ENZIMAS: CONCEPTOS BÁSICOS Y CINÉTICA

Enzimas: catalizadores de naturaleza proteica

Seis clases de enzimas

Clase	Tipo de reacción	Ejemplo
1. Oxidoreductasas	Oxidación-Reducción	Lactato deshidrogenasa
2. Transferasas	Transferencia de Grupo	Nucleósido monofosfato quinasa (NMP quinasa)
3. Hidrolasas	Reacciones de Hidrólisis (transferencia de un grupo funcional al agua)	Quimotripsina
4. Liasas	Adición o remoción de grupos para formar dobles enlaces	Fumarasa
5. Isomerasas	Isomerisación (transferencia intramolecular de un grupo funcional)	Triosa fosfato isomerasa
6. Ligasas	Ligación de dos substratos a expensas de la hidrólisis de ATP	Aminoacil-tRNA sintetasa

Las enzimas requieren de cofactores enzimáticos

Cofactores Enzimáticos

Cofactor	Enzima
Coenzima	
Tiamina pirofosato	Piruvato deshidrogenasa
Flavina adenina nucleótido	Monoamino oxidasa
Nicotinamida adenina dinucleotido	Lactato dehydrogenasa
Pyridoxal fosfato	Glicogeno fosforilasa
Coenzima A (CoA)	Acetil CoA carboxilasa
Biotina	Pyruvato carboxilasa
5'-Deoxyadenosil cobalamina	Methylmalonil mutasa
Tetrahidrofolate	Timidilato sintetasa
Metal	
Zn^{2+}	Anhidrasa carbónica
Zn^{2+}	Carboxipeptidasa
Mg^{2+}	*Eco*RV
Mg^{2+}	Hexoquinasa
Ni^{2+}	Ureasa
Mo	Nitrato reductasa
Se	Glutatión peroxidasa
Mn^{2+}	Superoxido dismutasa
K^{+}	Propionil CoA carboxilasa

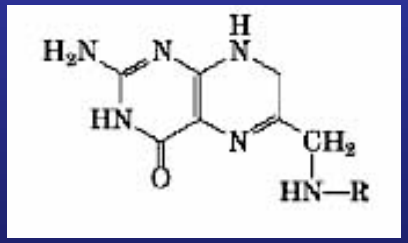
Cinética enzimática:

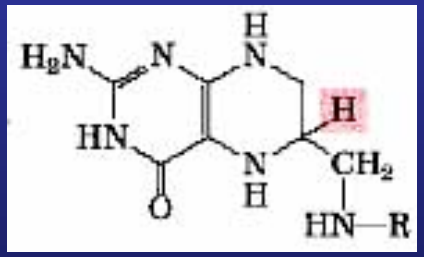
Formación de un complejo entre la enzima y el sustrato (complejo ES).

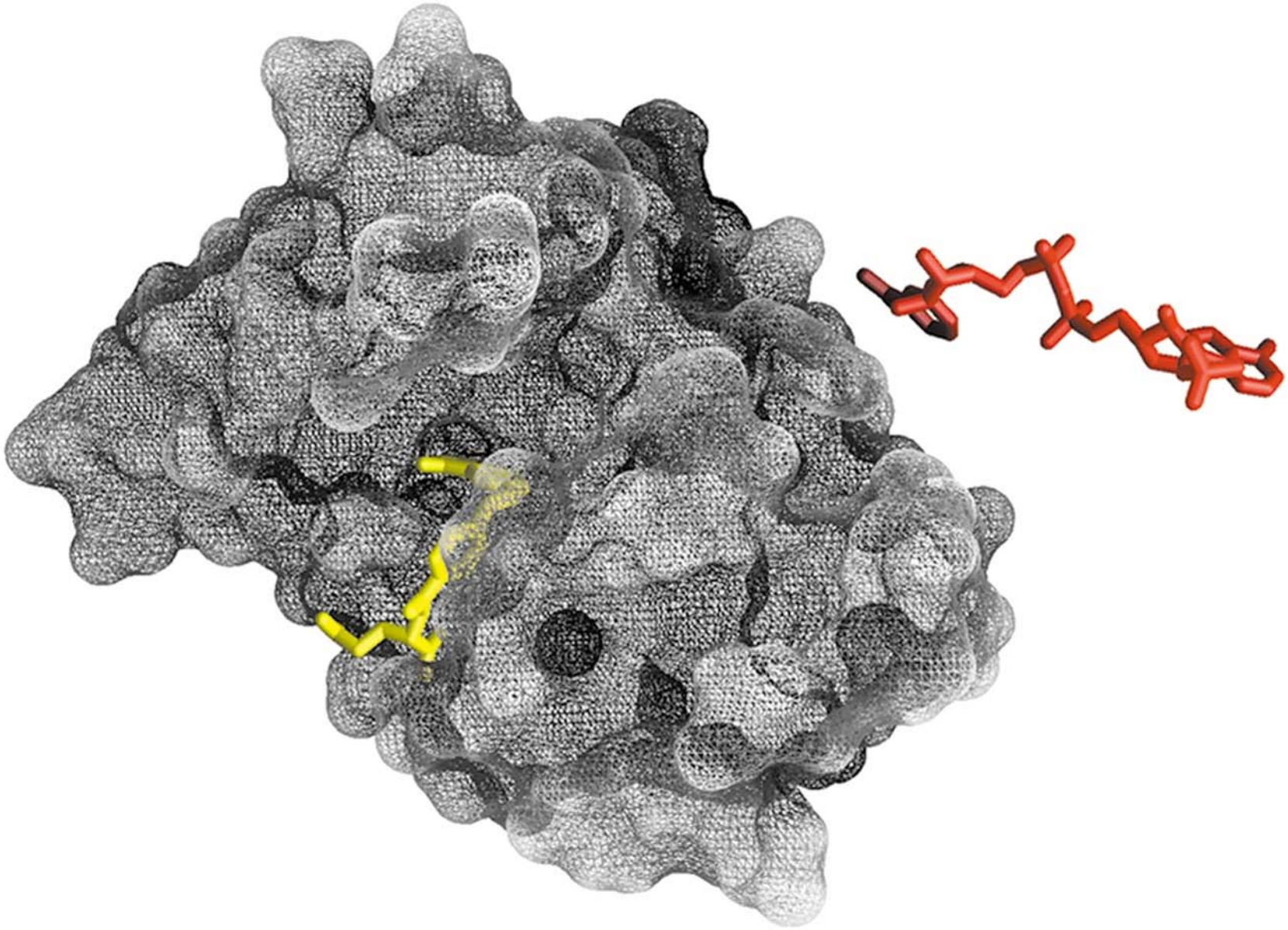
Dihidrofolato reductasa

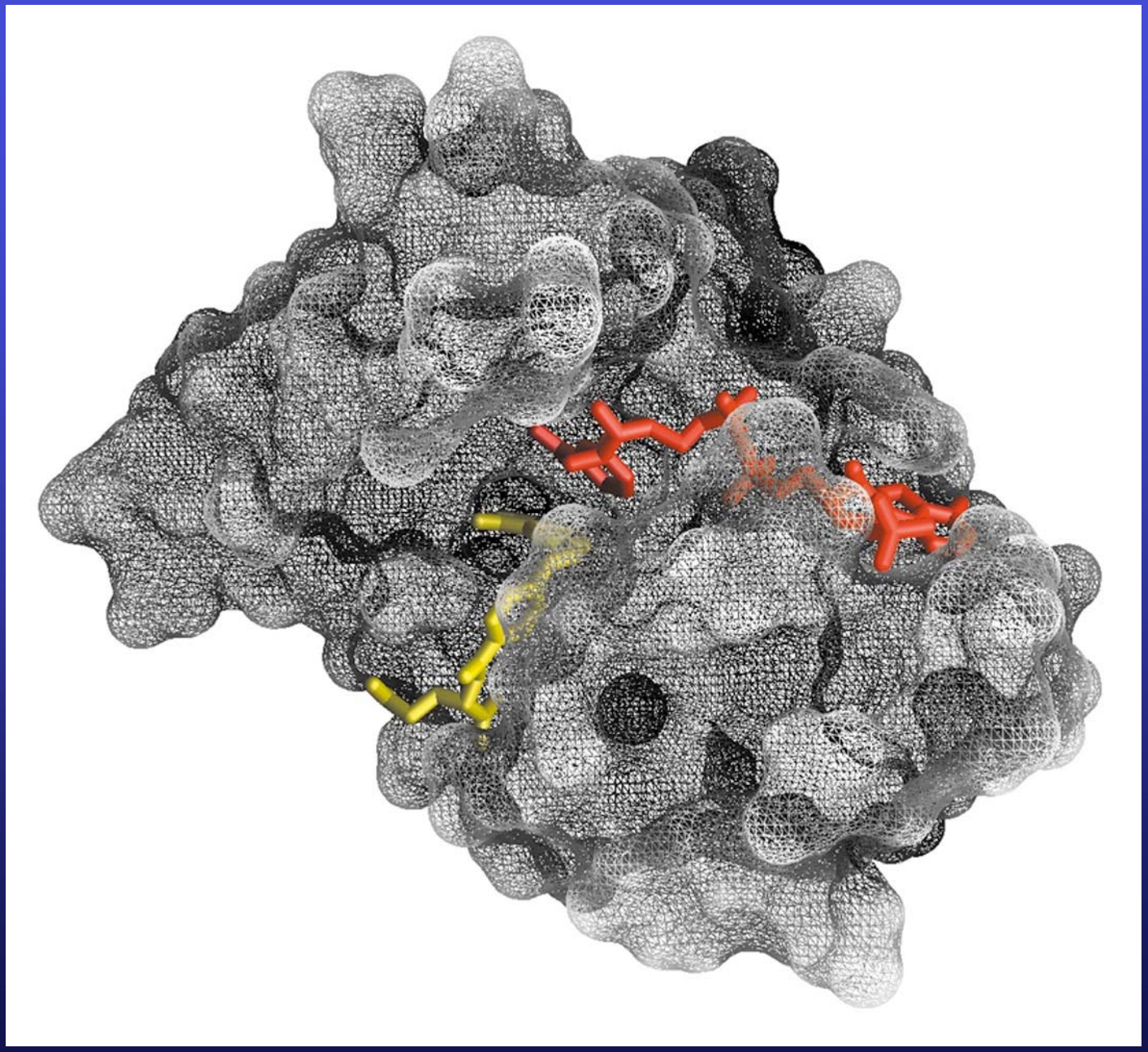
Reacción catalizada:

dihidrofolato + NADPH + H^+ ⇆ tetrahidrofolato + $NADP^+$









Las enzimas no modifican la posición del equilibrio de la reacción química.

