Proteínas como blancos farmacológicos.
Enzimas

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Estructura de proteínas
Estructura primaria

Secuencia de aminoácidos en una proteína

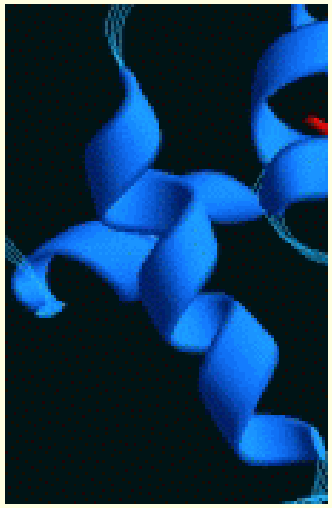
```
     1   2   3   4   5    126 127 128 129
     Lys-Val-Phe-Gly-Arg...Gly-Cys-Arg-Leu

NH2-terminal                  COO- terminal
```

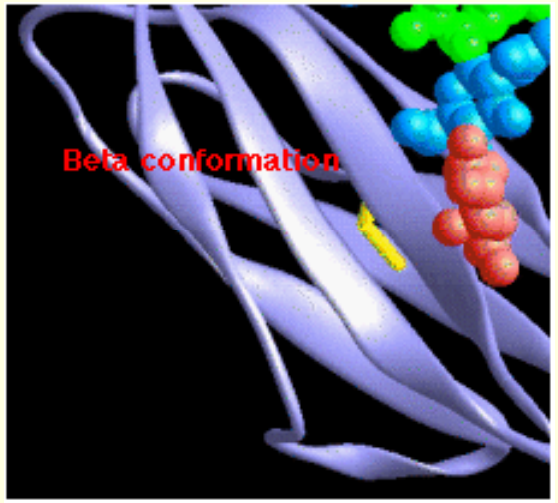
Estructura Secundaria

Segmentos de proteínas mantenidos por puentes de hidrógeno

α-helice



Estructura-β

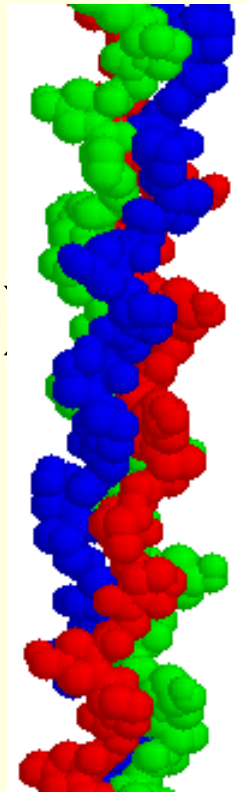


Estructura terciaria

Estructura tridimensional de una proteína. Estos tipos puedenj ser por ejemplo globulares o fibrosos.

Globular

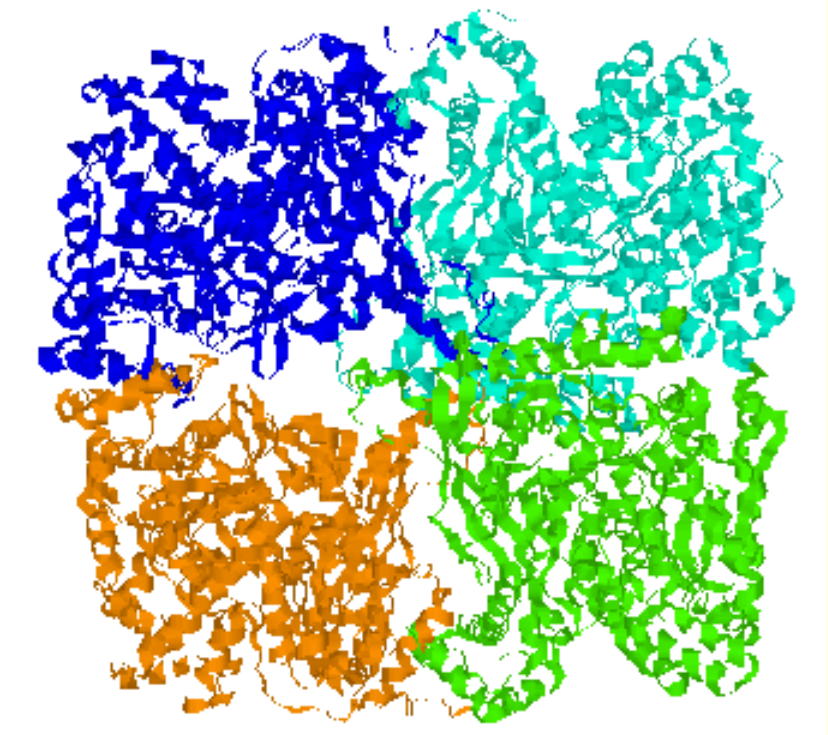
Fibroso (Colágeno)



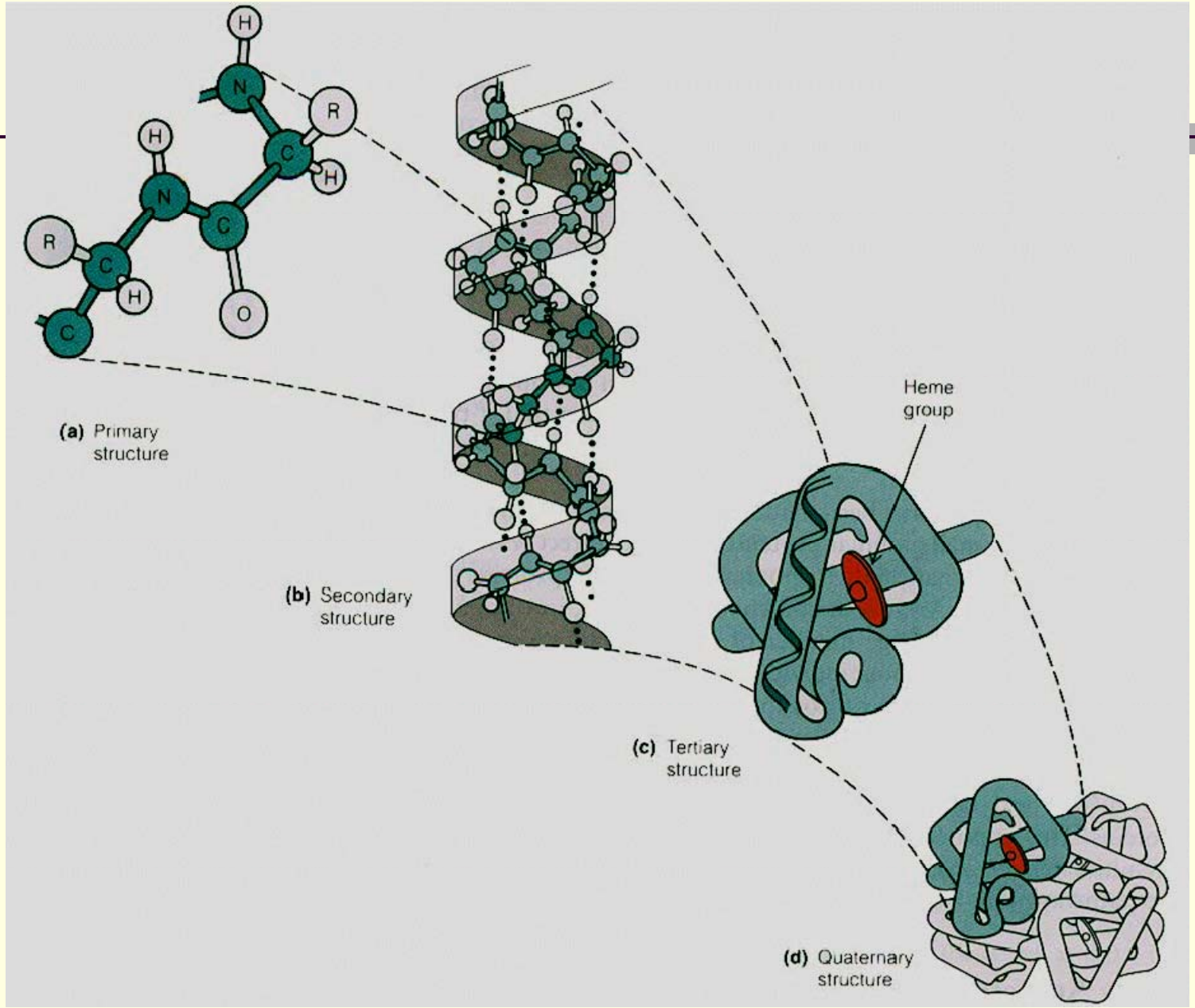
Estructura Cuaternaria

Complejos de proteínas

glicógeno
fosforilasa



Niveles de estructura



ENZIMAS

- Las enzimas son en su mayoría proteínas o RNA (riboenzimas).
- Son catalizadores que catalizan una reacción química que involucra la formación o la ruptura de enlaces químicos.
- No se consume ni se modifica en las reacciones.
- Al finalizar la reacción la enzima se libera y queda disponible para otra reacción.

Mecanismo de acción de fármacos

- **Modificación de proteínas estructurales**

Alcaloides para el tratamiento del cáncer

- **Enzimas**

Estreptokinasas para la disolución de trombos

- **Unión covalente a macromoléculas**

ciclofosfamida para el cancer

- **Reacción con moléculas pequeñas**

antiácidos

- **Unión a moléculas o átomos**

Unión a metales pesados

Catálisis por enzimas

- Actúan sobre un sustrato formando un complejo **enzima-sustrato** al unirse en un lugar específico del sustrato, el **sitio activo de la enzima** **(en donde se hallan los grupos químicos responsables de la catálisis).**

- Las enzimas son selectivas, pocas moléculas pueden interactuar con el sitio activo y formar el complejo.

Enzimas (características)

Proteínas de alto peso molecular (macromoléculas, 10^4 a 10^6 g/mol, 10^2 a 10^4 aminoácidos)

Su actividad depende de la integridad de su conformación proteica.

Metales pueden formar parte de su centro activo, o de otra parte de la enzima (co-factor).

Los reactivos de la reacciones catalizadas por enzimas se denominan sustratos.

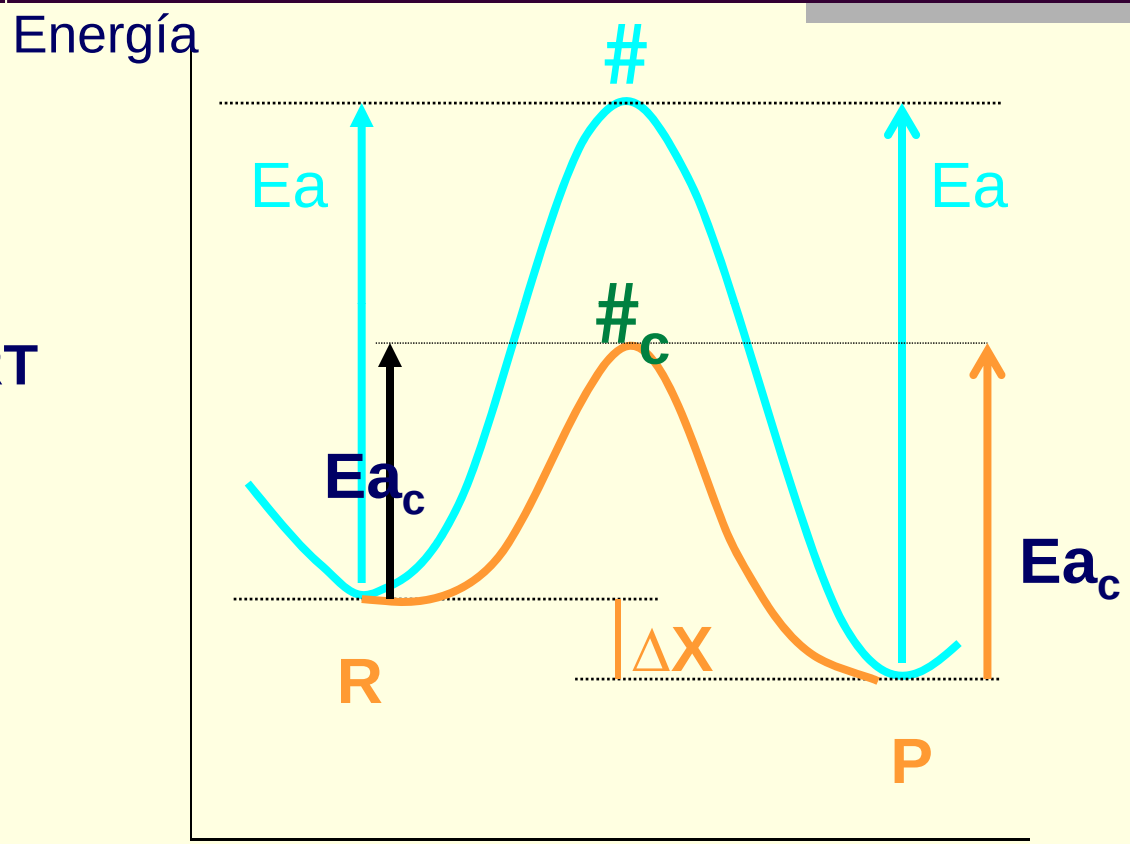
Características de un catalizador

- Incrementa la velocidad de reacción al $\downarrow E_A$.
- Forma un complejo transitorio con el sustrato.

