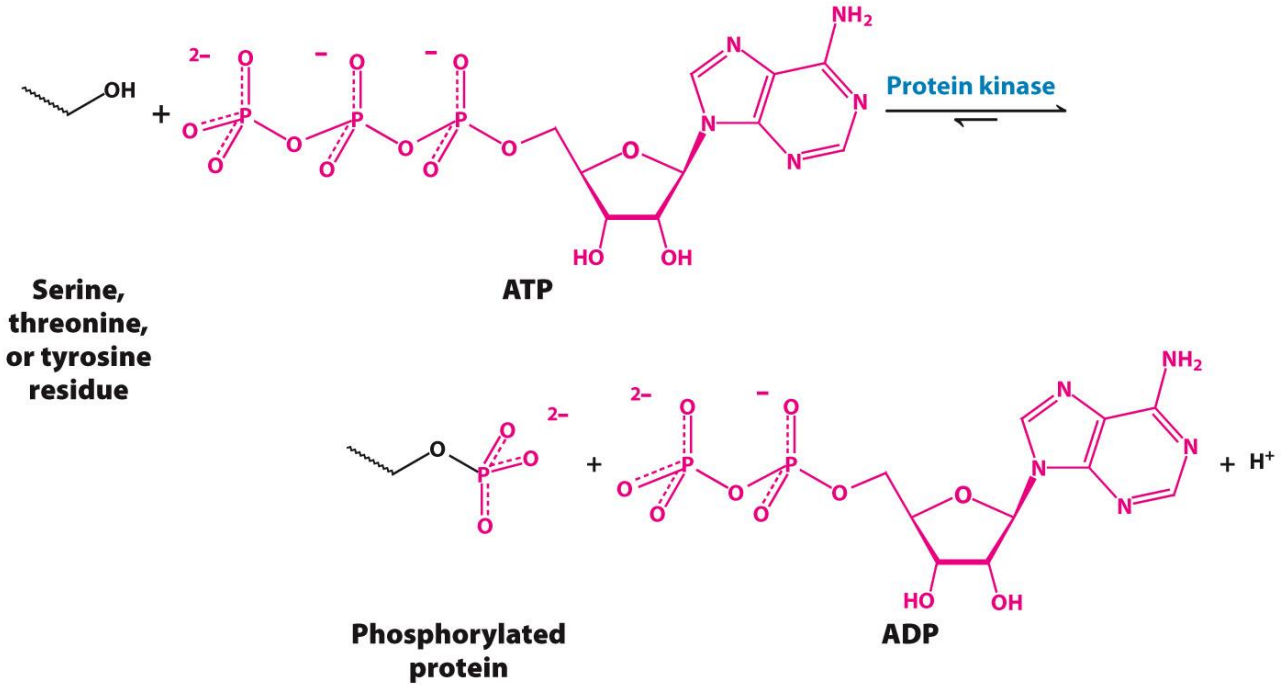
Kinases

Protein kinases modify proteins by attaching a phosphate to a serine, threonine, or tyrosine residue. ATP serves as the phosphate donor.



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Tyr kinases: unique to multicellular organisms
involved in growth regulation
mutation in Tyr kinases is often observed in cancer cells

TABLE 10.2 Examples of serine and threonine kinases and their activating signals

Signal	Enzyme
Cyclic nucleotides	Cyclic AMP-dependent protein kinase Cyclic GMP-dependent protein kinase
Ca^{2+} and calmodulin	Ca^{2+}—calmodulin protein kinase Phosphorylase kinase or glycogen synthase kinase 2
AMP	AMP-activated kinase
Diacylglycerol	Protein kinase C
Metabolic intermediates and other "local" effectors	Many target-specific enzymes, such as pyruvate dehydrogenase kinase and branched-chain ketoacid dehydrogenase kinase

Source: Information from D. Fell, *Understanding the Control of Metabolism* (Portland Press, 1997), Table 7.2.