Aceleran la velocidad de la reacción debido a que modifican el mecanismo de la reacción.

Dos posibles mecanismos para la misma reacción

1) reacción no catalizada:

$$S \leftrightarrows P$$

2) reacción catalizada por una enzima:

$$S + E \leftrightarrows ES \leftrightarrows EP \leftrightarrows E + P$$

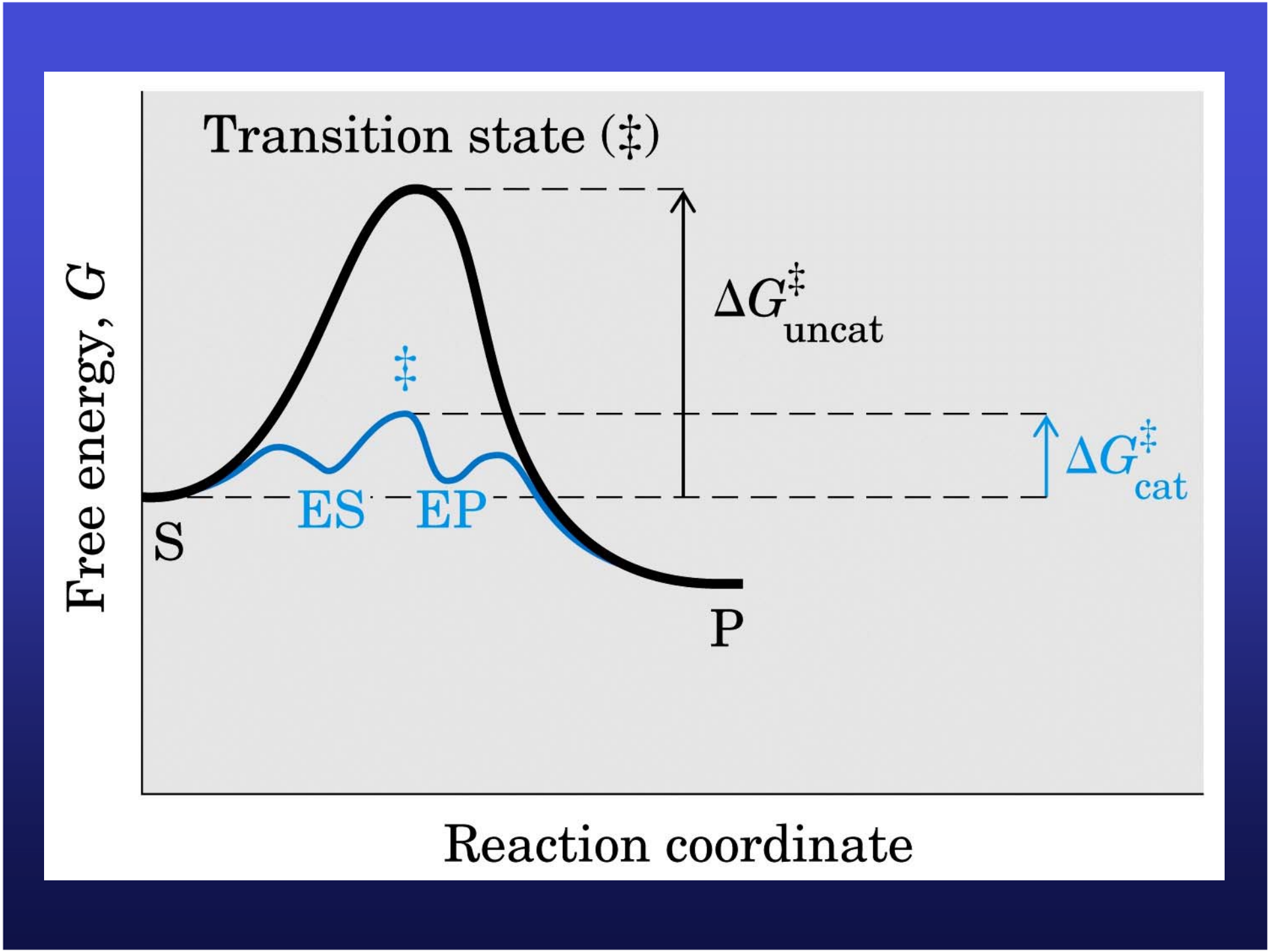

Transition state (‡)
Free energy, G
$\Delta G^{\ddagger}_{\text{uncat}}$
‡
$\Delta G^{\ddagger}_{\text{cat}}$
ES
EP
S
P
Reaction coordinate

Table 8.1. Rate enhancement by selected enzymes

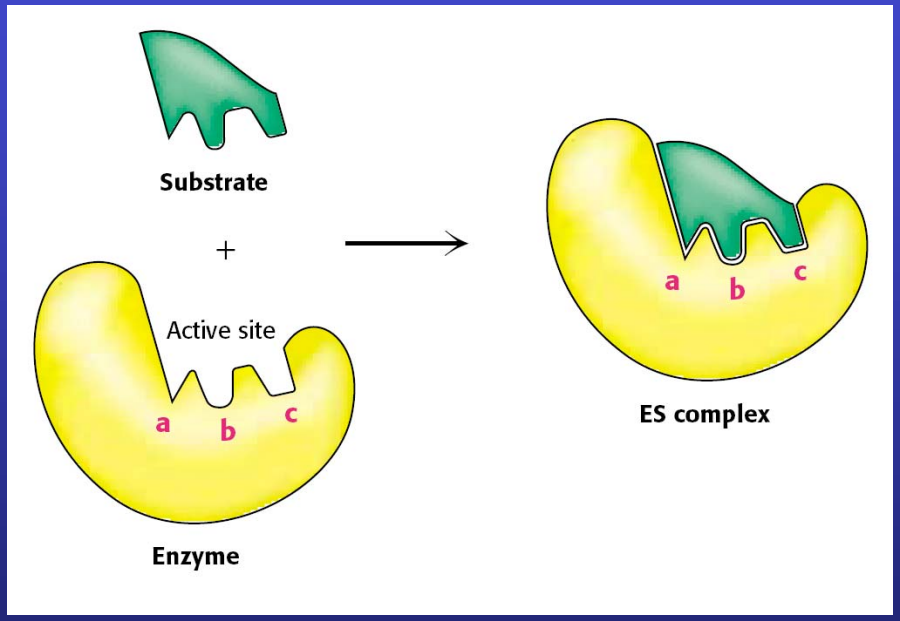
Enzyme	Nonenzymatic half-life	Uncatalyzed rate (k_{un}, s^{-1})	Catalyzed rate (k_{cat}, s^{-1})	Rate enhancement (k_{cat}/k_{un})
OMP decarboxylase	**78,000,000 years**	**2.8×10^{-16}**	**39**	**1.4×10^{17}**
Staphylococcal nuclease	**130,000 years**	**1.7×10^{-13}**	**95**	**5.6×10^{14}**
AMP nucleosidase	**69,000 years**	**1.0×10^{-11}**	**60**	**6.0×10^{12}**
Carboxypeptidase A	**7.3 years**	**3.0×10^{-9}**	**578**	**1.9×10^{11}**
Ketosteroid isomerase	**7 weeks**	**1.7×10^{-7}**	**66,000**	**3.9×10^{11}**
Triose phosphate isomerase	**1.9 days**	**4.3×10^{-6}**	**4,300**	**1.0×10^{9}**
Chorismate mutase	**7.4 hours**	**2.6×10^{-5}**	**50**	**1.9×10^{6}**
Carbonic anhydrase	**5 seconds**	**1.3×10^{-1}**	**1×10^{6}**	**7.7×10^{6}**

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.
Source: After A. Radzicka and R. Wolfenden. *Science* 267 (1995):90–93.

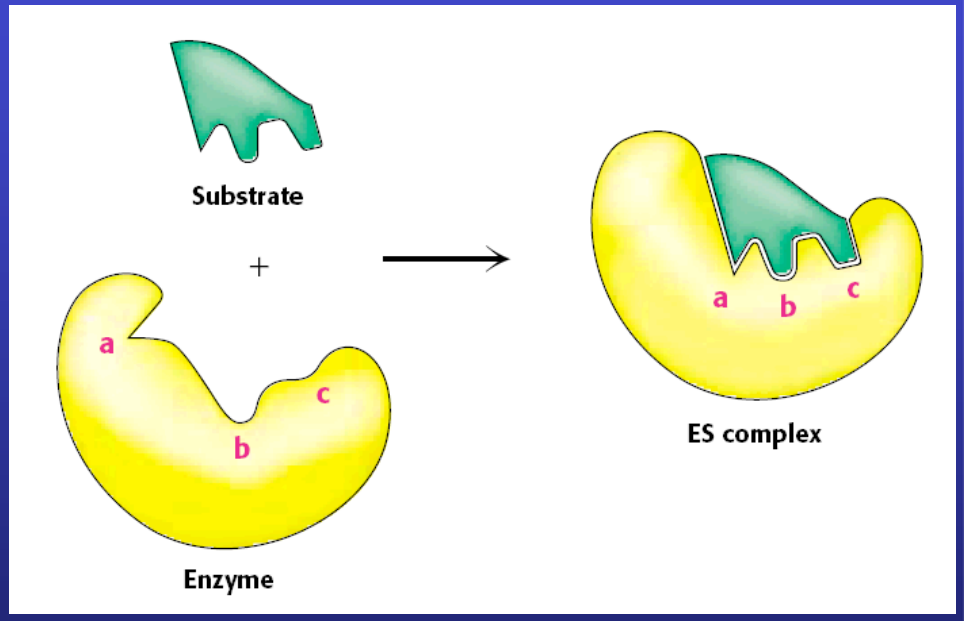
Modelos de unión de una enzima al sustrato:

a) **Llave-cerradura**
b) **Encaje inducido**

llave-cerradura

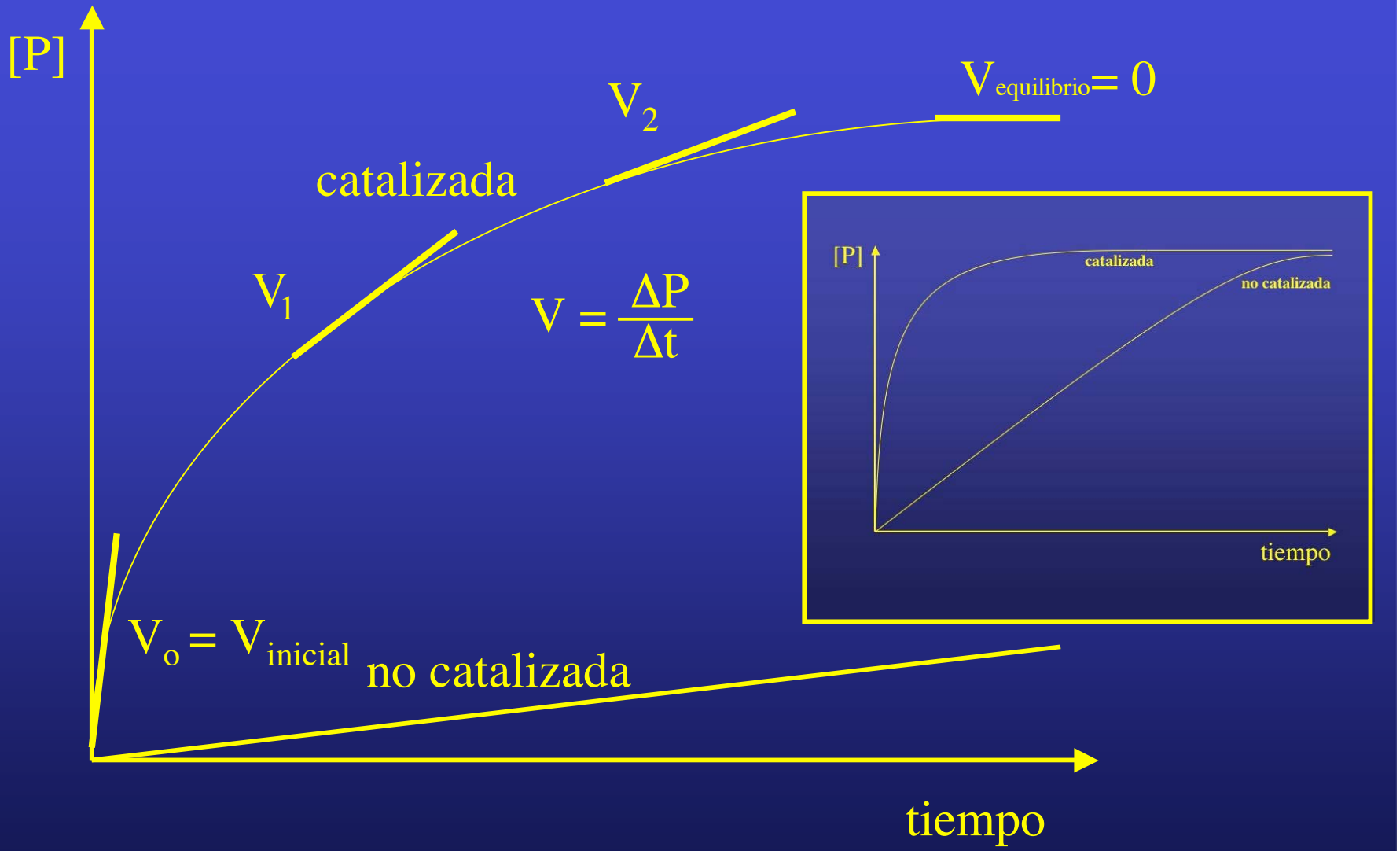


encaje inducido

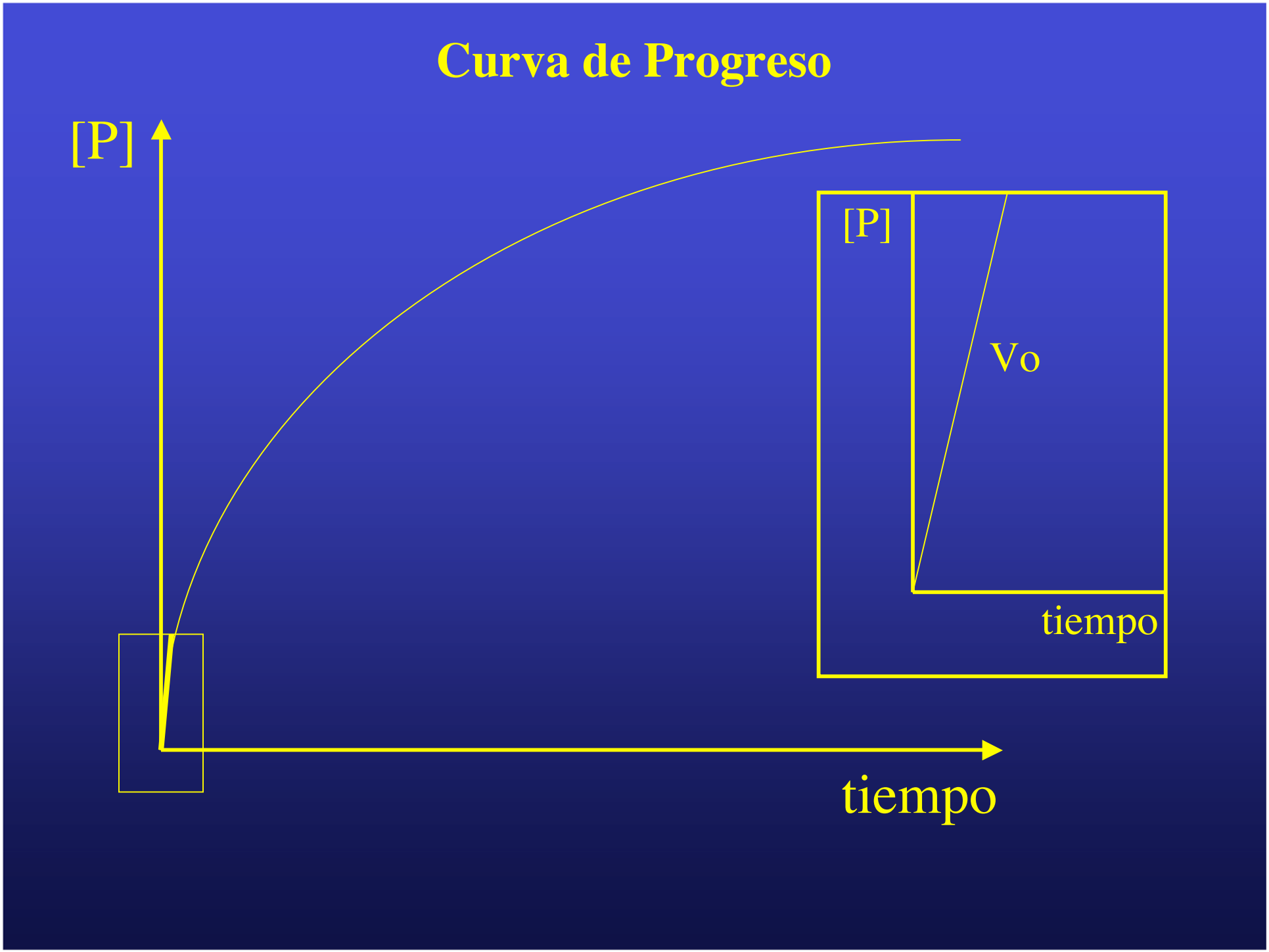


Cinética enzimática

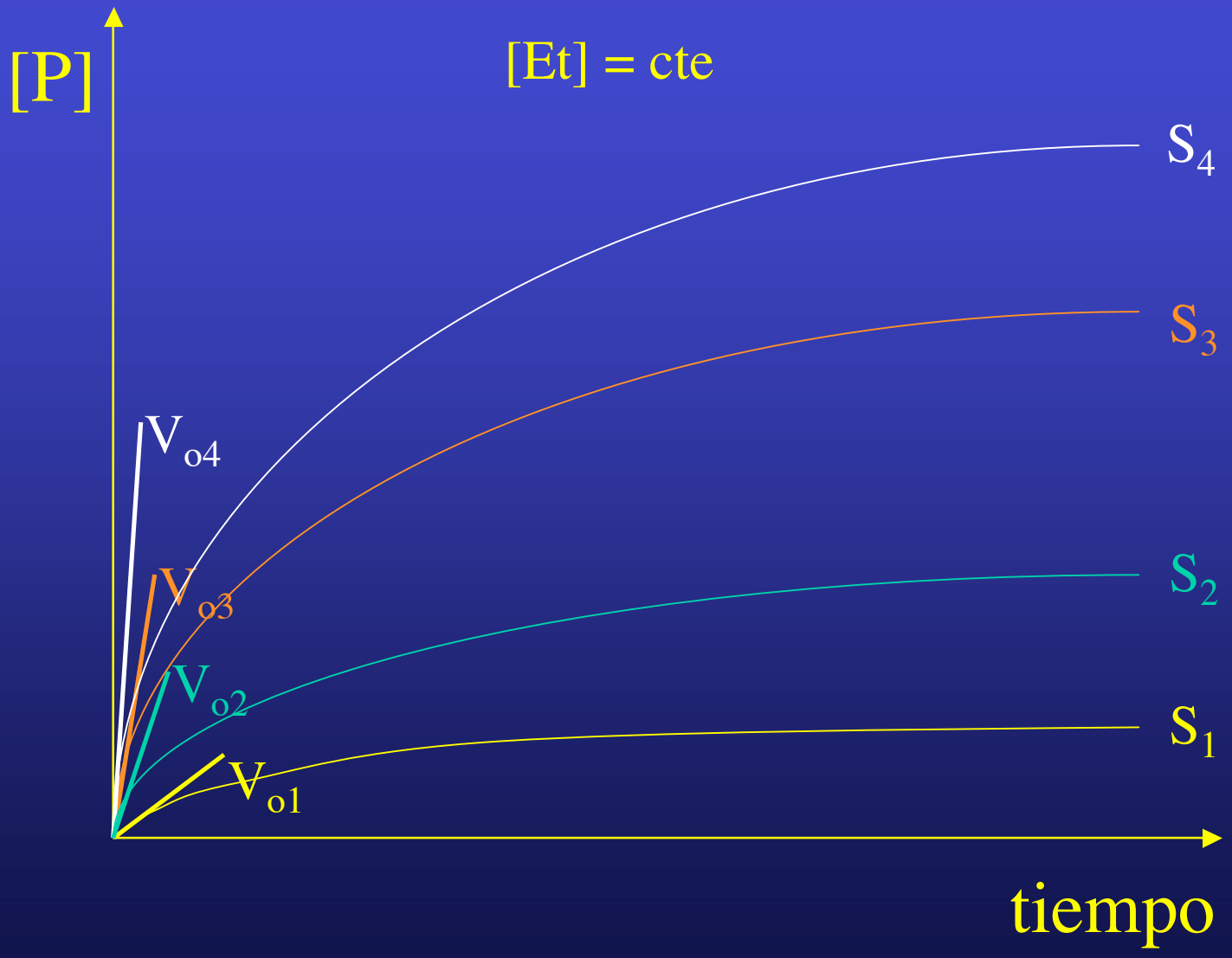
CURVA DE PROGRESO

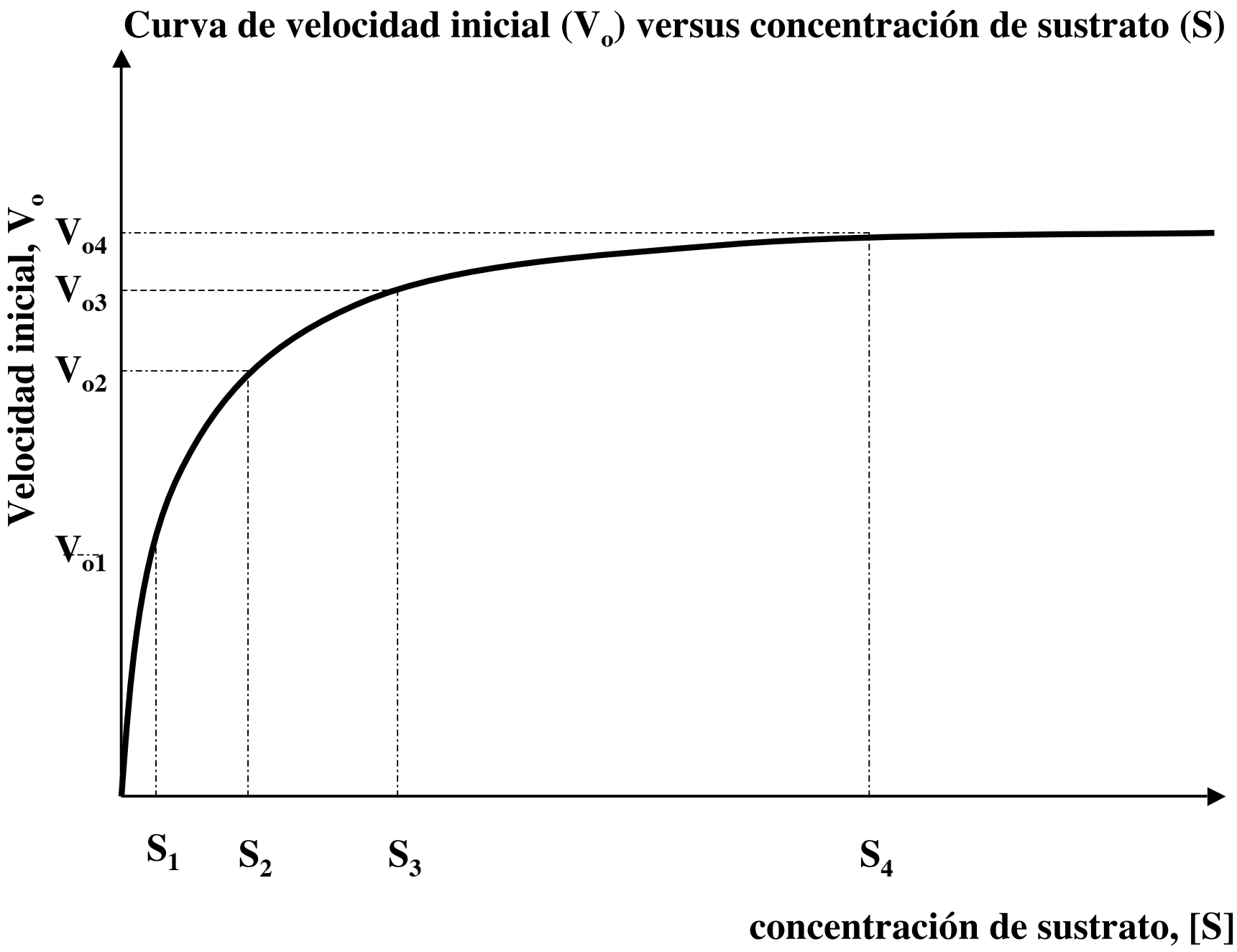


Curva de Progreso



Curva de progreso a diferentes concentraciones de sustrato




Curva de velocidad inicial (V_o) versus concentración de sustrato (S)
Velocidad inicial, V_o
V_{o4}
V_{o3}
V_{o2}
V_{o1}
S_1
S_2
S_3
S_4
concentración de sustrato, [S]

Análisis de Estado Estacionario

Mecanismo Michaeliano (Michaelis y Menten)

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + P$$

[P] ≈ 0, k_4 no se considera para el análisis

y escribimos: $E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$