$$E + S \longrightarrow ES \longrightarrow E + P$$

Efecto de un catalizador sobre la Ea

$$k_r = A.\ e^{-Ea/RT}$$



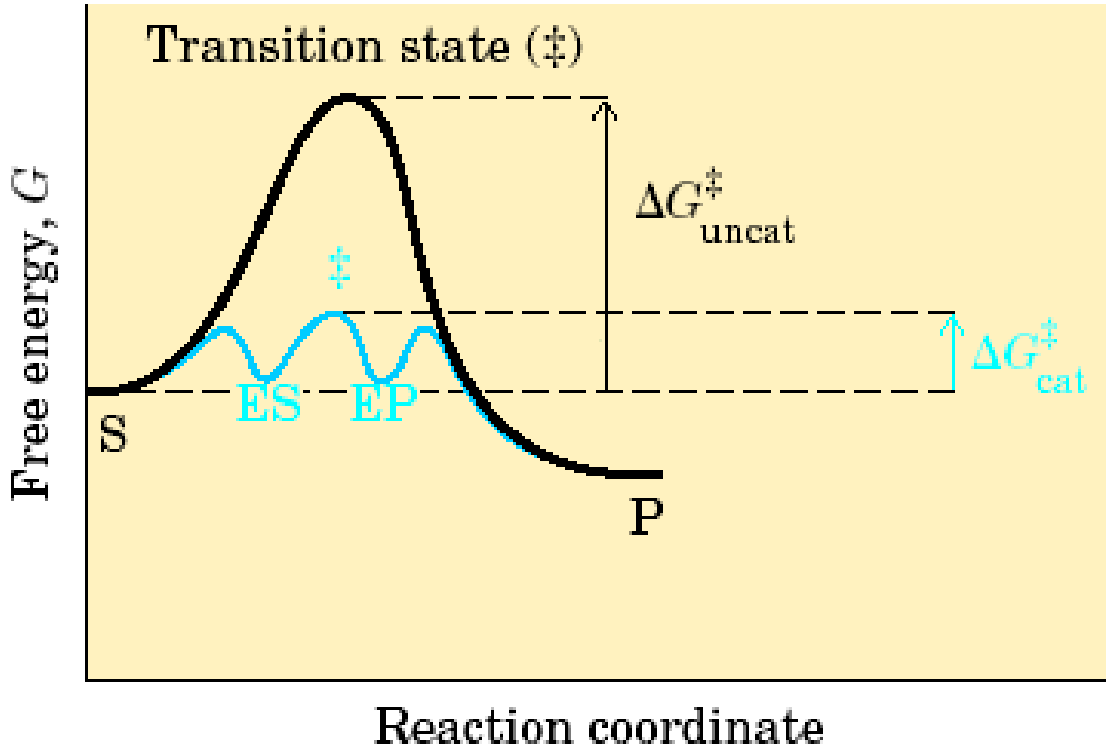
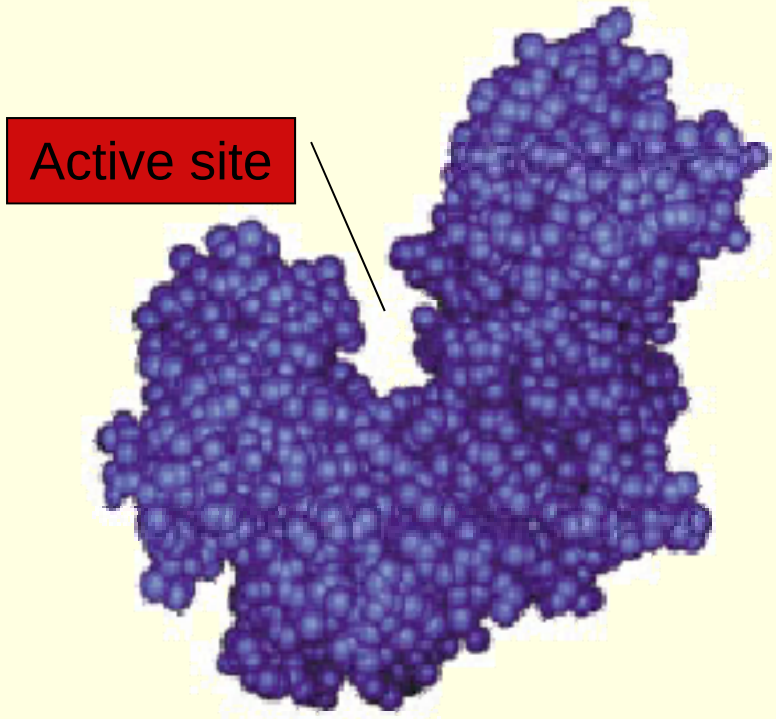


FIGURE 6–3 Reaction coordinate diagram comparing enzyme-catalyzed and uncatalyzed reactions. In the reaction S ⟶ P, the ES and EP intermediates occupy minima in the energy progress curve of the enzyme-catalyzed reaction. The terms $\Delta G^{\ddagger}_{uncat}$ and $\Delta G^{\ddagger}_{cat}$ correspond to the activation energy for the uncatalyzed reaction and the overall activation energy for the catalyzed reaction, respectively. The activation energy is lower when the enzyme catalyzes the reaction.

Estructura de las enzimas

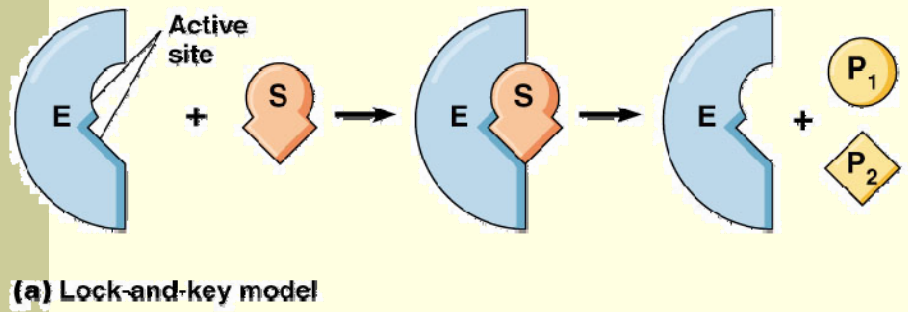
- Sitio activo
  - Algunos residuos aminoacídicos.
  - C, H, S, D, E, & K
  - Cisteina, histidina, serina, glicina, glutamico, lisina



Estructura de las enzimas

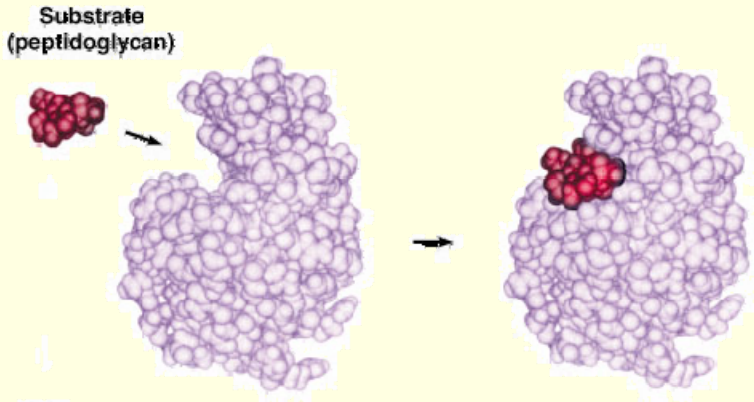
- Grupo prostético
  - No proteico
    - Cofactor
    - Coenzima
- Apoenzima
  - Enzima sin grupo prostético.
- Holoenzima
  - Enzima completa.

Especificidad enzimática

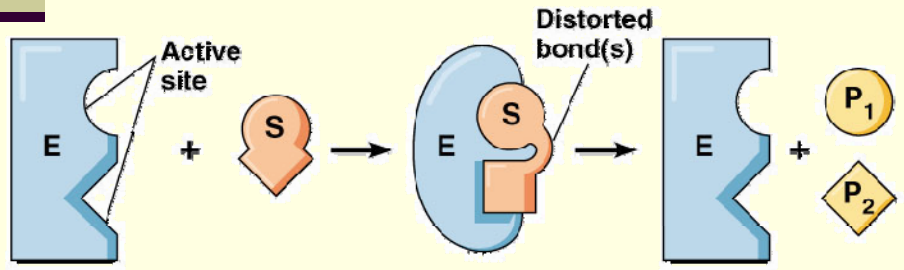


(a) Lock-and-key model

Emil Fischer (1890)

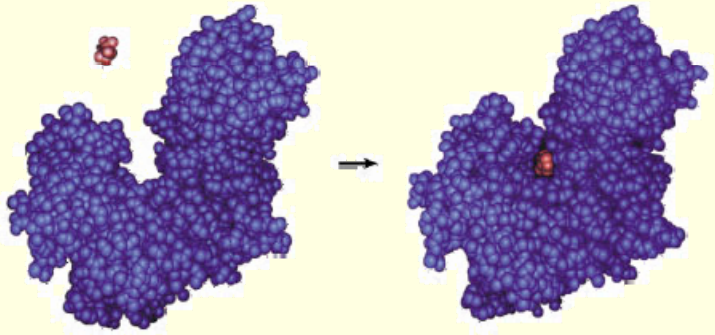


(a) Lysozyme



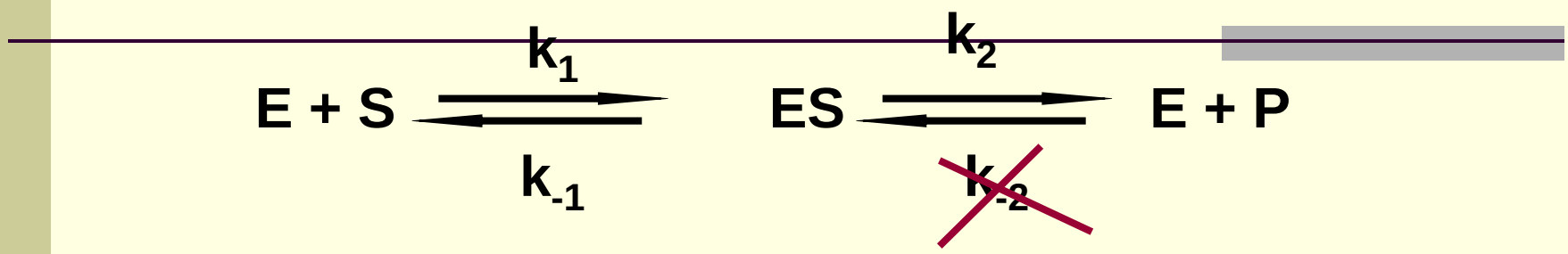
(b) Induced-fit model

Daniel Koshland (1958)



(b) Hexokinase

Mecanismo de Michaelis-Menten



$$v = - d(S)/dt = d(P)/dt = k_2 (ES)$$

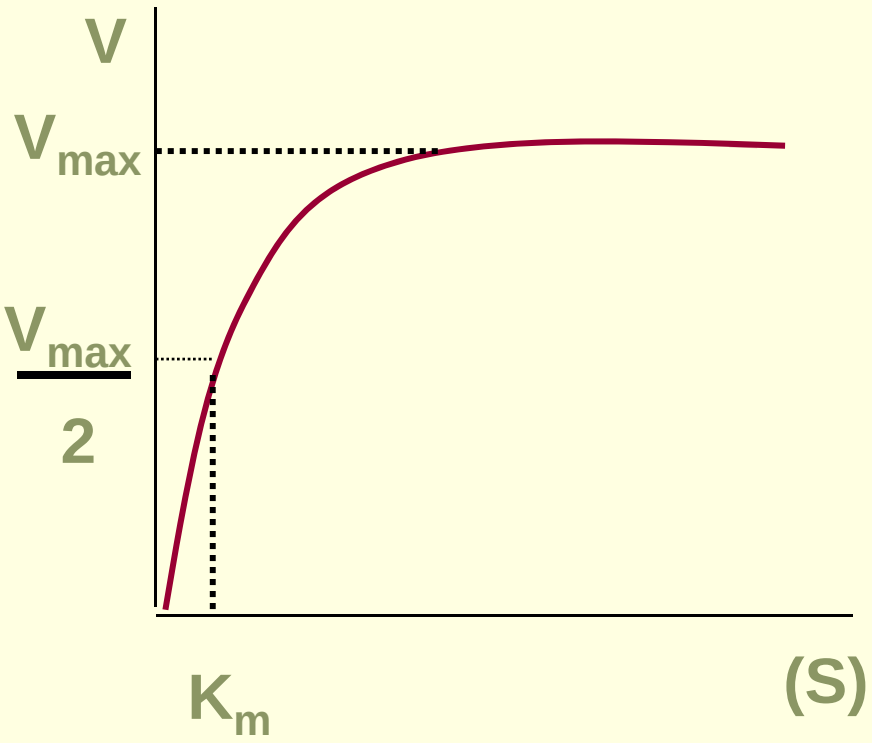
suposiciones: $(E) << S$ $d(ES)/dt = 0$

$$k_1(E)(S) = (k_2 + k_{-1})(ES)$$

$$(ES) = \frac{k_1(E)(S)}{(k_2 + k_{-1})} \qquad Km = \frac{(k_2 + k_{-1})}{k_1}$$