Table 10.2
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Substrate specificity

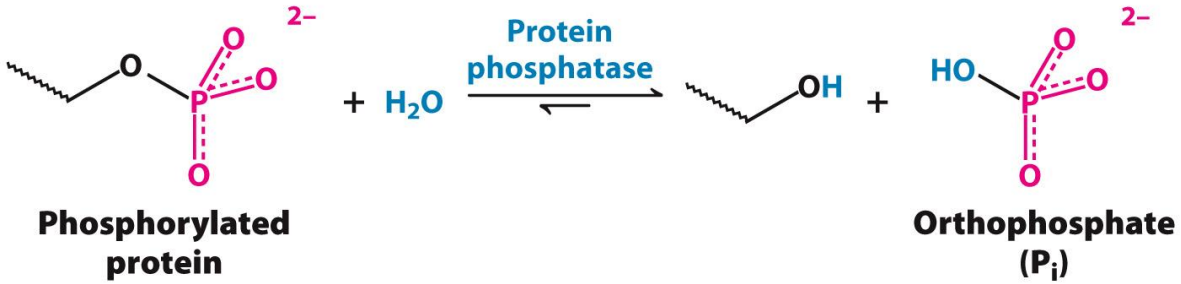
- Dedicated protein kinases: phosphorylate single protein or related proteins
- Multifunctional protein kinases: phosphorylates different targets by recognizing specific sequence:

protein kinase A: Arg-Arg-X-*Ser*-Z where X stands for a small residue and Z for a large residue

Kinases and phosphatases control the extent of protein phosphorylation

Protein phosphatases remove phosphates added by kinases.
Swithc off mechanism

The reactions catalyzed by kinases and phosphatases are irreversible under cellular conditions.



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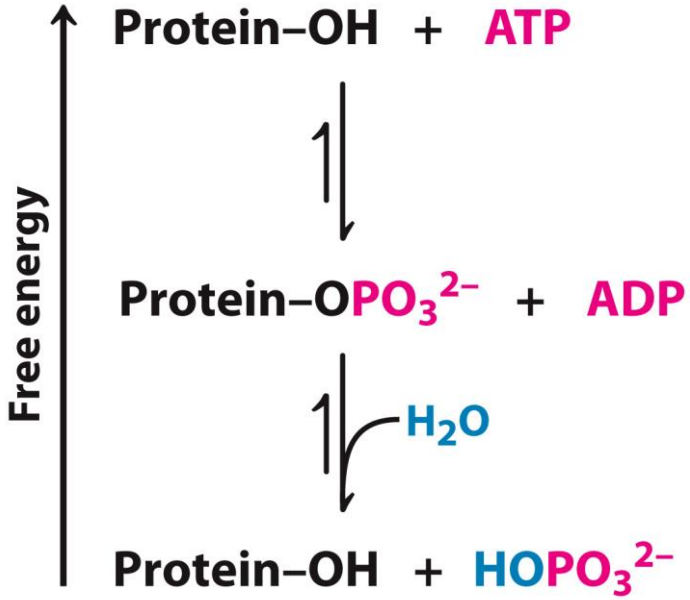

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Phosphorylation is a highly effective means of regulating the activities of target proteins

Key features of regulation by phosphorylation are:

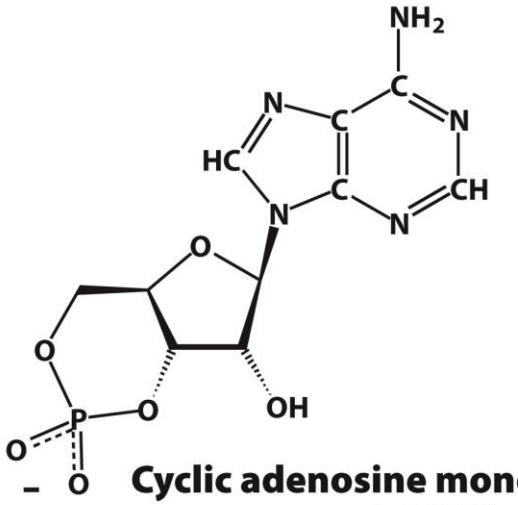
1. The free energy of phosphorylation is large (-12 kcal mol^{-1})
2. The addition of the phosphoryl group alters electrostatic interactions.
3. A phosphoryl group can form hydrogen bonds.
4. Phosphorylation and dephosphorylation can occur rapidly.
5. Phosphorylation can be used to amplify signals.
6. ATP is the cellular energy currency; link between the energy status of the cell and metabolism regulation

Cyclic AMP activates protein kinase A by altering the quaternary structure

Epinephrine (adrenaline) signifies the “fight or flight” response in muscles. In muscle cells exposed to epinephrine, cAMP is synthesized.