donde k_3 es la constante de velocidad de la etapa limitante o lenta

Teoría del estado estacionario

Permite deducir una ecuación de velocidad en función de la concentración de sustrato.

Si k_3 es la etapa limitante,

$V_o = k_3[ES]$

Si suponemos que la [ES] es constante, entonces:
velocidad de formación de ES = velocidad de disociación de ES

Substituyendo en:

$$V_o = k_3[ES]$$

$$V_o = \frac{k_3[Et][S]}{[S] + (k_2 + k_3)/k_1}$$

donde $\frac{k_2 + k_3}{k_1} = K_m$, ó constante de Michaelis

Entonces:

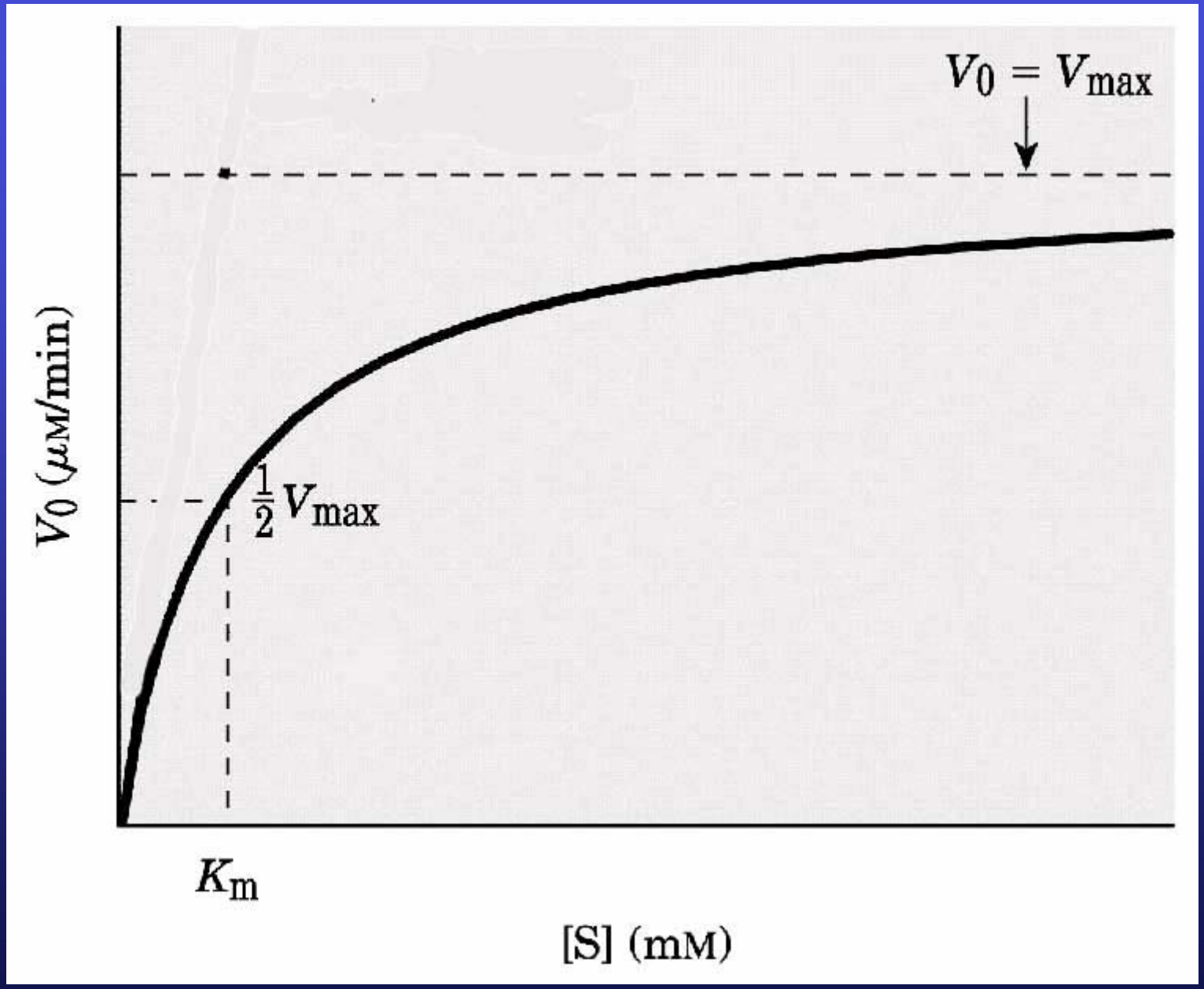
$$V_o = \frac{k_3[Et][S]}{K_m + [S]}$$

Si la [Et] = [ES] entonces

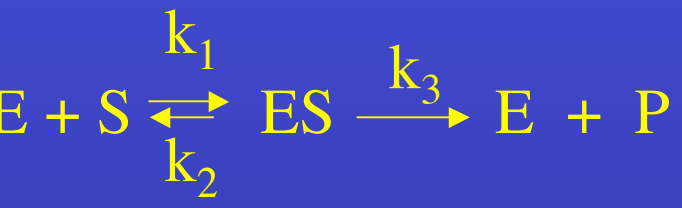
$$V_o = k_3[Et] = V_{max}$$

Ecuación de Michaelis y Menten

$$V_o = \frac{V_{max}\,[S]}{K_m + [S]}$$


$V_0 = V_{max}$
V_0 (μM/min)
$\frac{1}{2}V_{max}$
K_m
[S] (mM)