Ecuación de Michaelis-Menten

$$v = \frac{V_{max}\,(S)}{[Km + (S)]}$$

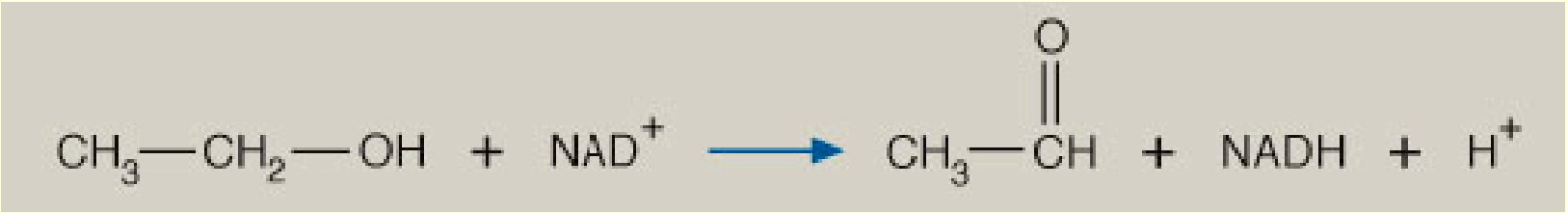


Categorías de enzimas

- Oxidoreductasas
- Tranferasas
- Hidrolases
- Liases
- Isomerasas
- Ligasas

Oxidoreductasas (E.C. 1)

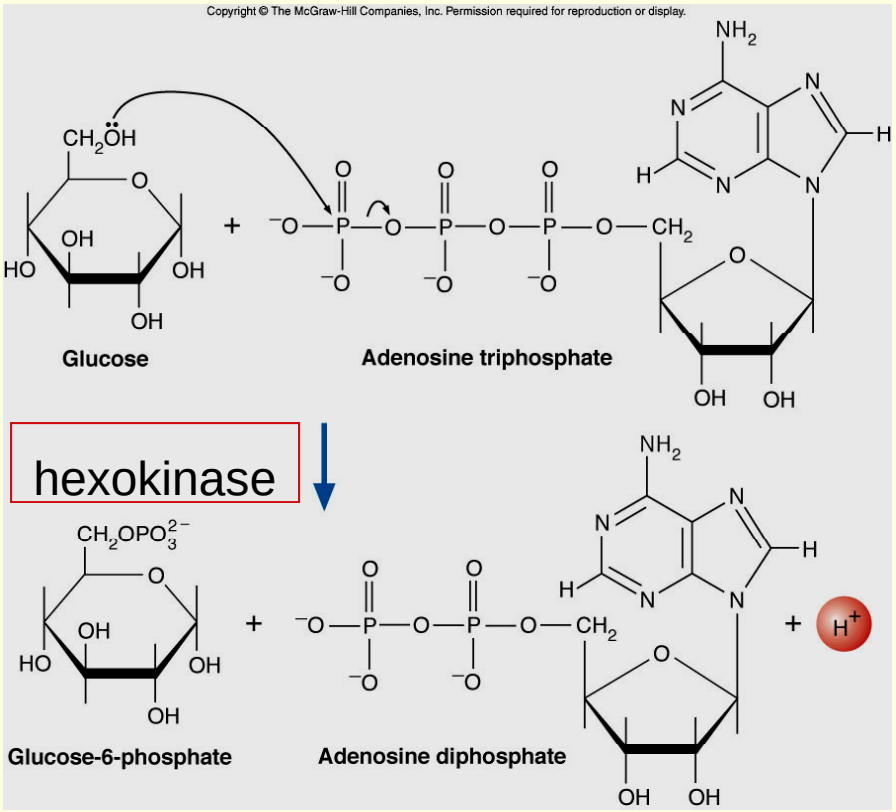
- Catalizan reacciones de oxido-reducción.
  - Dehidrogenasas
  - Oxidasas
  - Oxigenasas
  - Reductasas
  - Peroxidasas
  - hidroxilases



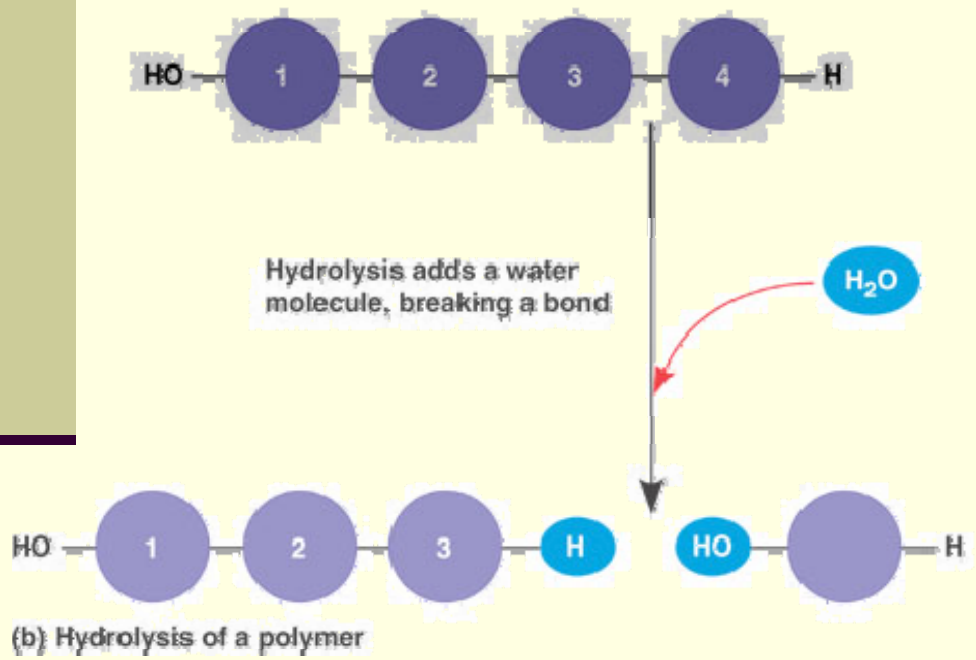
Alcohol dehydrogenase

Transferasas (E.C. 2)

- Catalizan la transferencia de un grupo funcional de una molécula a otra.
  - Transaminasas
  - Transmetilasas
  - Transcarboxilasas
  - Kinasas



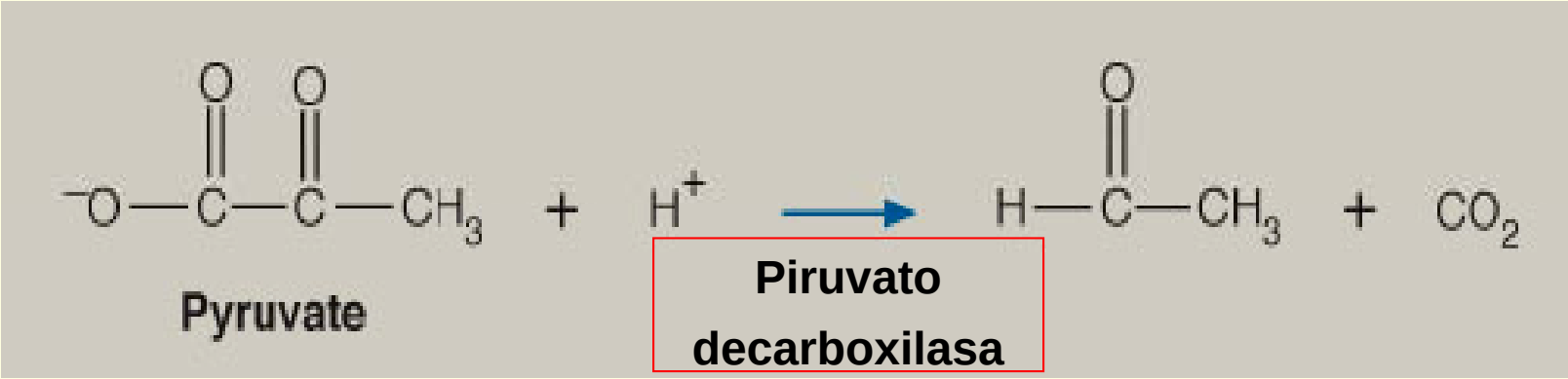
Hidrolasas (E.C. 3)



- Catalizan reacciones de hidrólisis
  - Esterasas
  - Fosfatasas
  - Peptidasas

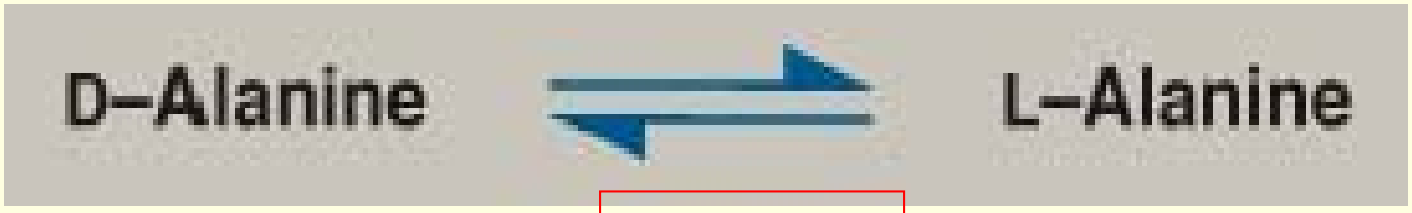
Lyases (E.C. 4)

- Catalizan grupos son removidos o agregados a un doble enlace.
  - Decarboxilasas
  - Hidratasas
  - Deaminasas



Isomerasas (E.C. 5)

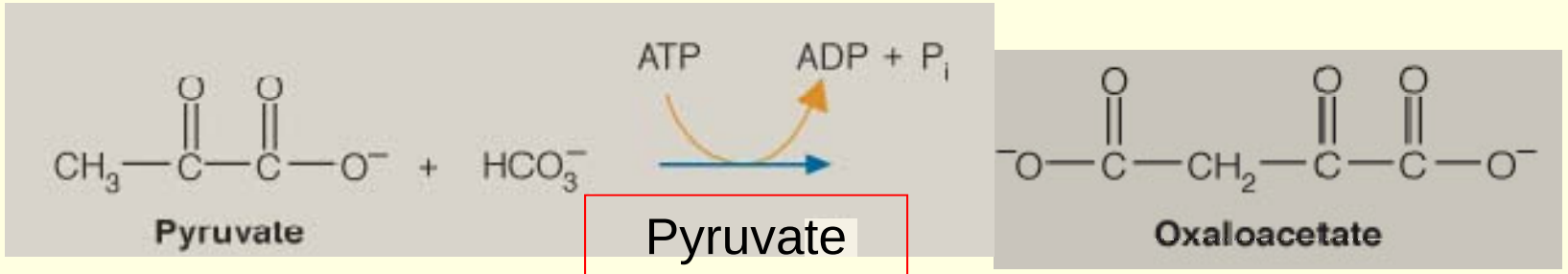
- Catalizan rearreglos intramoleculares.
  - Epimerasas
  - Racemasas
  - Mutasas



Alanina racemasa

Ligasas (E.C. 6)