Cyclic AMP stimulates protein kinase A (PKA) by binding to the regulatory subunits of PKA, causing their dissociation from the catalytic subunits. The free catalytic subunits are active.

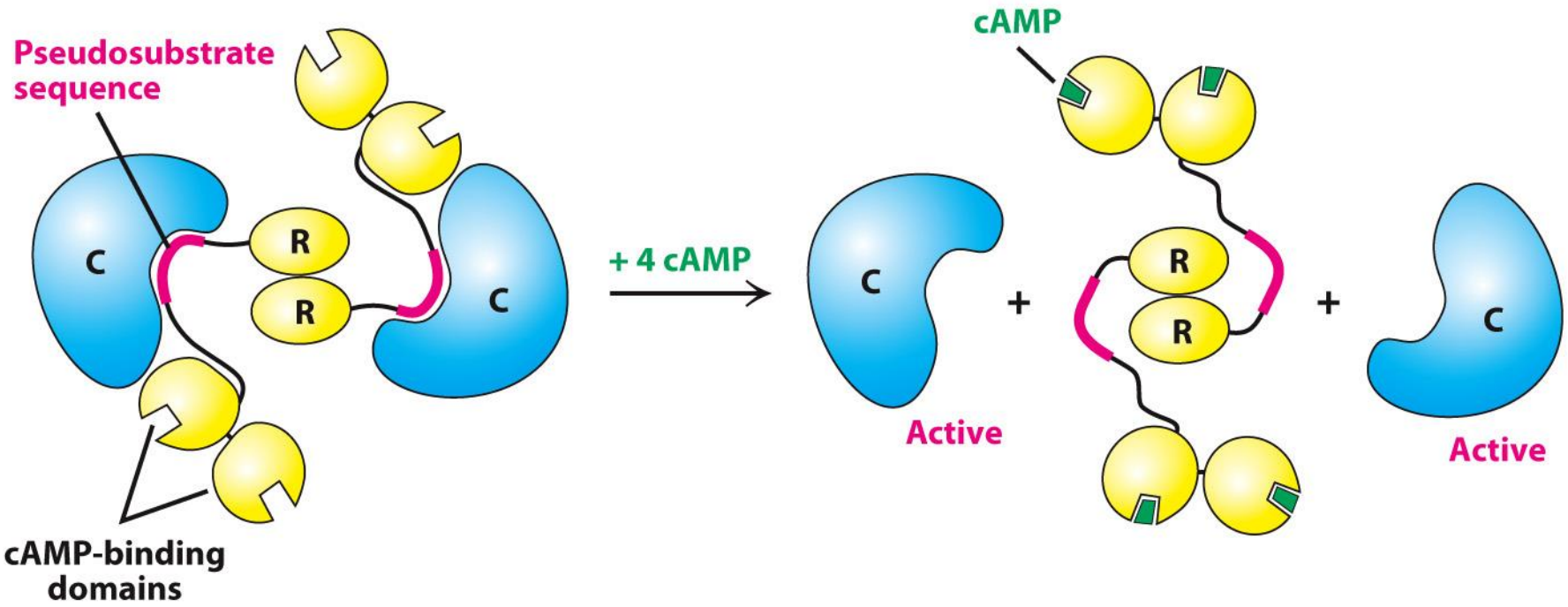
A pseudosubstrate sequence of the R subunit blocks the active site of the C subunit when the subunits are bound to each other.



Cyclic adenosine monophosphate (cAMP)

Resting state

Active state



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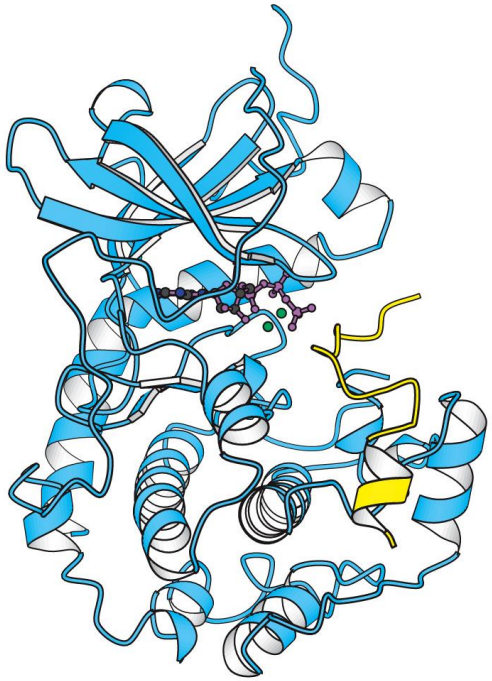
Upon cAMP binding the enzyme dissociates into a regulatory subunit and two active catalytic subunits