SIGNIFICADO DE LA K_m



$K_m = (k_2 + k_3)/k_1$

Si k_3 es la etapa lenta, entonces:

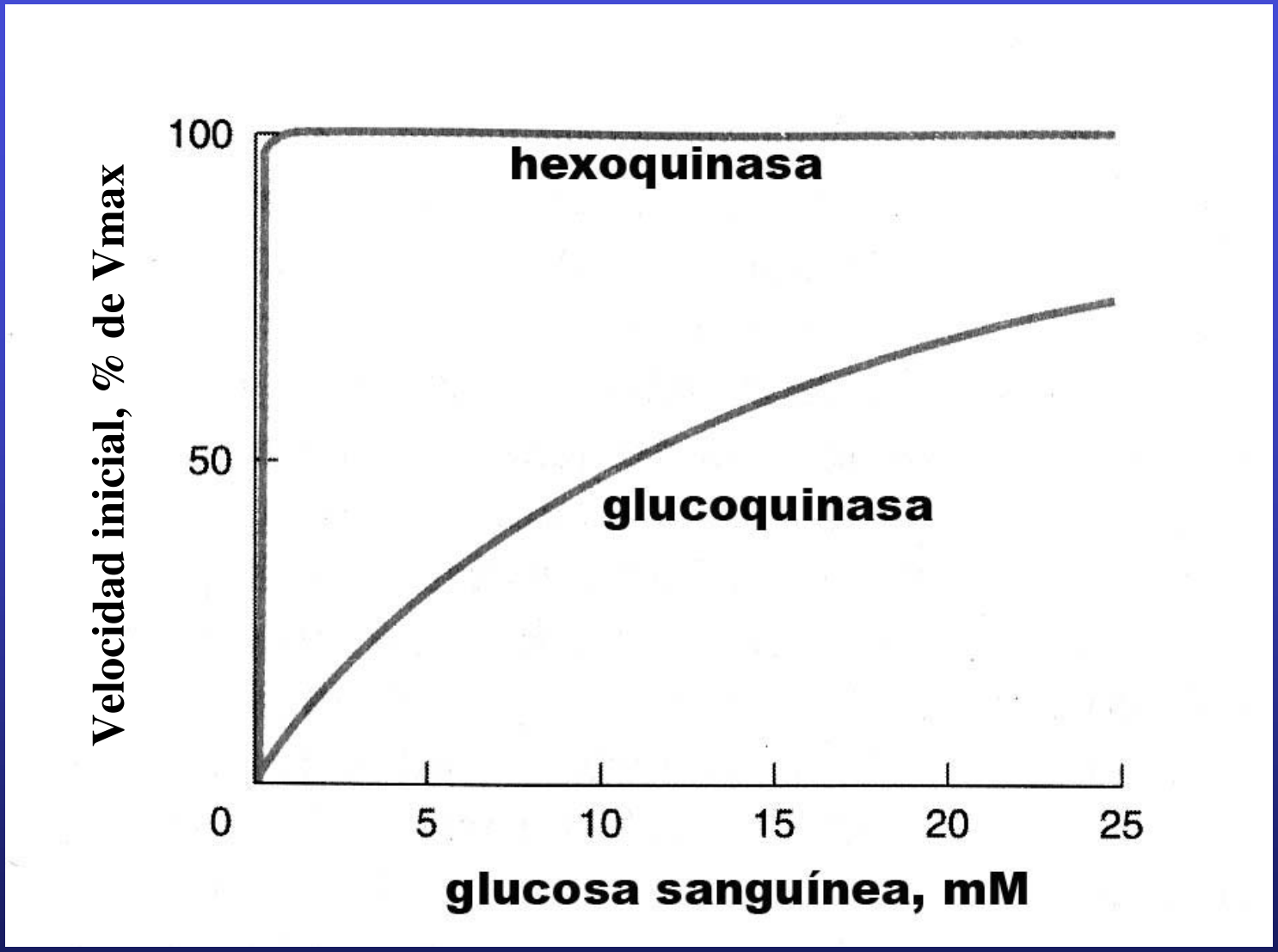
$k_2 + k_3 \approx k_2$, de donde **$K_m = k_2/k_1$**

Operacionalmente K_m = [S] con la que se obtiene $V_{max}/2$

En estas condiciones K_m es una medida de la afinidad de la enzima por el sustrato

Km para algunas enzimas y sustratos

Enzima	Sustrato	Km (mM)
Catalasa	H_2O_2	25
Hexoquinasa (cerebro)	ATP	0,4
	D-glucosa	0,05
	D-fructosa	0,9
	D-manosa	0,06
Glucoquinasa (hepática)	D-glucosa	10
Anhidrasa carbónica	HCO_3^-	26
Quimotripsina	Gliciltirosinilglicina	108
	N-Benzoiltirosinamida	2,5
β-galactosidada	D-lactosa	4,0
Treonina deshidratasa	L-treonina	5.0



	Hexoquinasa	Glucoquinasa
Km glucosa	0,05mM	10 mM

En forma general, para una enzima V_{max} es función de la etapa limitante y por tanto

$V_{max} = k_{cat}[Et]$, de donde

$$k_{cat} = \frac{V_{max}}{[Et]}$$

número de recambio (tiempo^{-1})

SIGNIFICADO DEL NÚMERO DE RECAMBIO ó k_{cat}:

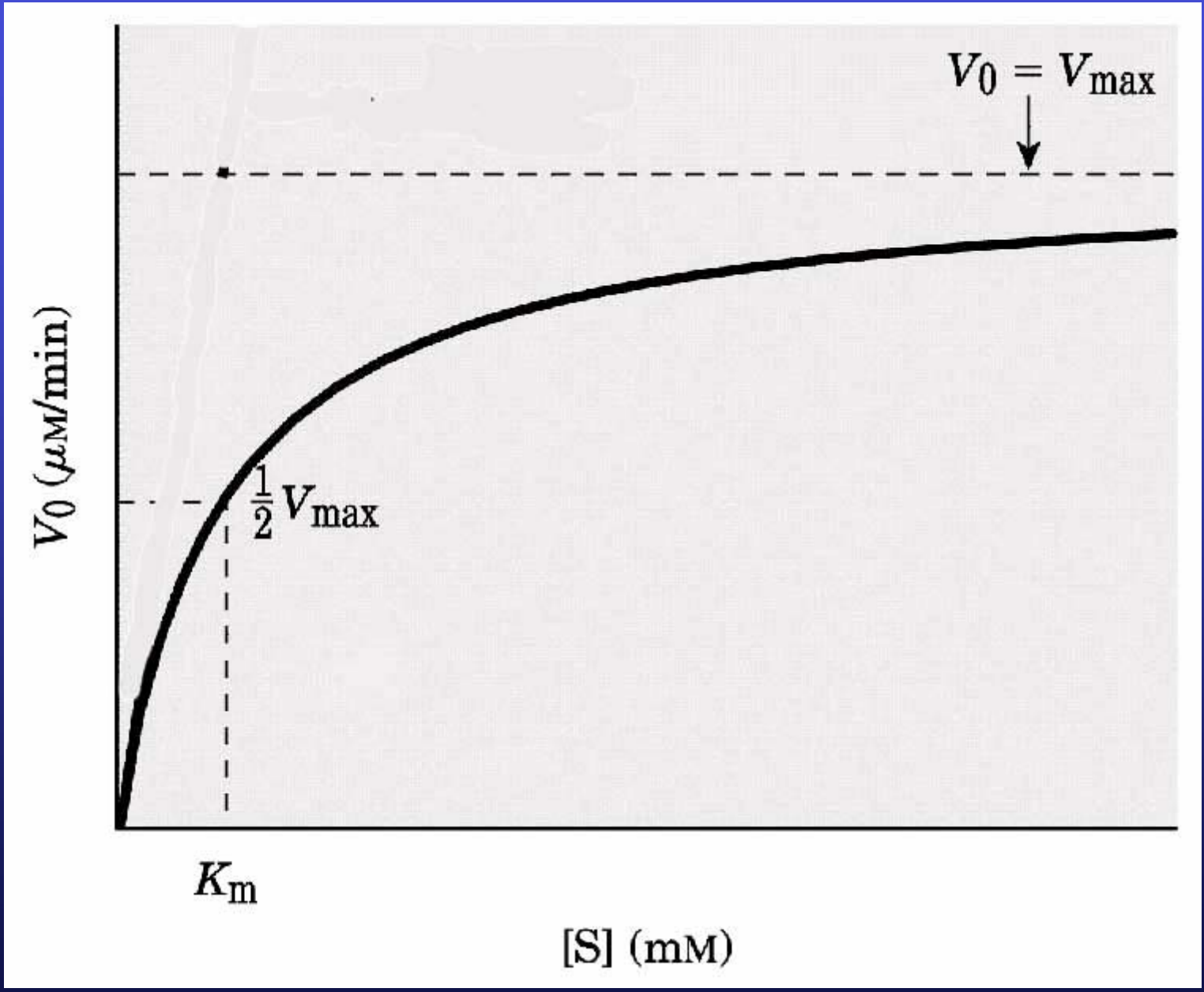
número de moléculas de sustrato convertidas a producto por unidad de tiempo y por molécula de enzima