- Catalizan la formación de enlaces.
  - Sintetasas
  - Carboxilasas

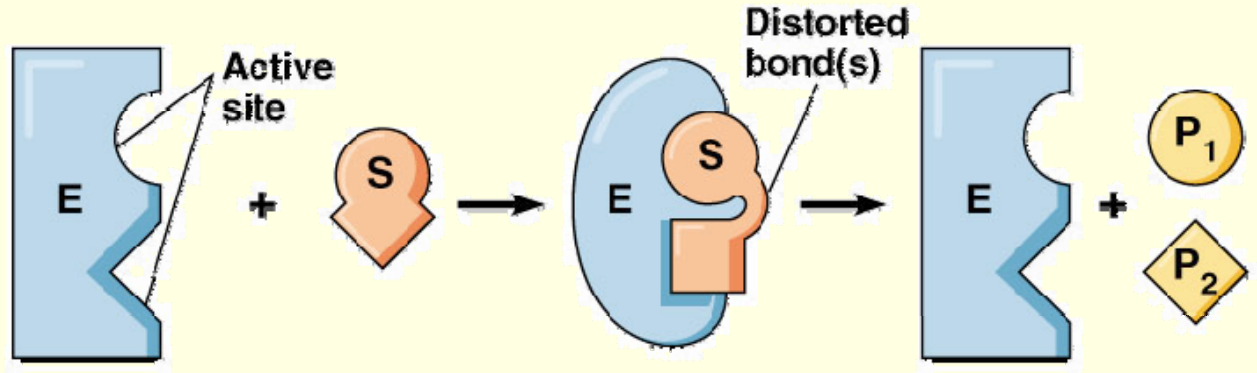


Catálisis enzimática

- Mecanismo catalítico
  - Efecto de proximidad
  - Efectos electroestáticos
  - Catálisis ácido-base
  - Catálisis covalente

Efecto de proximidad

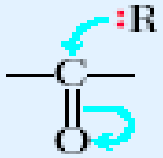
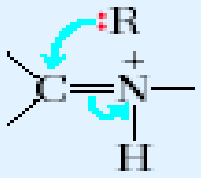
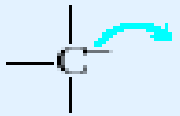
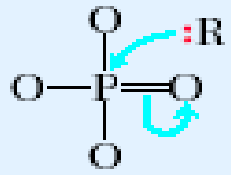
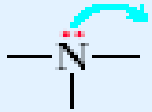
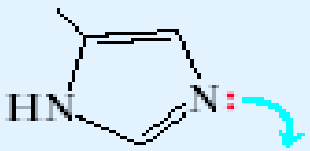
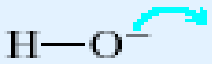
- Distorción del sustrato, modelo del encaje inducido



(b) Induced-fit model

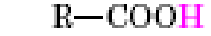
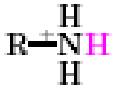
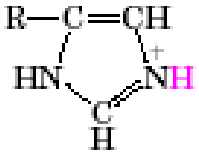
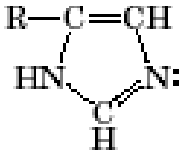
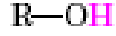
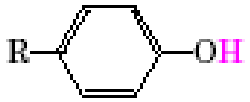
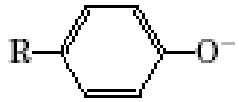
Efectos Electrostáticos

- El agua es excluida del sitio activo.
- Permite que el sustrato se una mas eficientemente a la enzima, disminuyendo la E_A.

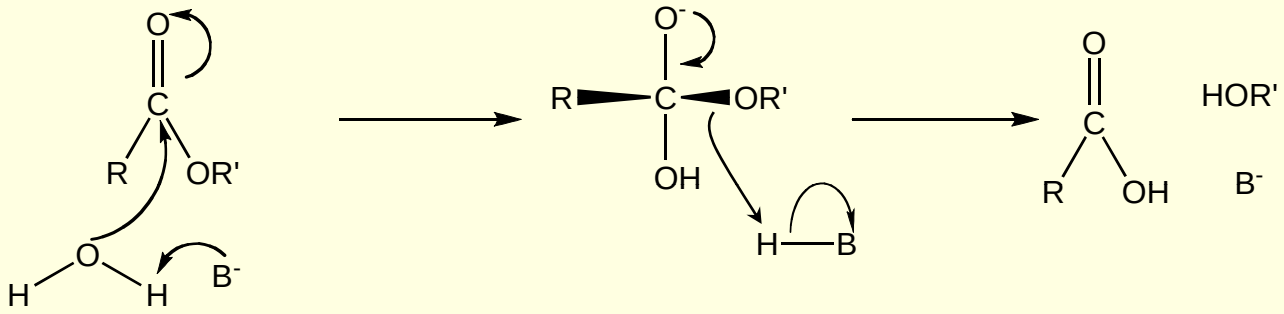
Nucleophiles	Electrophiles
 Negatively charged oxygen (as in an unprotonated hydroxyl group or an ionized carboxylic acid)	 Carbon atom of a carbonyl group (the more electronegative oxygen of the carbonyl group pulls electrons away from the carbon)
 Negatively charged sulfhydryl	 Pronated imine group (activated for nucleophilic attack at the carbon by protonation of the imine)
 Carbanion	 Phosphorus of a phosphate group
 Uncharged amine group	 Proton
 Imidazole	
 Hydroxide ion	

Catálisis Acido-Base

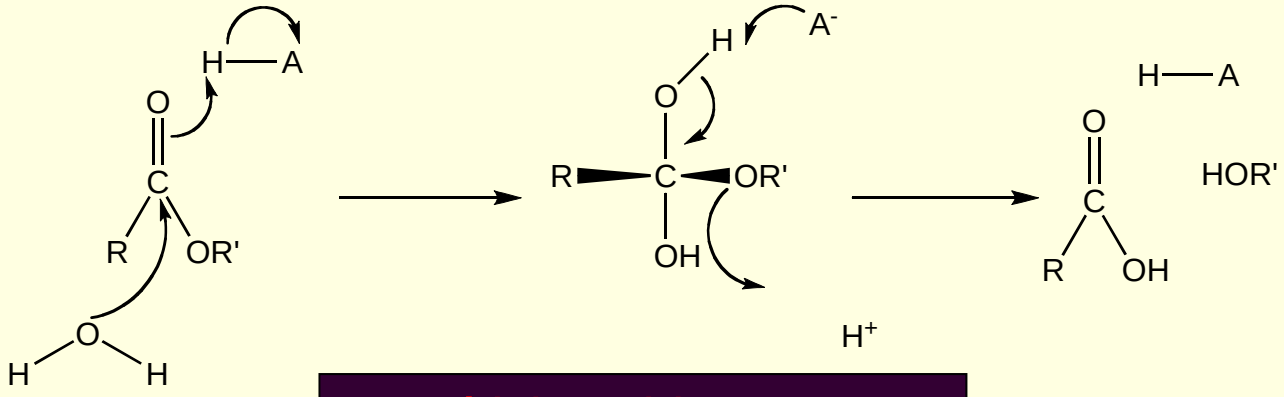
- Los grupos de los aminoácidos en el sitio activo actuan como dadores o aceptores de protones.
- La adición y remoción de protones puede hacer que un grupo sea mas reactivo, disminuyendo la E_A.

Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp		
Lys, Arg		
Cys		
His		
Ser		
Tyr		

Catálisis Acido-Base



Catálisis Básica General



Catálisis Acida General

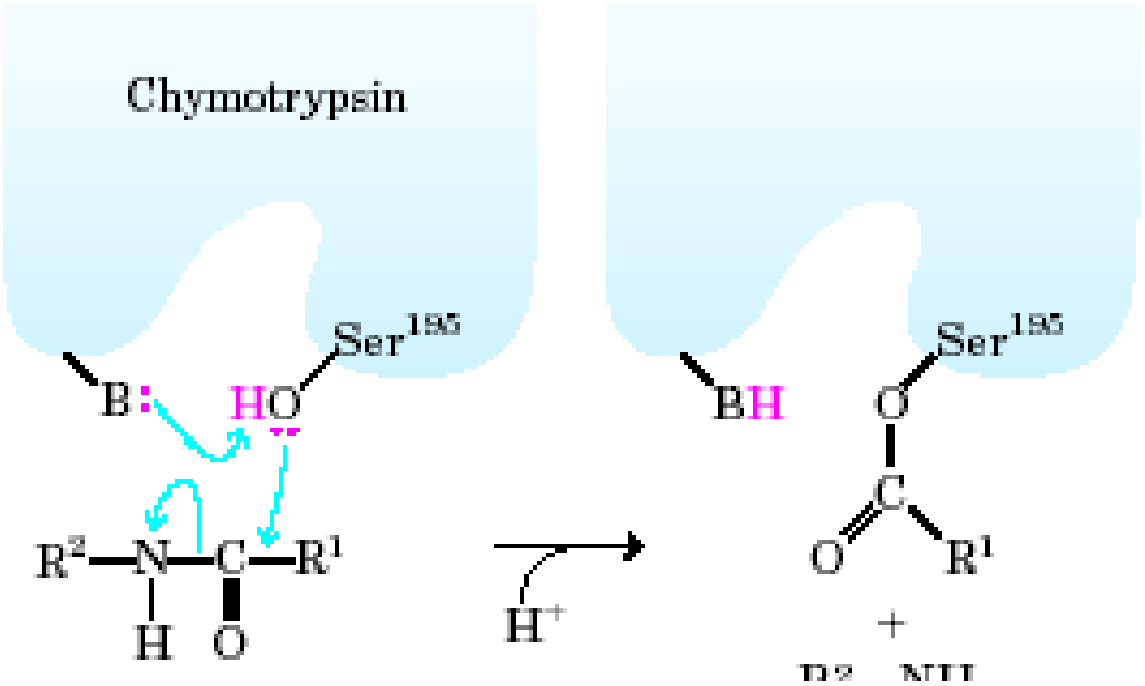
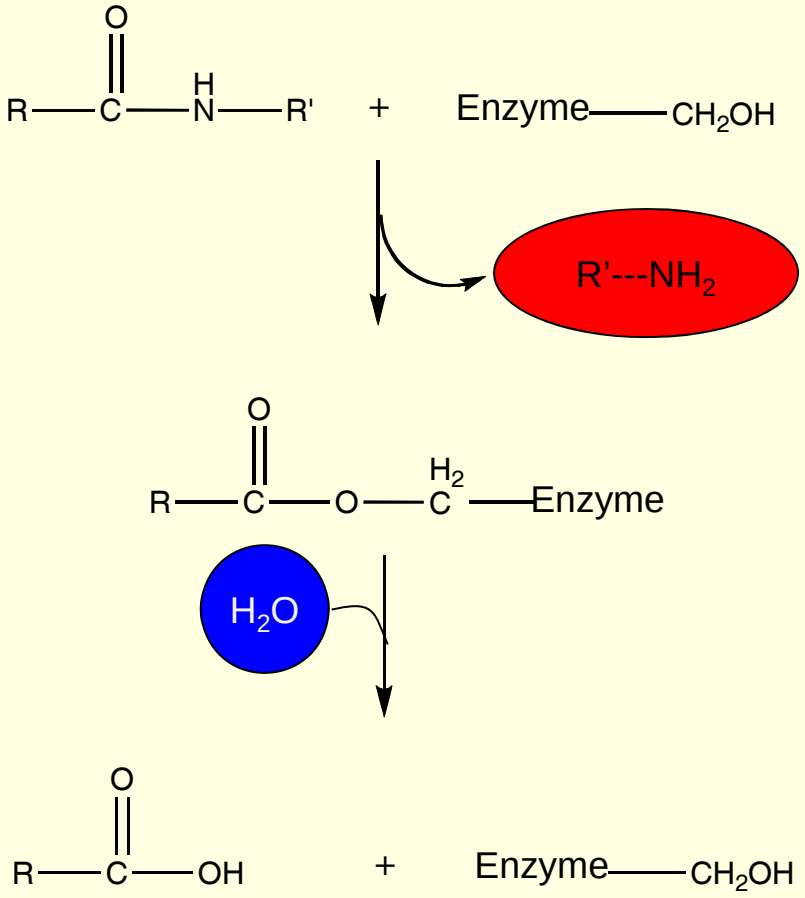


FIGURE 6–10 **Covalent and general acid-base catalysis.** The first step in the reaction catalyzed by chymotrypsin is the acylation step. The hydroxyl group of Ser^{195} is the nucleophile in a reaction aided by general base catalysis (the base is the side chain of His^{57}). This provides a new pathway for the hydrolytic cleavage of a peptide bond. Catalysis occurs only if each step in the new pathway is faster than the uncatalyzed reaction. The chymotrypsin reaction is described in more detail in Figure 6–21.

Catálisis Covalente

- Un residuo del aminoácido forma un enlace inestable con el sustrato.
  - Substitución Nucleofílica, la Enzima dona electrones al substrato
  - Substitution electrofílica, el Substrato dona electrones a la enzima. Generalmente requiere de un cofactor.