Pseudo-substrate sequence: Arg-Arg-Gly-Ala-Ile
Consensus sequence: Arg-Arg-Gly-Ser-Ile

Structure of protein kinase A

The catalytic subunit consists of two lobes. ATP and the target protein sequence (or the pseudosubstrate) occupy the cleft between the lobes.

Such a structural arrangement is common to all known protein kinases.
The substrate sequence is recognize through several contacts between the peptide and the protein



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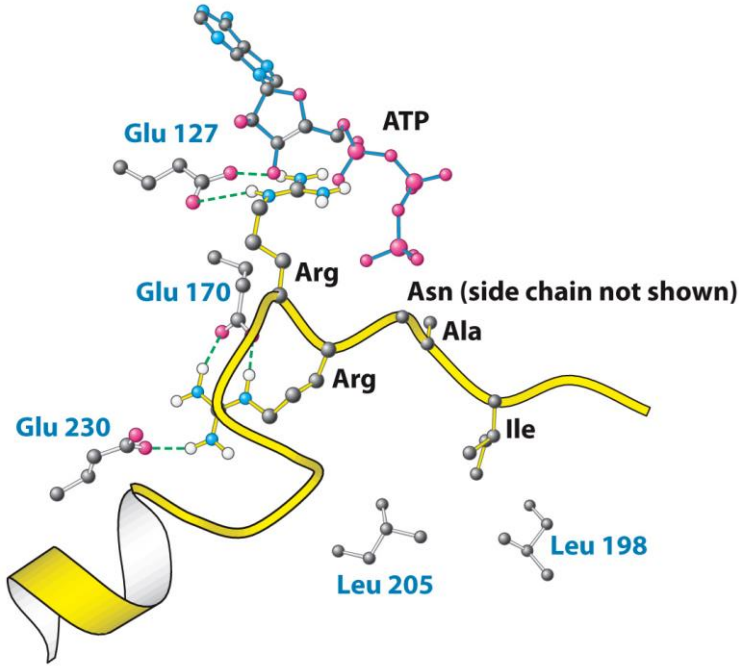

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Activation through a proteolytic cleavage

Proteolytic cleavage plays a key role in a number of biochemical processes.

1. Activation of digestive enzymes. The inactive form of the enzyme is called a zymogen or proenzyme.

2. Blood clotting.

3. Hormone activation.

4. Collagen formation.

5. Developmental processes.

6. Programmed cell death.

Chymotrypsinogen is activated by specific cleavage of a single peptide bond

The digestive enzyme chymotrypsin is synthesized as an inactive precursor called chymotrypsinogen.

A specific cleavage generates an active enzyme, π-chymotrypsin.

Two subsequent cleavages yields the mature enzyme, α-chymotrypsin.

TABLE 10.3 Gastric and pancreatic zymogens

Site of synthesis	Zymogen	Active enzyme
Stomach	Pepsinogen	Pepsin
Pancreas	Chymotrypsinogen	Chymotrypsin
Pancreas	Trypsinogen	Trypsin
Pancreas	Procarboxypeptidase	Carboxypeptidase