table 8–7

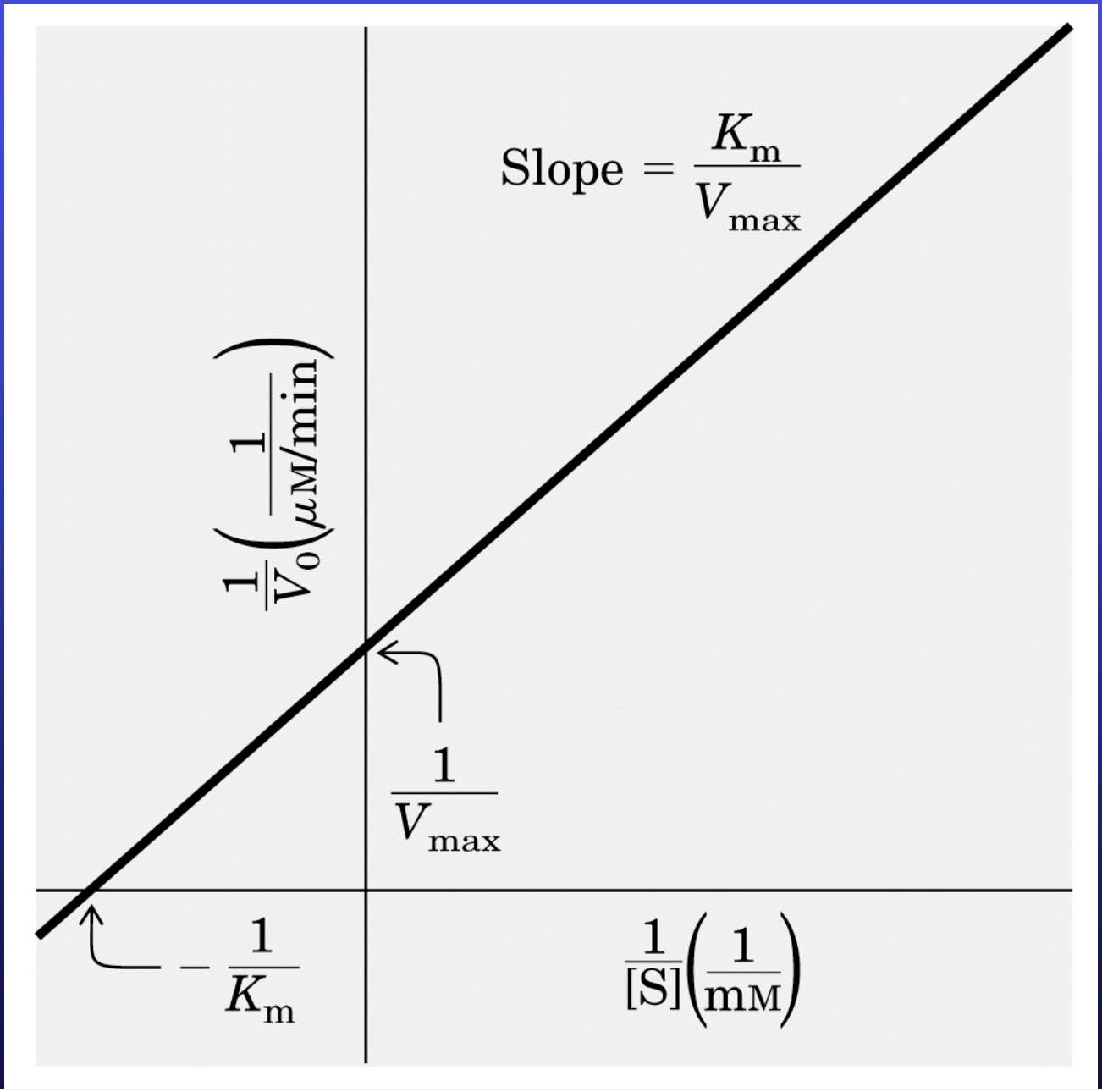
Turnover Numbers (k_{cat}) of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	140,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

Determinación experimental de Km y Vmax

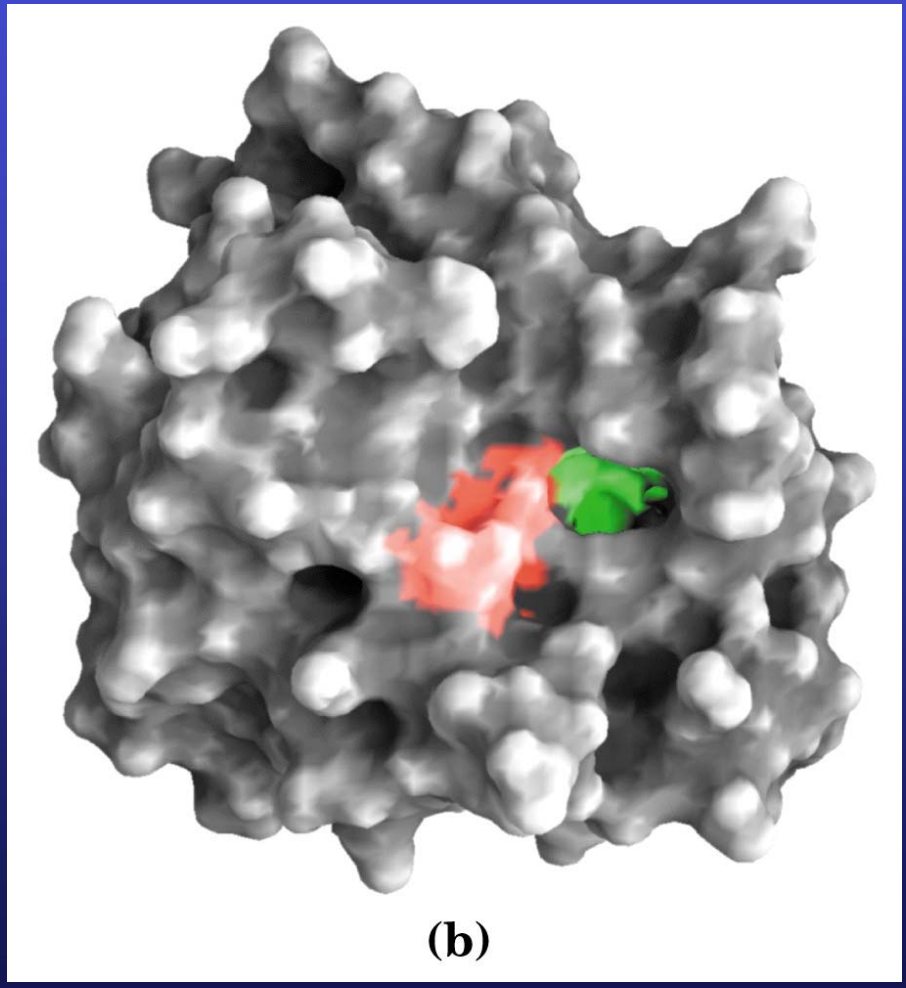


Determinación experimental de Km y Vmax



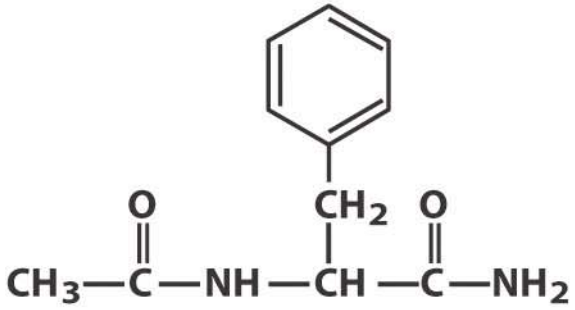
¿Cuál es la importancia de la razón k_{cat}/K_m ó eficiencia catalítica?

Quimotripsina

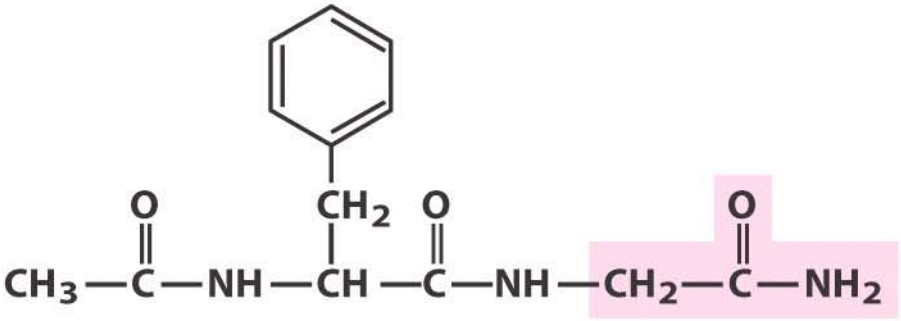


(b)