Catálisis Covalente

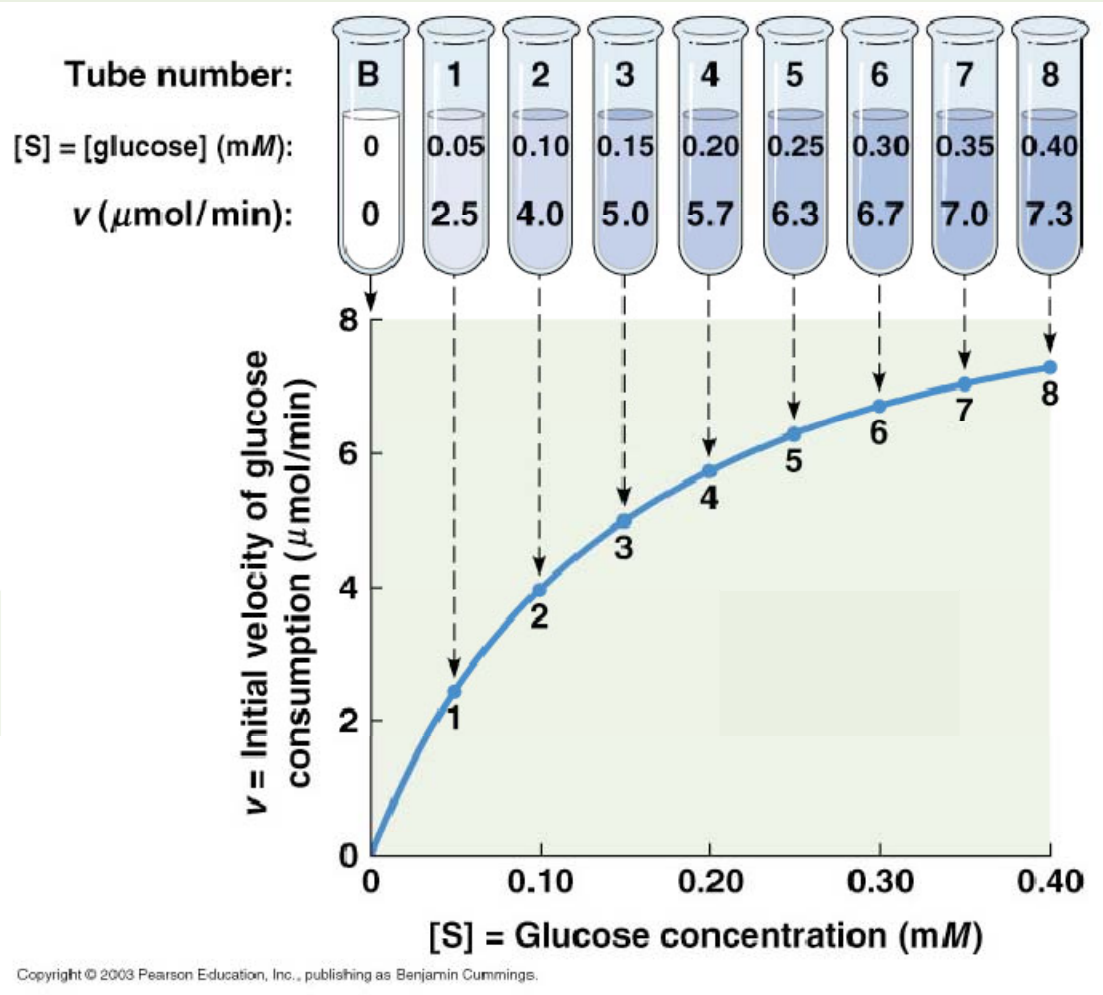


Parámetros que afectan la función enzimática

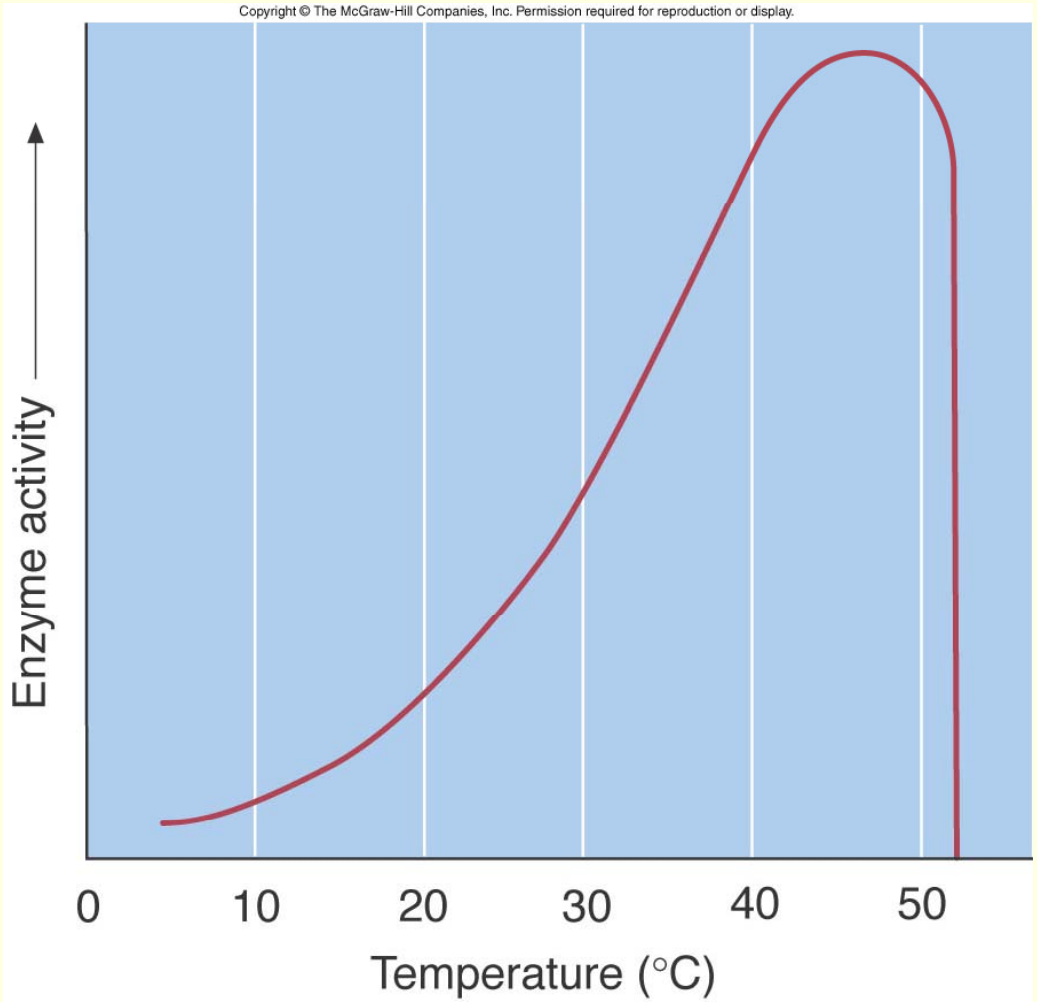
- Concentracion de Substrato o enzima
- Temperatura
- pH
- concentracion de Sales
- Grupos Prostéticos
  - cofactores
  - coenzimas
- Inhibidores
- Reguladores

Concentración de Substrato

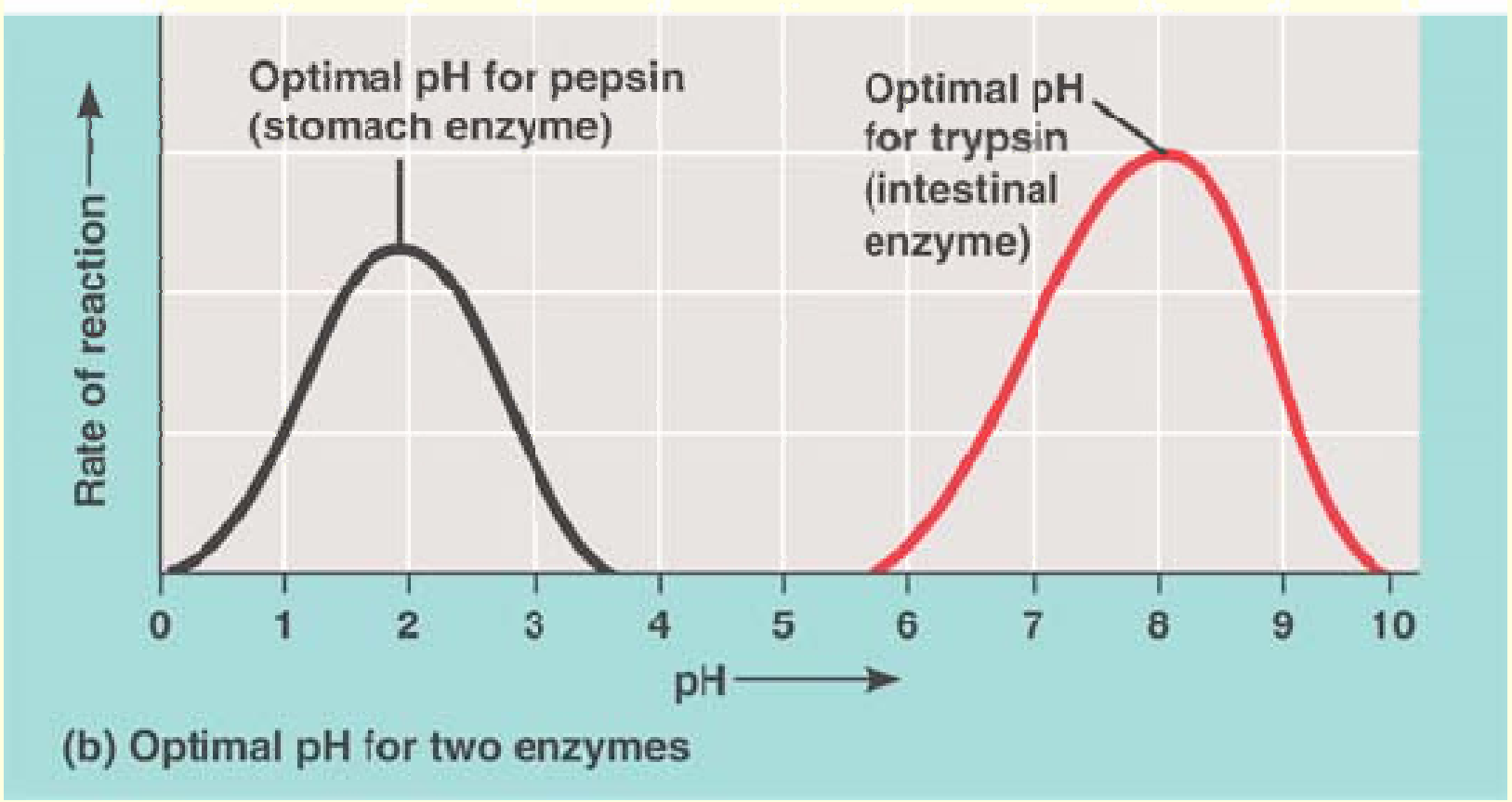
↑[S] ↑Velocidad



Temperatura

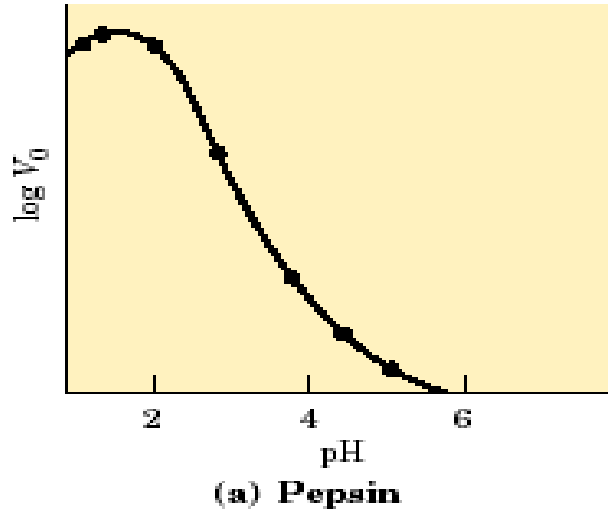


pH



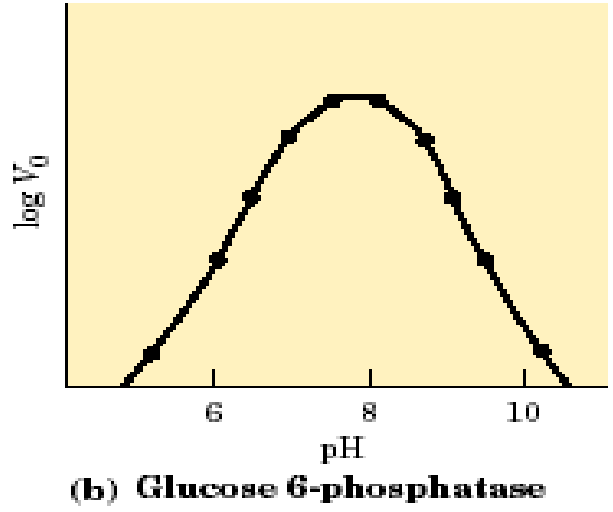
(b) Optimal pH for two enzymes

Efecto del pH en la catálisis enzimática



(a) Pepsin

Proteasa



(b) Glucose 6-phosphatase

Fosfatasa

Cofactores

Activadores que cambian la conformación de la enzima para formar el complejo enzima-sustrato reciben el nombre de **cofactores**:

- iones metálicos
- orgánicos se llaman **coenzimas**.