Table 10.3
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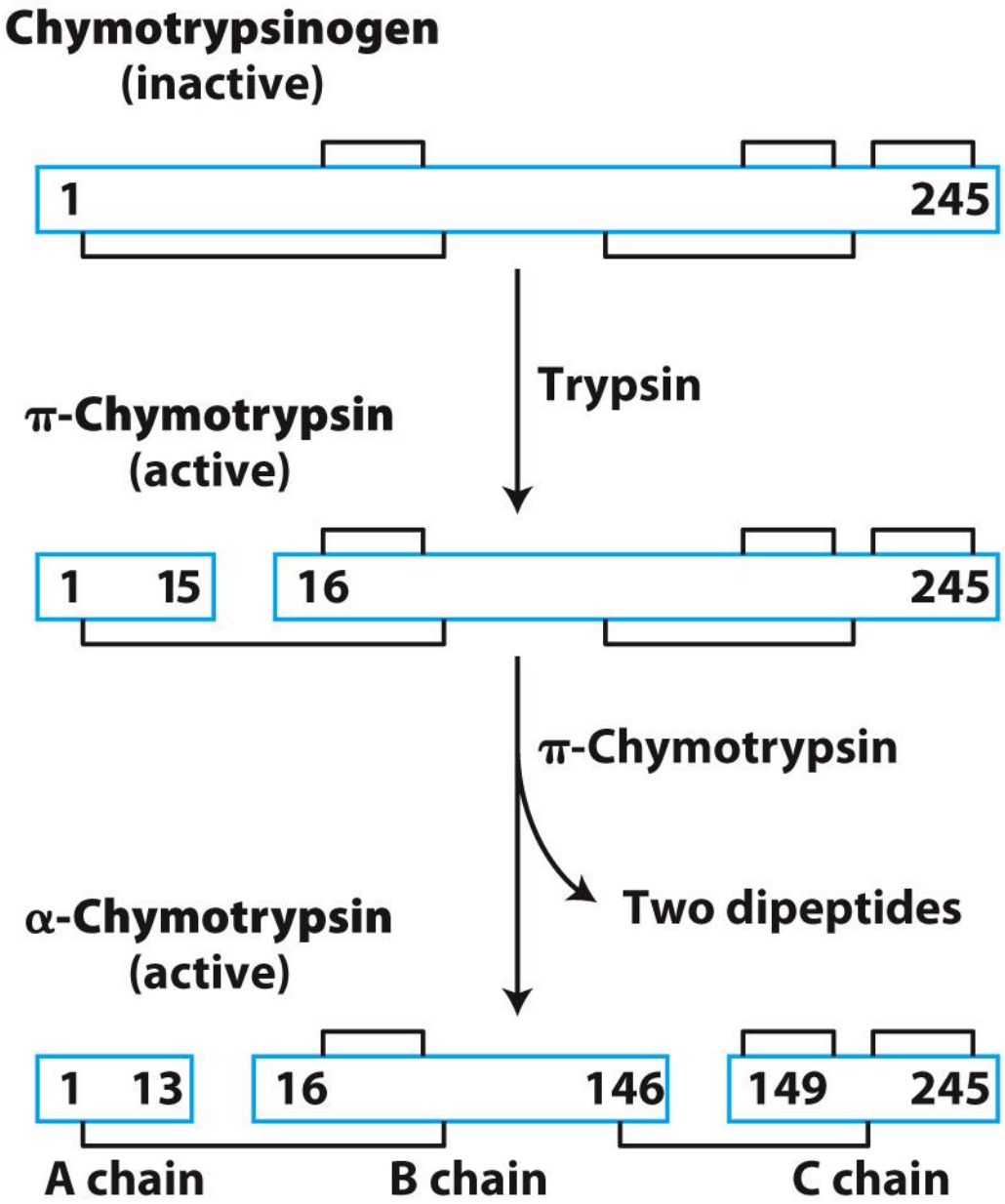