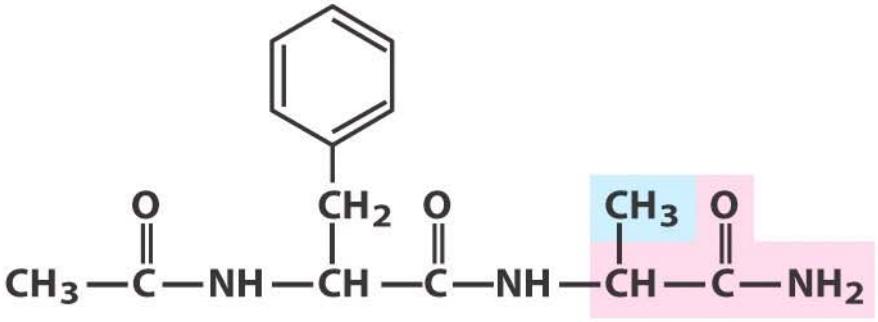
Substrate A



Substrate B



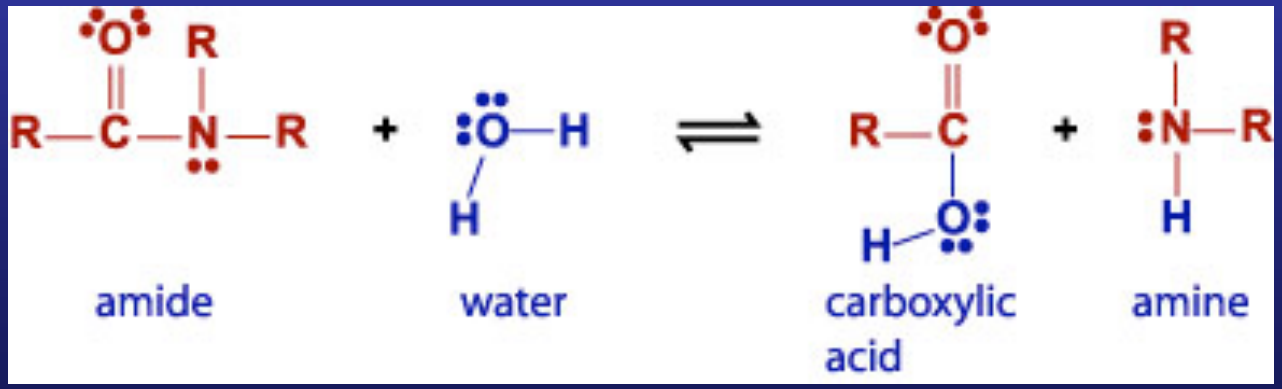
Substrate C

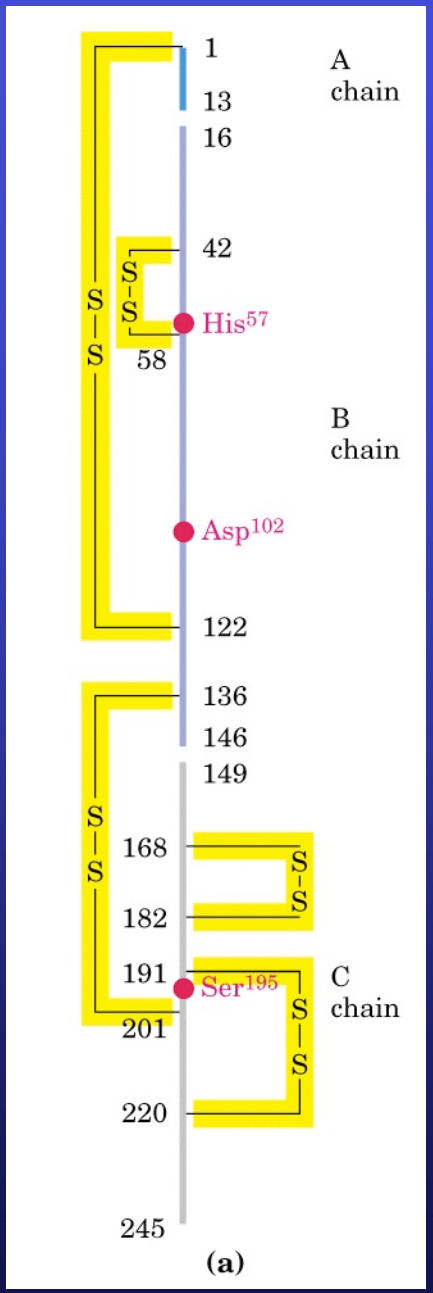


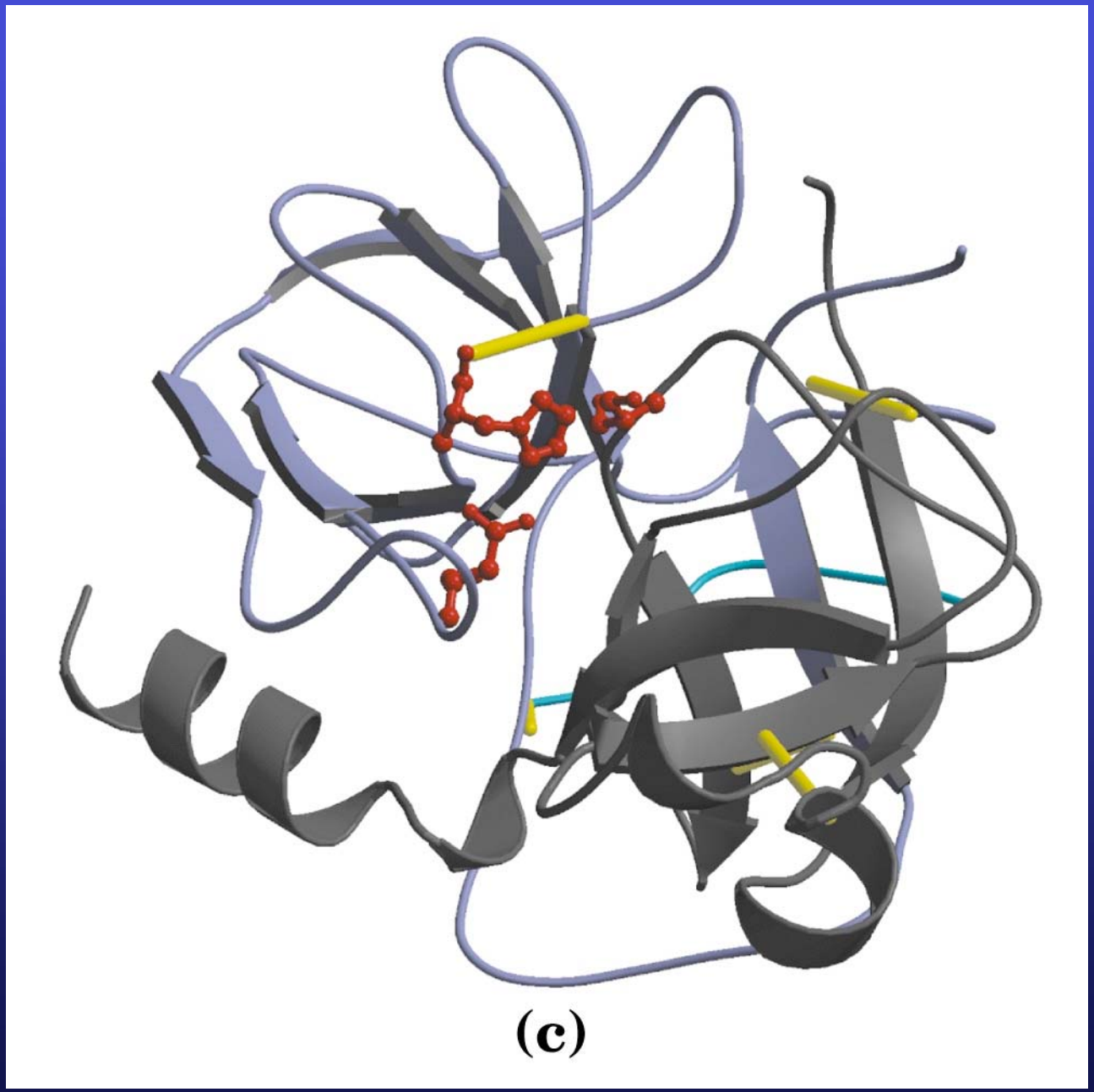
k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}\ s^{-1}$)
0.06	31	2
0.14	15	10
2.8	25	114