Cofactores

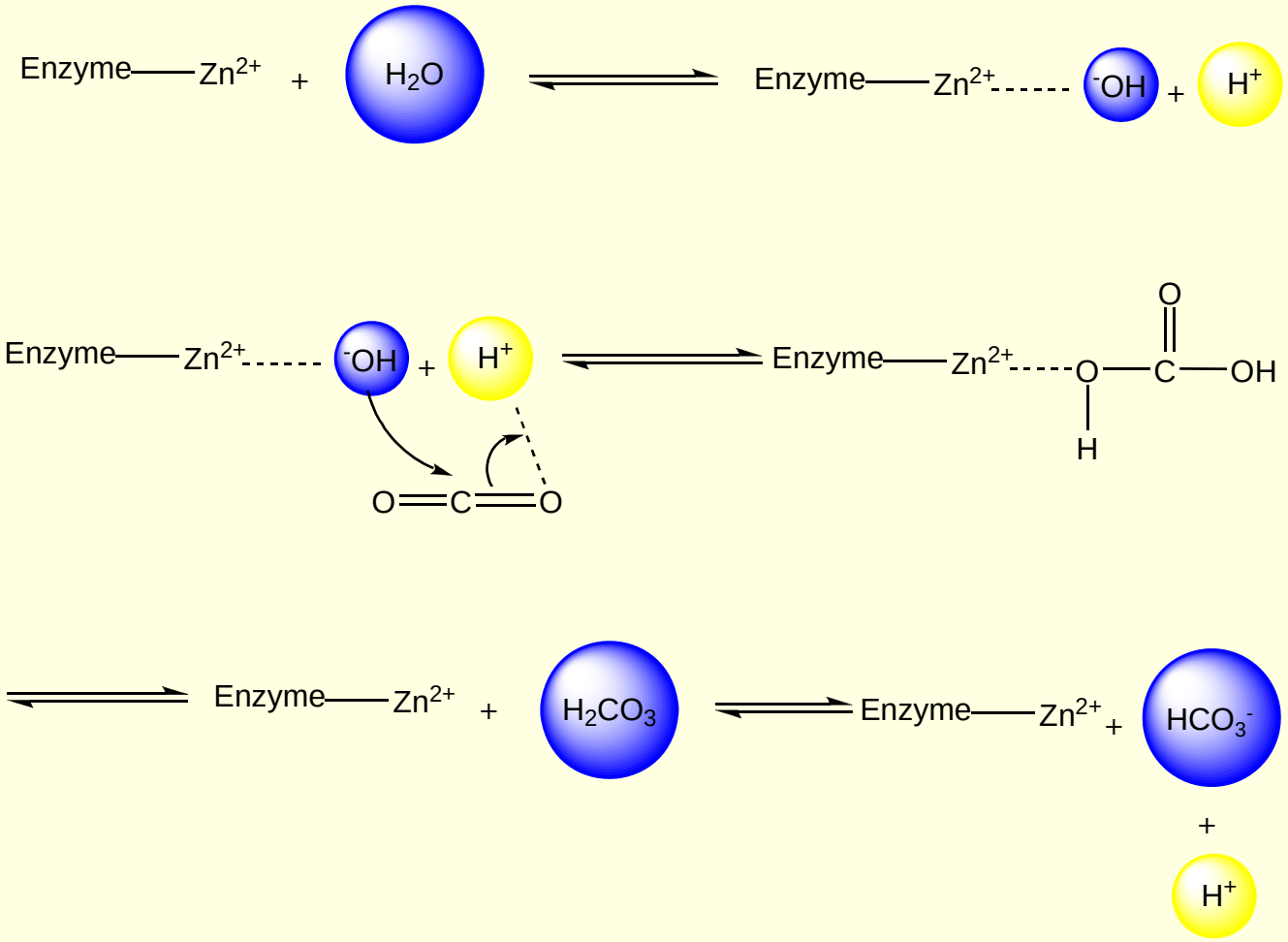
Muchas enzimas necesitan **co-factores** (co-reactivos, co-sustratos) para cumplir su función catalítica.

TABLE 6–1 Some Inorganic Elements That Serve as Cofactors for Enzymes

Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Cofactores metálicos

- Por que metales de transición
  - Buenos electróficos
  - Pueden interaccionar con mas de un ligando para orientar el sustrato en el sitio activo.
  - Pueden participar en reacciones óxido-reducción.


Enzyme—Zn2+ + H2O
Enzyme—Zn2+ ------ -OH + H+
Enzyme—Zn2+ ------ -OH + H+
O=C=O
Enzyme—Zn2+ ------ O(H)—C(=O)—OH
Enzyme—Zn2+ + H2CO3
Enzyme—Zn2+ + HCO3-
+
H+

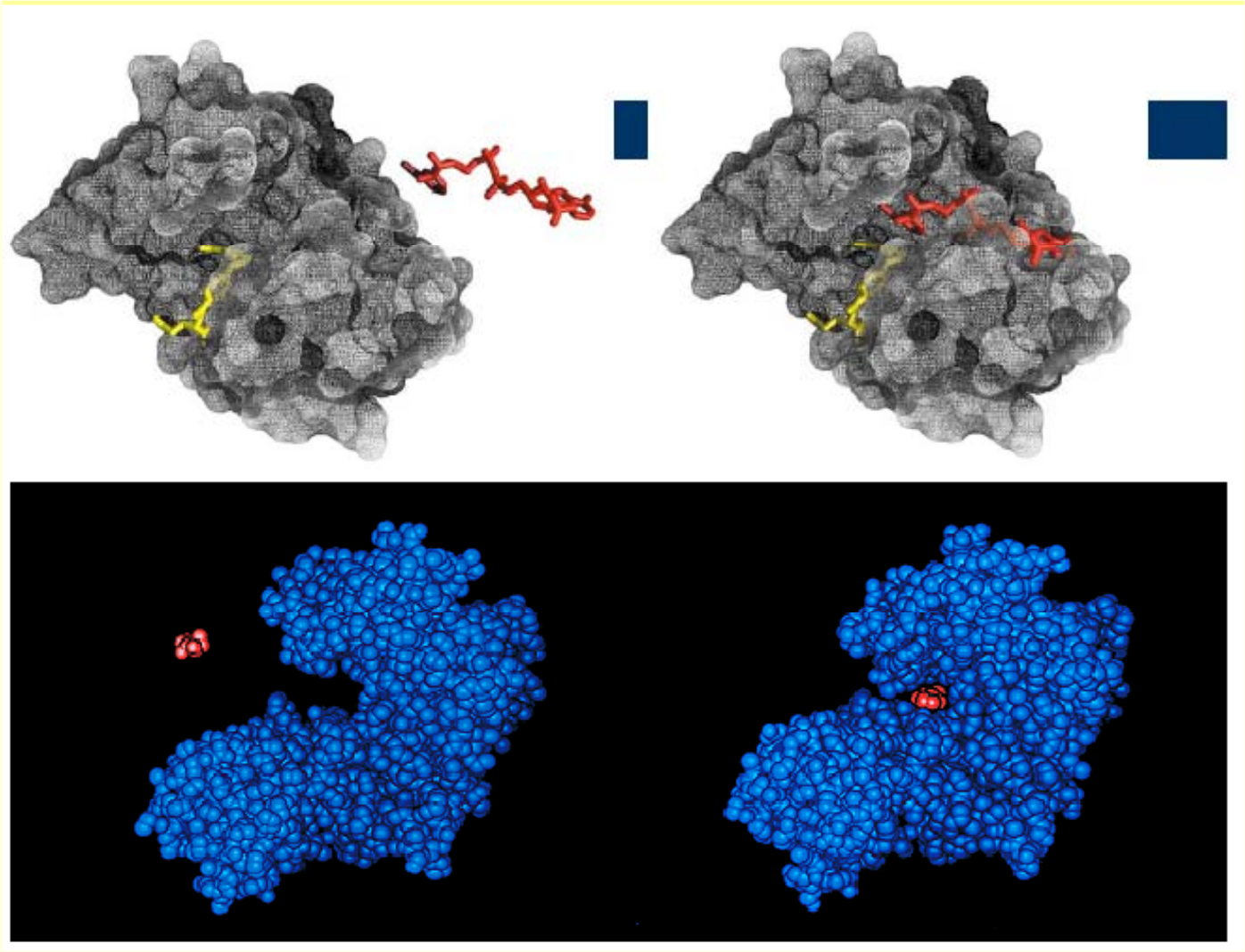
Coenzimas

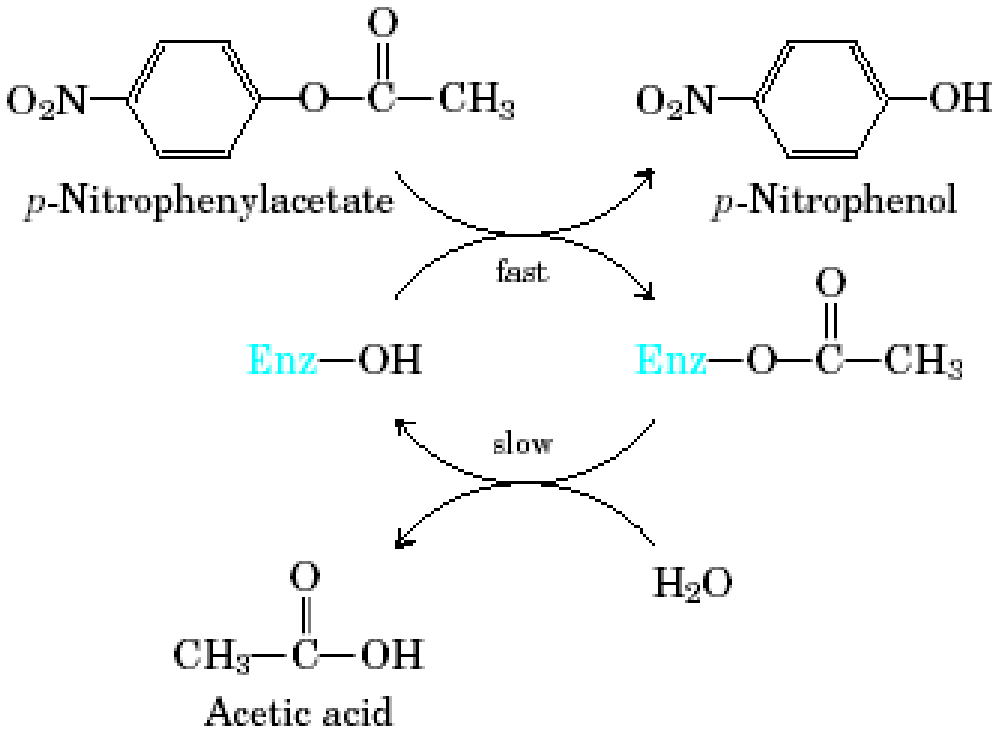
Coenzyme	Reaction Catalyzed
Biotin	Carboxylation
Cobalamin (B12)	Alkylation transfer
Coenzyme A	Acyl transfers
Flavin	Oxido-Reduction
Lipoic acid	Acyl transfers
Nicotinamide	Oxido-Reduction
Pyridoxal phosphate	Amino group transfers
Tetrahydrofolate	One carbon group transfers
Thiamine pyrophosphate	Aldehyde transfer

Estructura del sitio activo

- Bolsillo o surco en la superficie de la proteína donde el sustrato encaja.
- Especificidad de sustrato
- La enzima cambia de forma para posicionar los residuos para catalizar la reacción

- Orientación de sustrato
- Especificidad de sustrato





(a)