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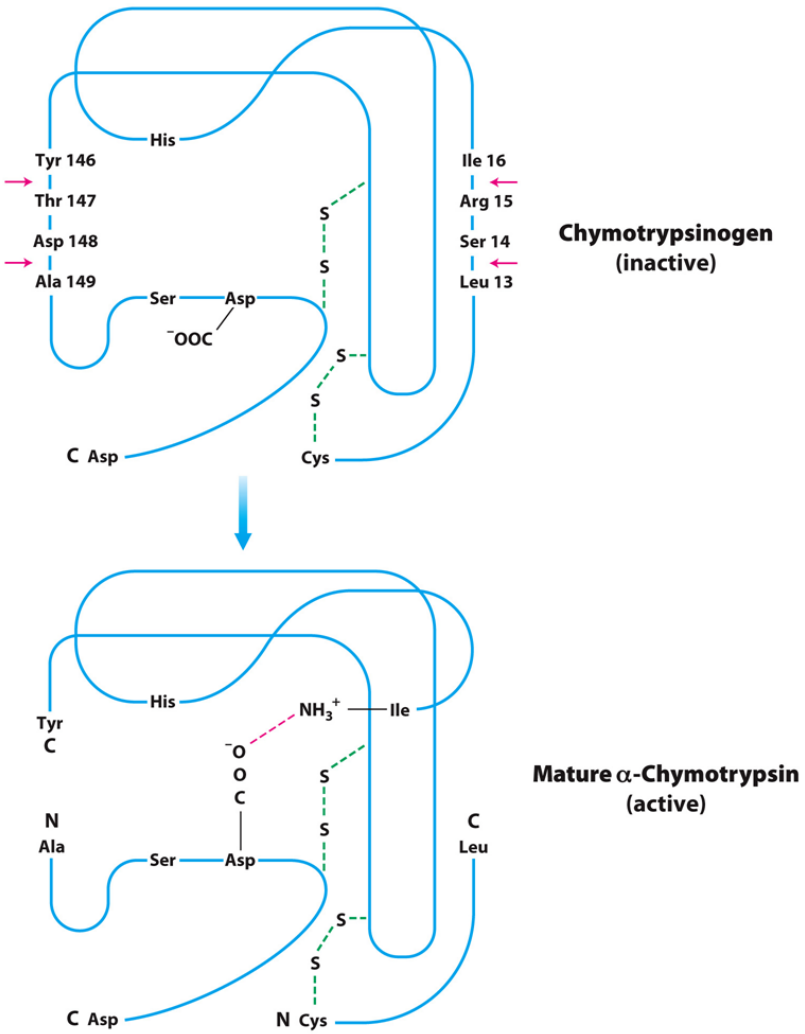
The conversion of chymotrypsinogen to chymotrypsin results in structural changes that generate the substrate-binding site and the oxyanion hole.



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How can cleavage of a peptide bond activate the enzyme?

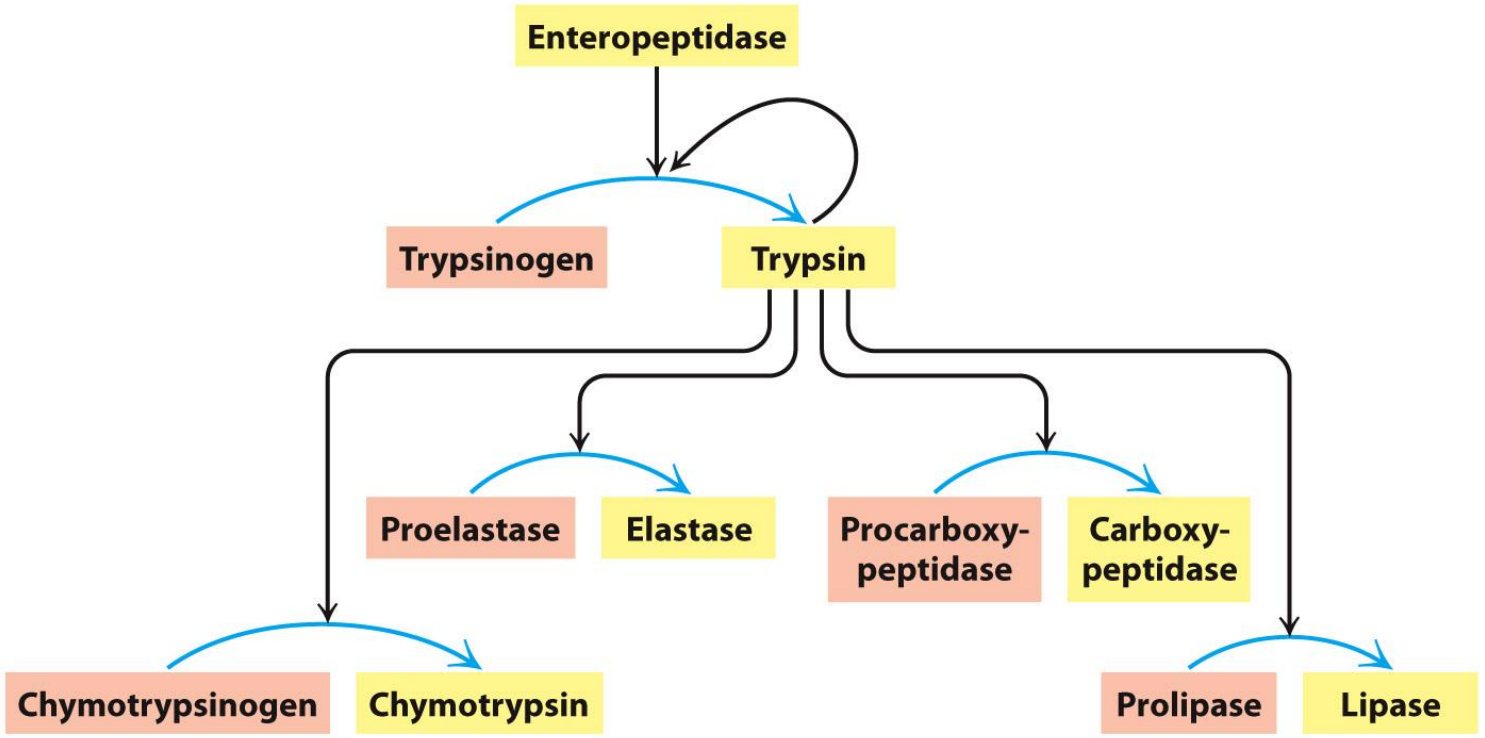
The newly form N- terminal group on Ile 16 forms a salt bridge with Asp 194.