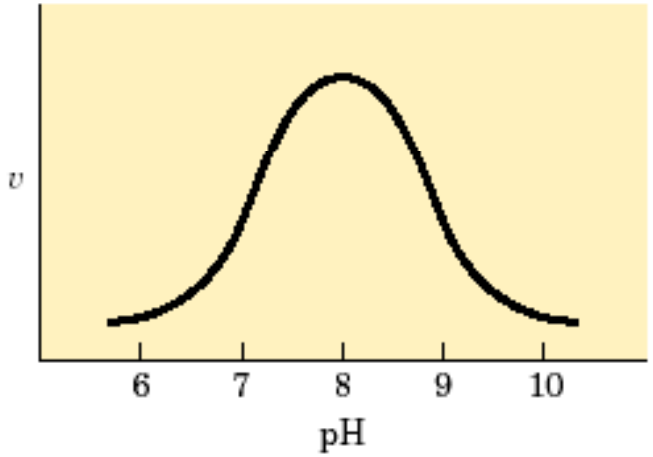
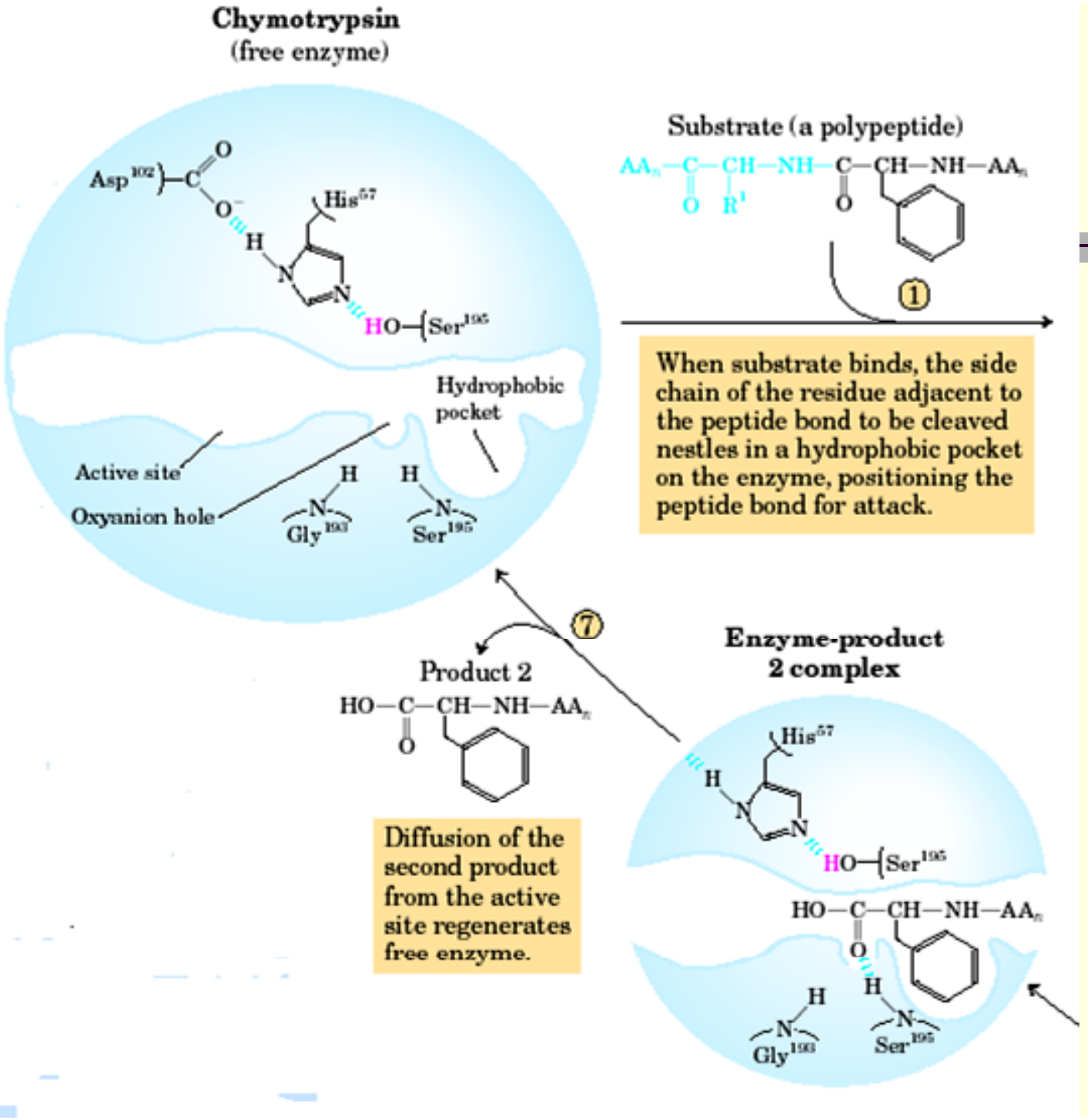
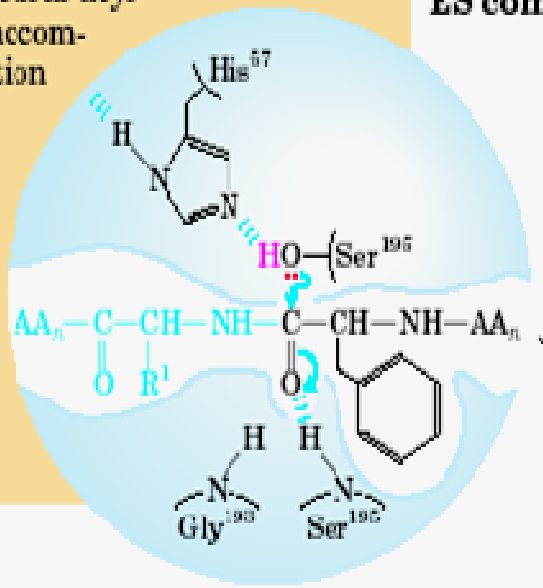


FIGURE 6–19 **Pre–steady state kinetic evidence for an acyl-er intermediate.** The hydrolysis of *p*-nitrophenylacetate by chymotrypsin is measured by release of *p*-nitrophenol (a colored product). Initially, the reaction releases a rapid burst of *p*-nitrophenol nearly stoichiometric with the amount of enzyme present. This reflects the fast acylation phase of the reaction. The subsequent rate is slower, because enzyme turnover is limited by the rate of the slower deacylation phase.


Chymotrypsin
(free enzyme)
Substrate (a polypeptide)
When substrate binds, the side chain of the residue adjacent to the peptide bond to be cleaved nestles in a hydrophobic pocket on the enzyme, positioning the peptide bond for attack.
Hydrophobic pocket
Active site
Oxyanion hole
Product 2
Diffusion of the second product from the active site regenerates free enzyme.
Enzyme-product 2 complex

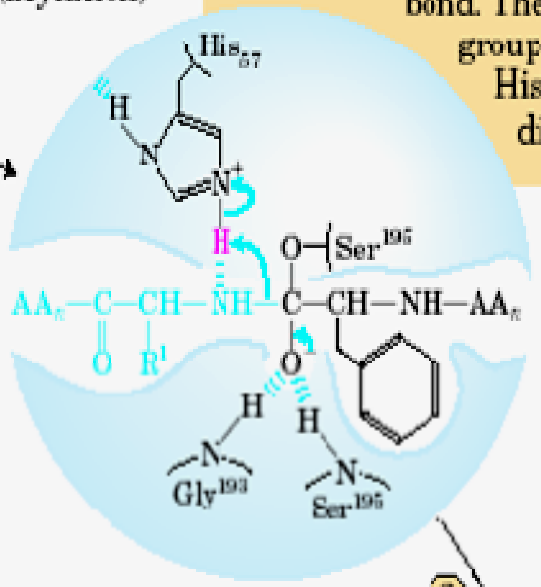
Interaction of Ser^{195} and His^{57} generates a strongly nucleophilic alkoxide ion on Ser^{195}; the ion attacks the peptide carbonyl group, forming a tetrahedral acyl-enzyme. This is accompanied by formation of a short-lived negative charge on the carbonyl oxygen of the substrate, which is stabilized by hydrogen bonding in the oxyanion hole.

ES complex



②

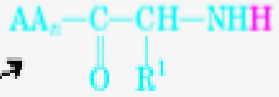
Short-lived intermediate (acylation)



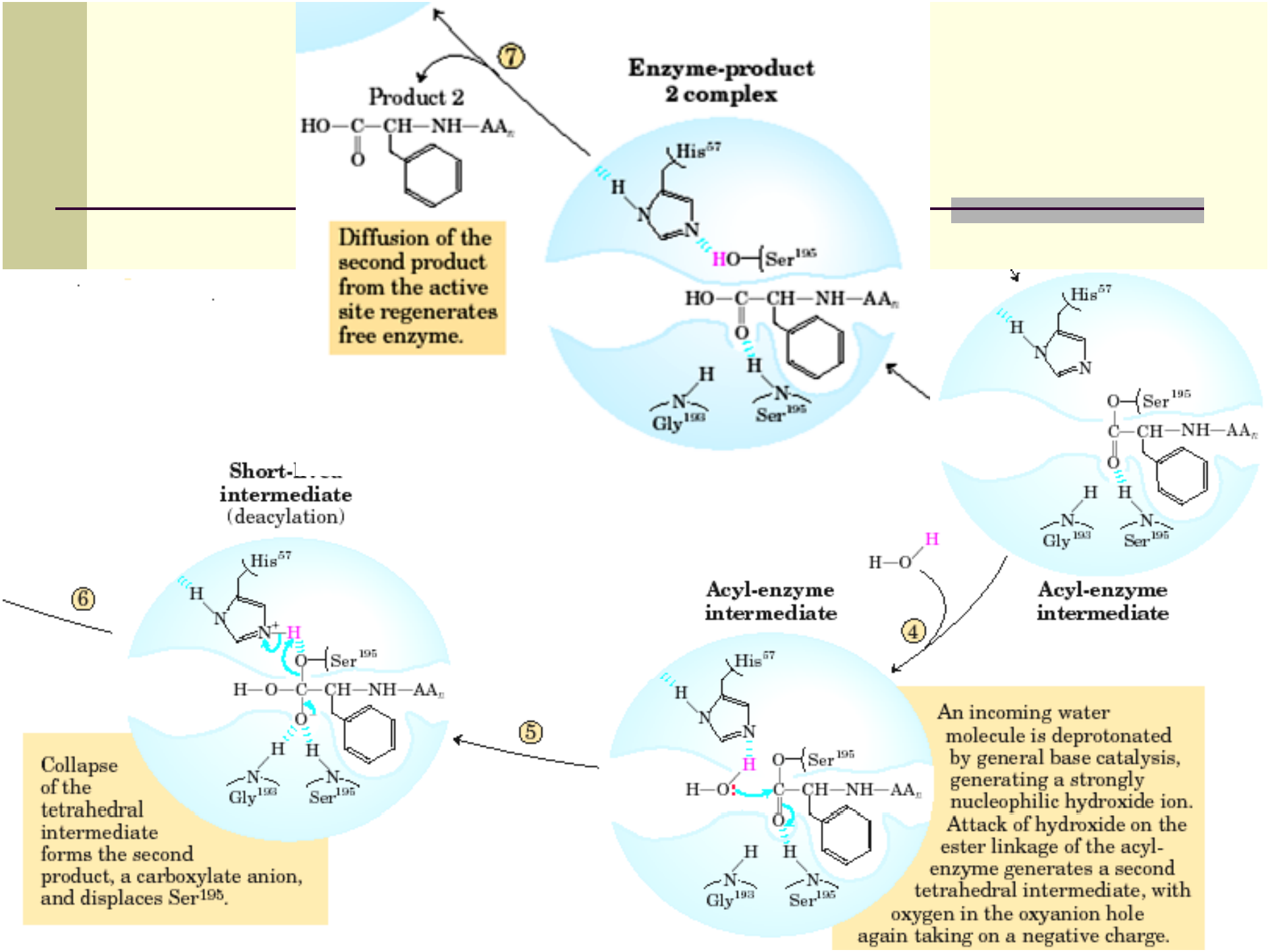
Instability of the negative charge on the substrate carbonyl oxygen leads to collapse of the tetrahedral intermediate; re-formation of a double bond with carbon displaces the bond between carbon and the amino group of the peptide linkage, breaking the peptide bond. The amino leaving group is protonated by His^{57}, facilitating its displacement.

③

Product 1

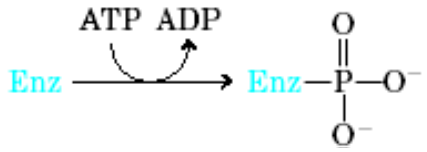


MECHANISM FIGURE 6-21 **Hydrolytic cleavage of a peptide bond by chymotrypsin.** The reaction has two phases. In the acylation phase (steps ① to ③), formation of a covalent acyl-enzyme intermediate is coupled to cleavage of the peptide bond. In the deacylation phase (steps ④ to ⑦), deacylation regenerates the free enzyme; this is essentially the reverse of the acylation phase, with water mirroring, in reverse, the role of the amine component of the substrate. Chymo-

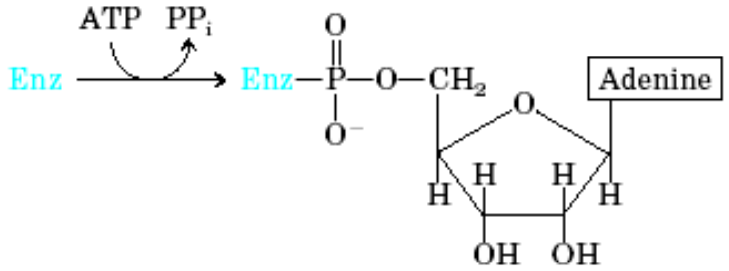

Product 2
HO—C—CH—NH—AA
Diffusion of the second product from the active site regenerates free enzyme.
7
Enzyme-product 2 complex
His57
Ser195
Gly193
Ser195
Acyl-enzyme intermediate
4
An incoming water molecule is deprotonated by general base catalysis, generating a strongly nucleophilic hydroxide ion. Attack of hydroxide on the ester linkage of the acyl-enzyme generates a second tetrahedral intermediate, with oxygen in the oxyanion hole again taking on a negative charge.
5
Short-
intermediate
(deacylation)
6
Collapse of the tetrahedral intermediate forms the second product, a carboxylate anion, and displaces Ser195.

Covalent modification
(target residues)

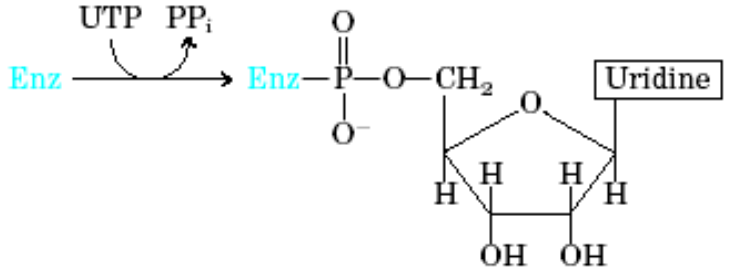
Phosphorylation
(Tyr, Ser, Thr, His)



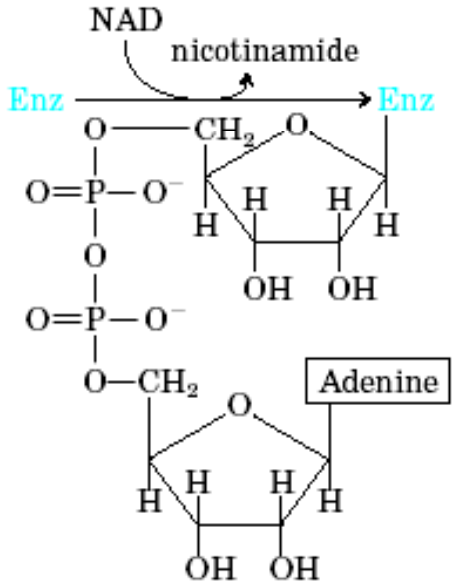
Adenylylation
(Tyr)



Uridylylation
(Tyr)



ADP-ribosylation
(Arg, Gln, Cys, diphthamide—a modified His)



Methylation
(Glu)

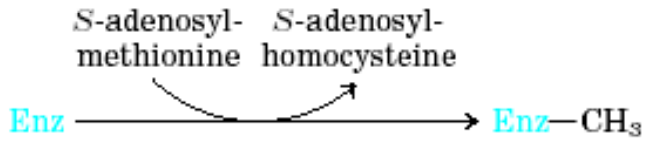
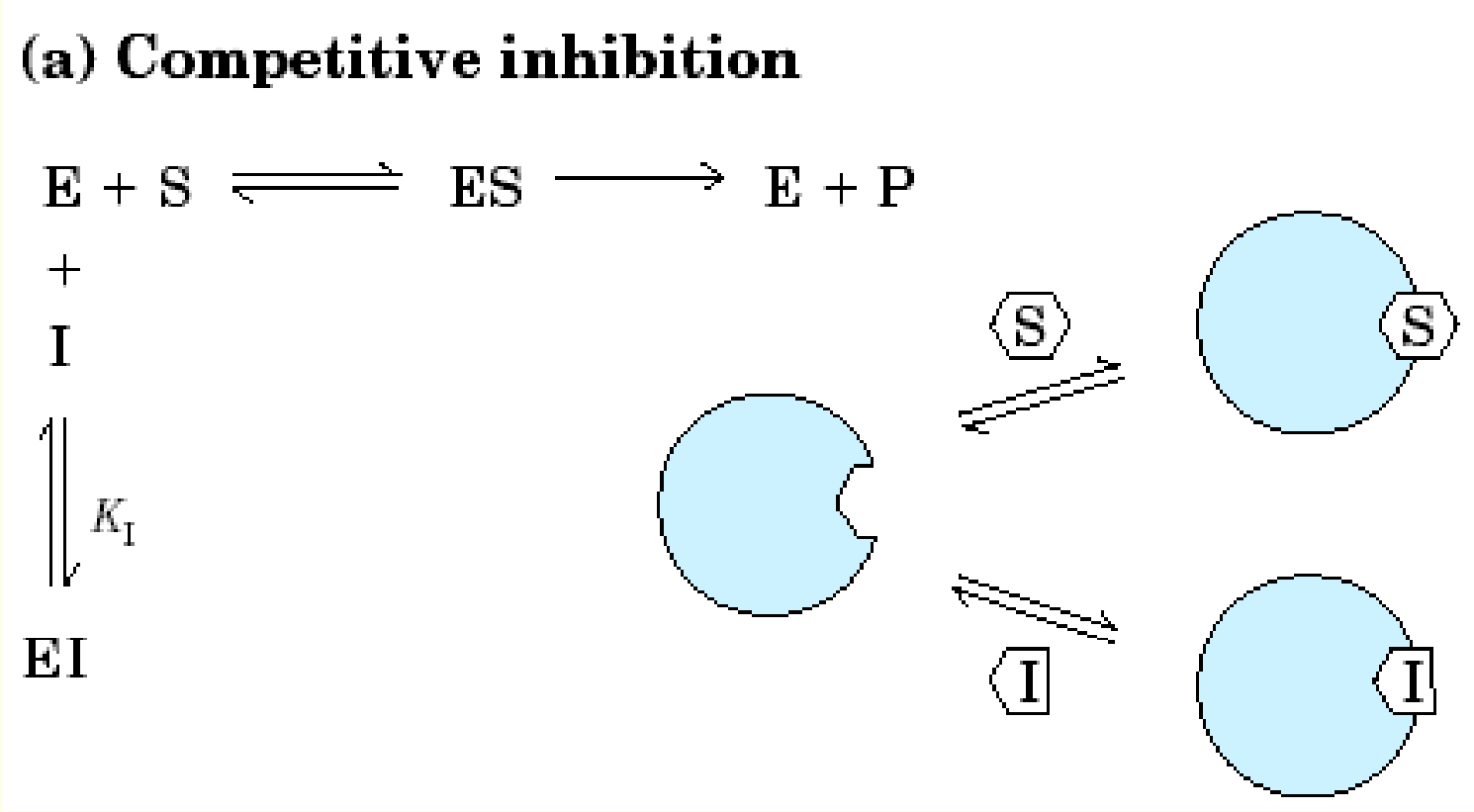


FIGURE 6–30 Some enzyme modification reactions.

Inhibidores

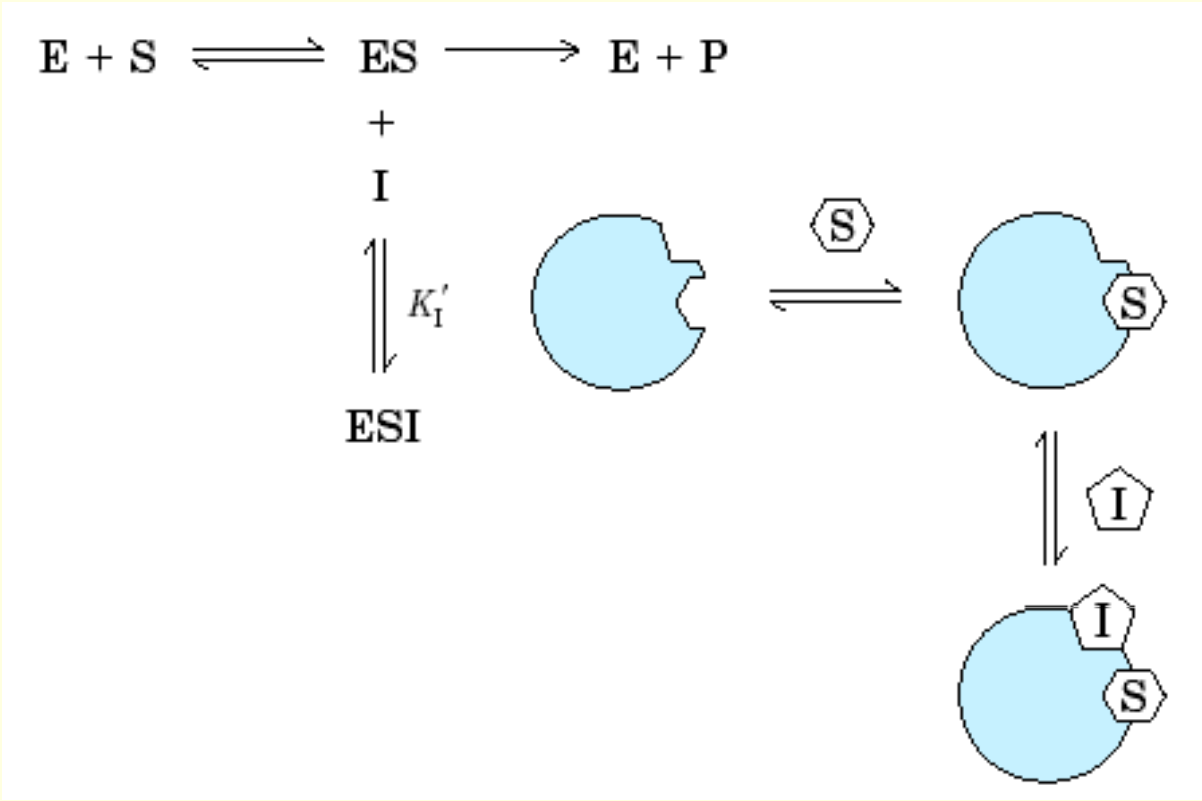
Los inhibidores impiden la actividad de la enzima. Pueden afectar el sitio activo de dos maneras:

- **Competitiva:** Bloquea el sitio activo.



Inhibición

- **No competitiva:** Altera indirectamente la forma del sitio activo, al pegarse en otro lugar de la enzima.



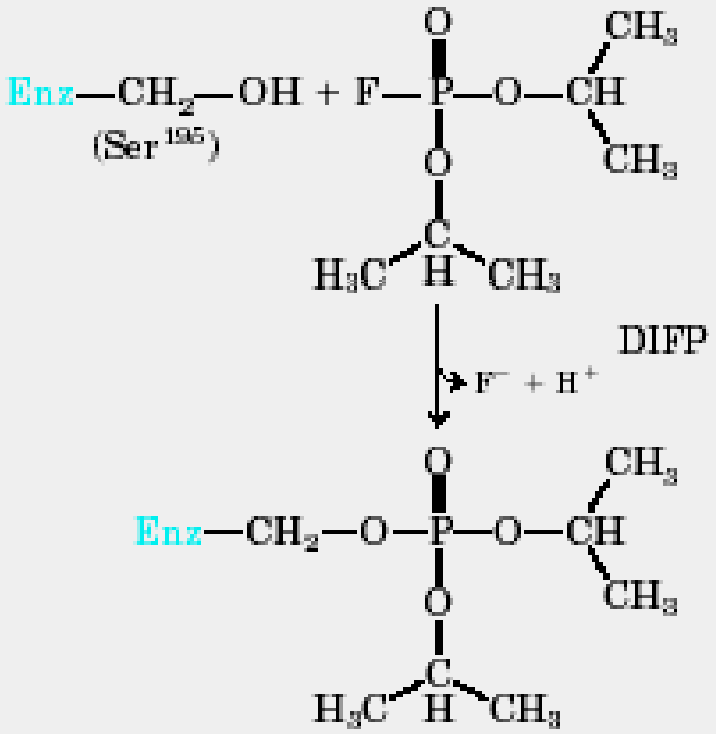
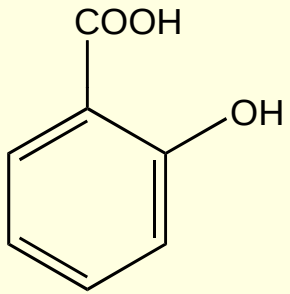


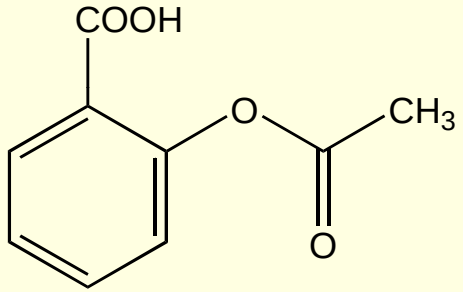
FIGURE 6–16 Irreversible inhibition. Reaction of chymotrypsin with diisopropylfluorophosphate (DIFP) irreversibly inhibits the enzyme. This has led to the conclusion that Ser^{195} is the key active-site Ser residue in chymotrypsin.

Aspirina

Inhibidor de la ciclooxigenasa



salicylic acid



acetylsalicylate (aspirin)