This conormational change leads to other conformational changes including movement of Met192 on the protein surface and residues 187 and 193 move apart forming the substrate binding site . Also oxyanion hole is fully formed

Trypsin

Trypsinogen is converted to active trypsin by enteropeptidase.

Trypsin then activates all of the pancreatic zymogens.



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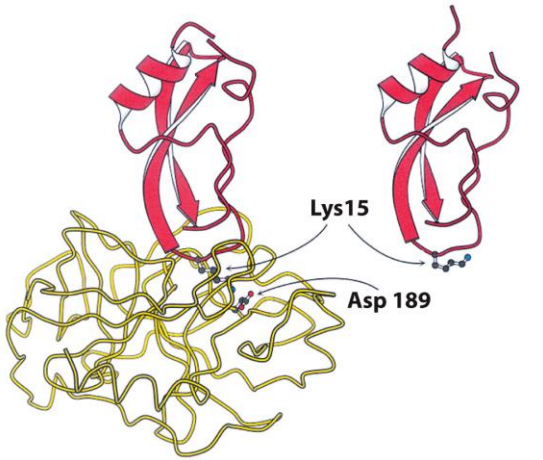
Some proteolytic enzymes have specific inhibitors

Pancreatic trypsin inhibitor protects against premature activation of trypsin in the pancreas.

Trypsin inhibitor is essentially a substrate that binds so tightly to the active site that it cannot progress to the transition state.

Lung elastase is a secretory product of white cells.
Mutations in a lung elastase inhibitor (α1 antitrypsin) can lead to lung diseases as chronic obstructive pulmonary disease
Cigarette smoke inactivates the inhibitor by oxidizing a methionine residue in the inhibitor.



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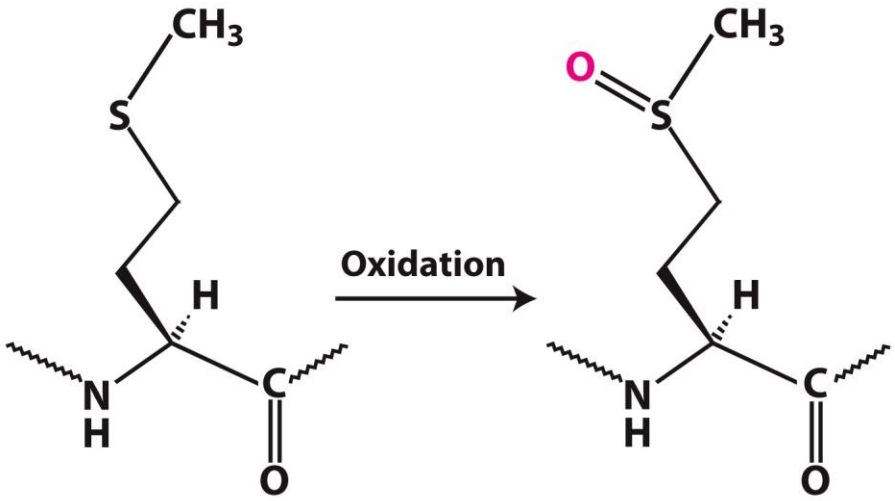

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