Sitio activo y mecanismo de catálisis

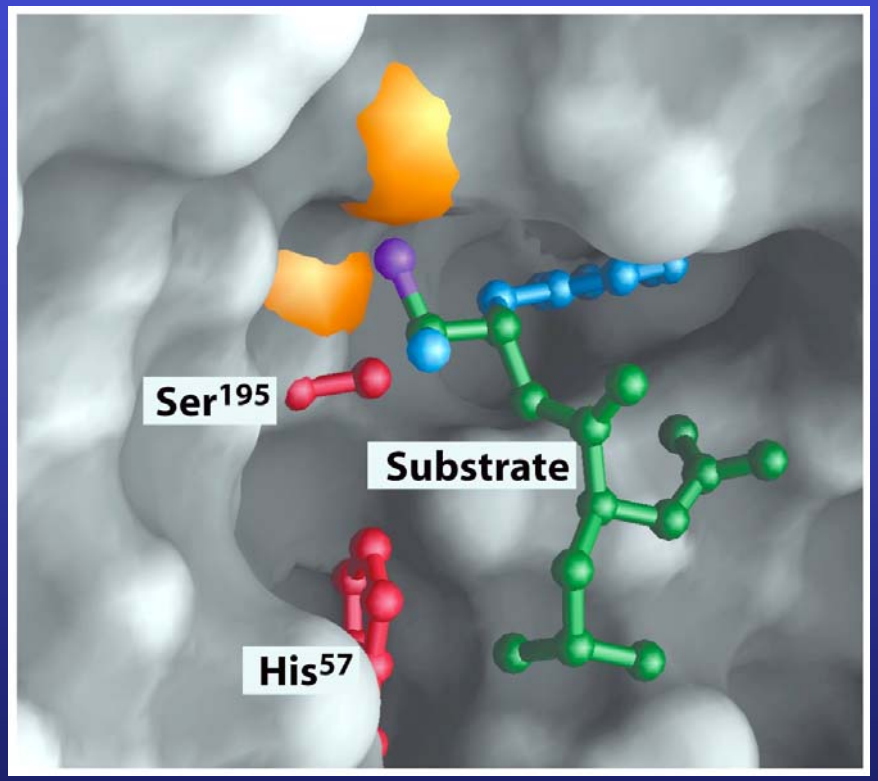
Quimotripsina

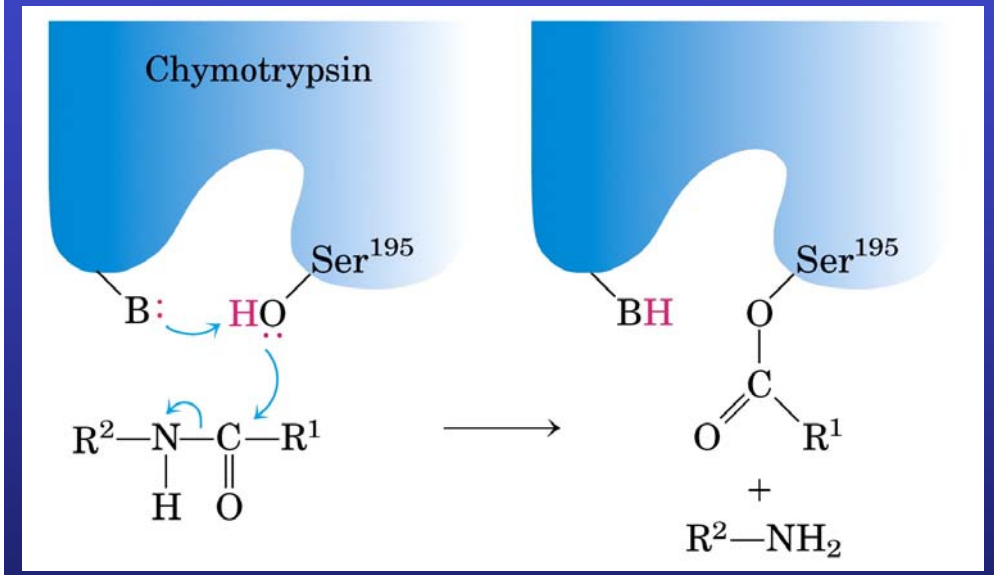


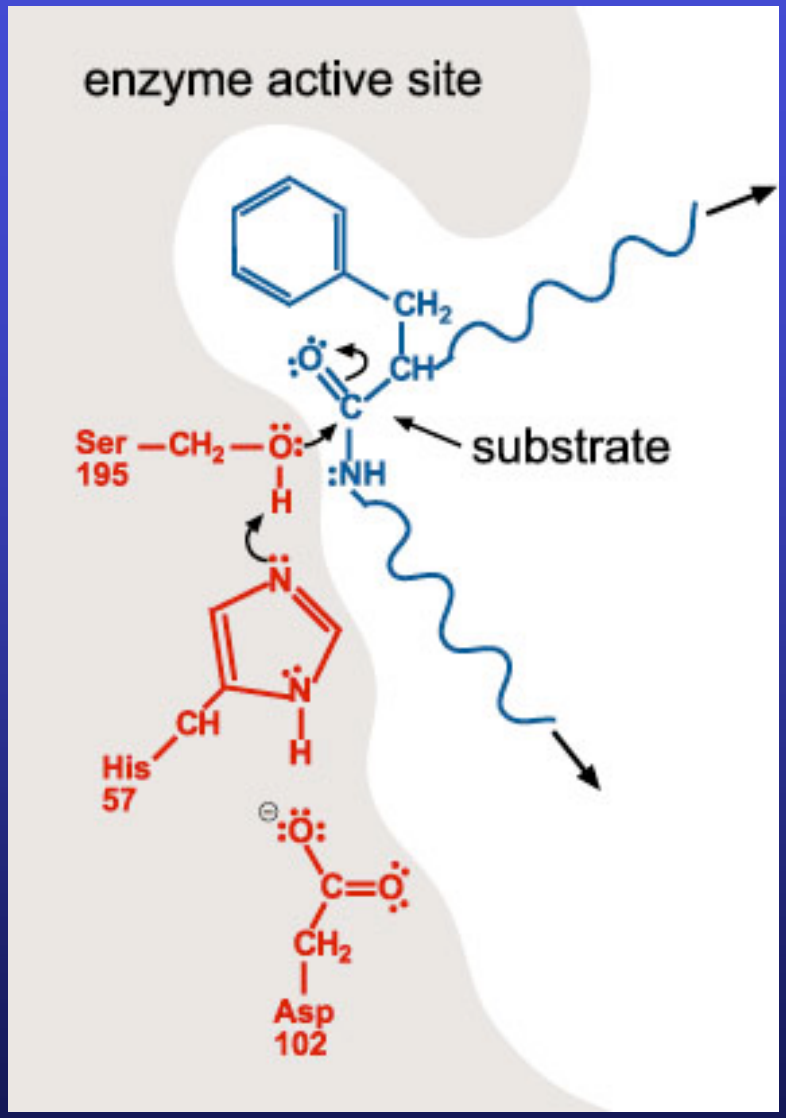

1
A chain
13
16
42
S–S
S—S
His57
58
B chain
Asp102
122
136
146
149
S—S
168
S–S
182
191
Ser195
C chain
S—S
201
220
245
(a)

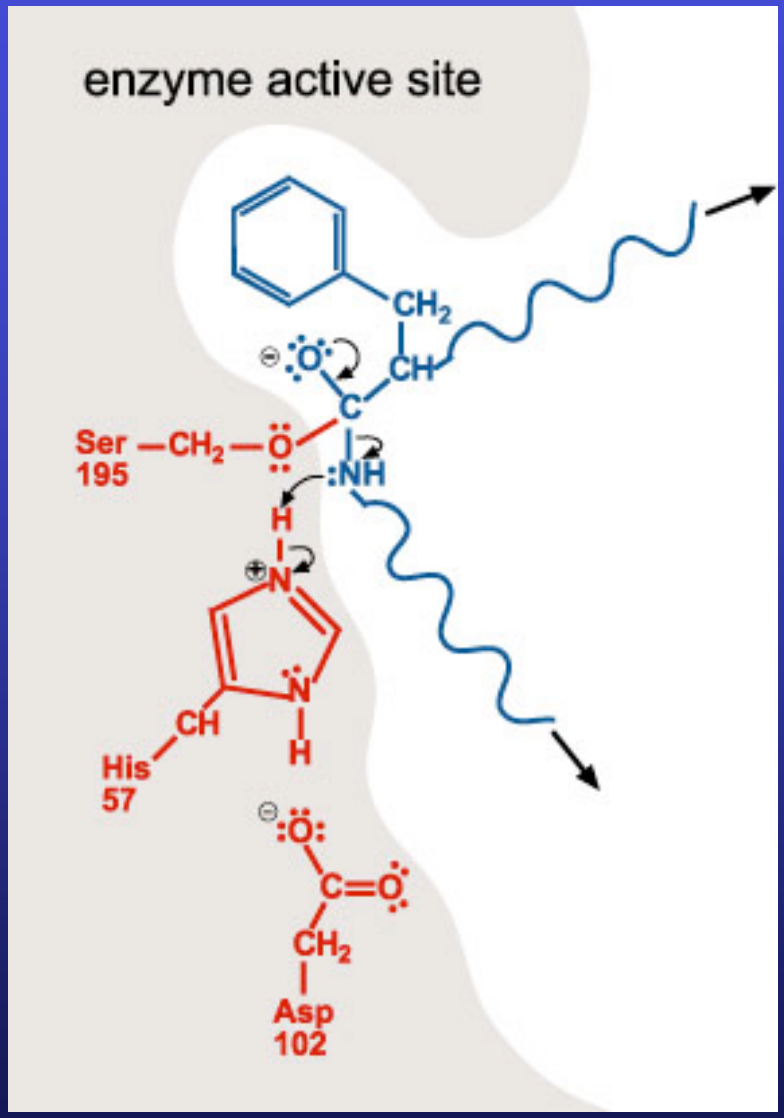

(c)

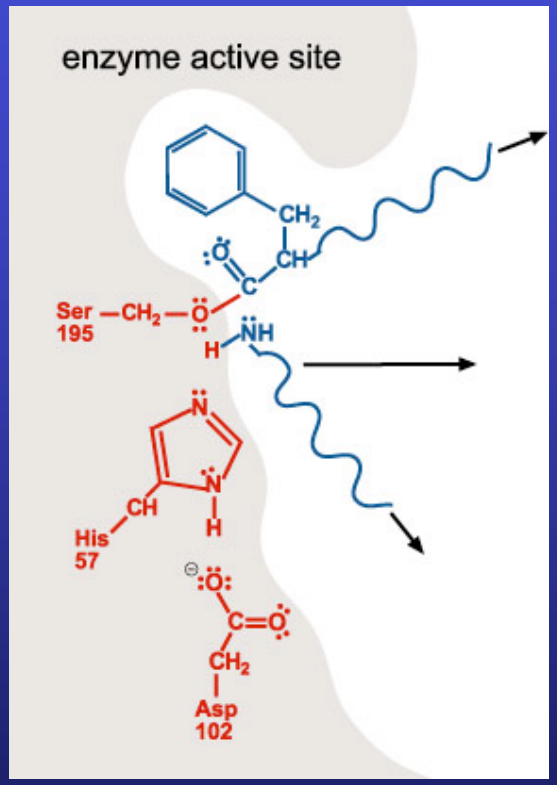
Sitio activo de la quimotripsina

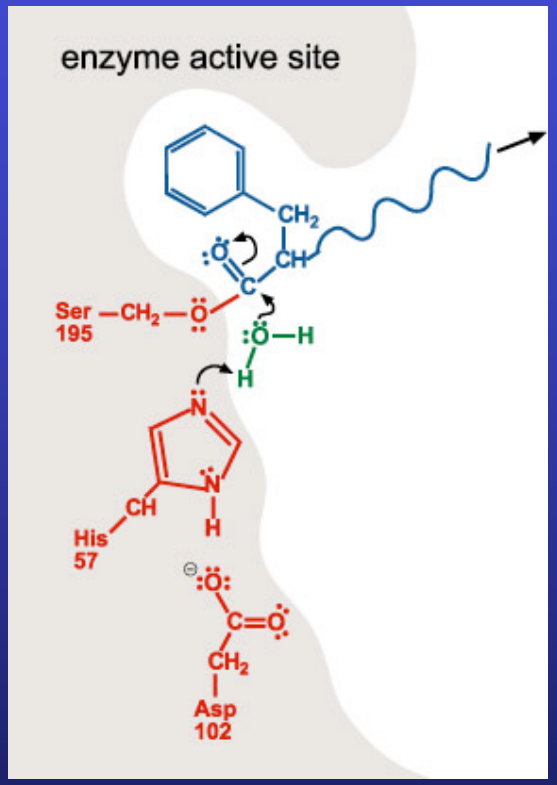


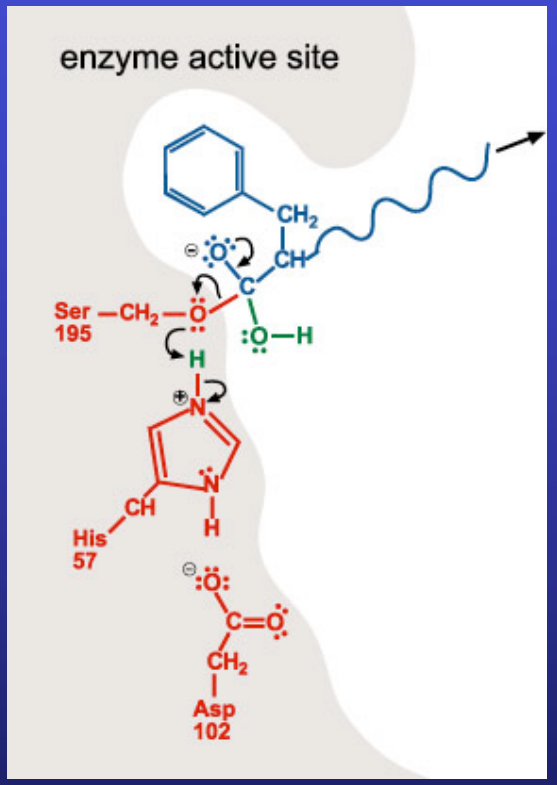



enzyme active site
substrate
Ser 195
His 57
Asp 102


enzyme active site
Ser 195
His 57
Asp 102

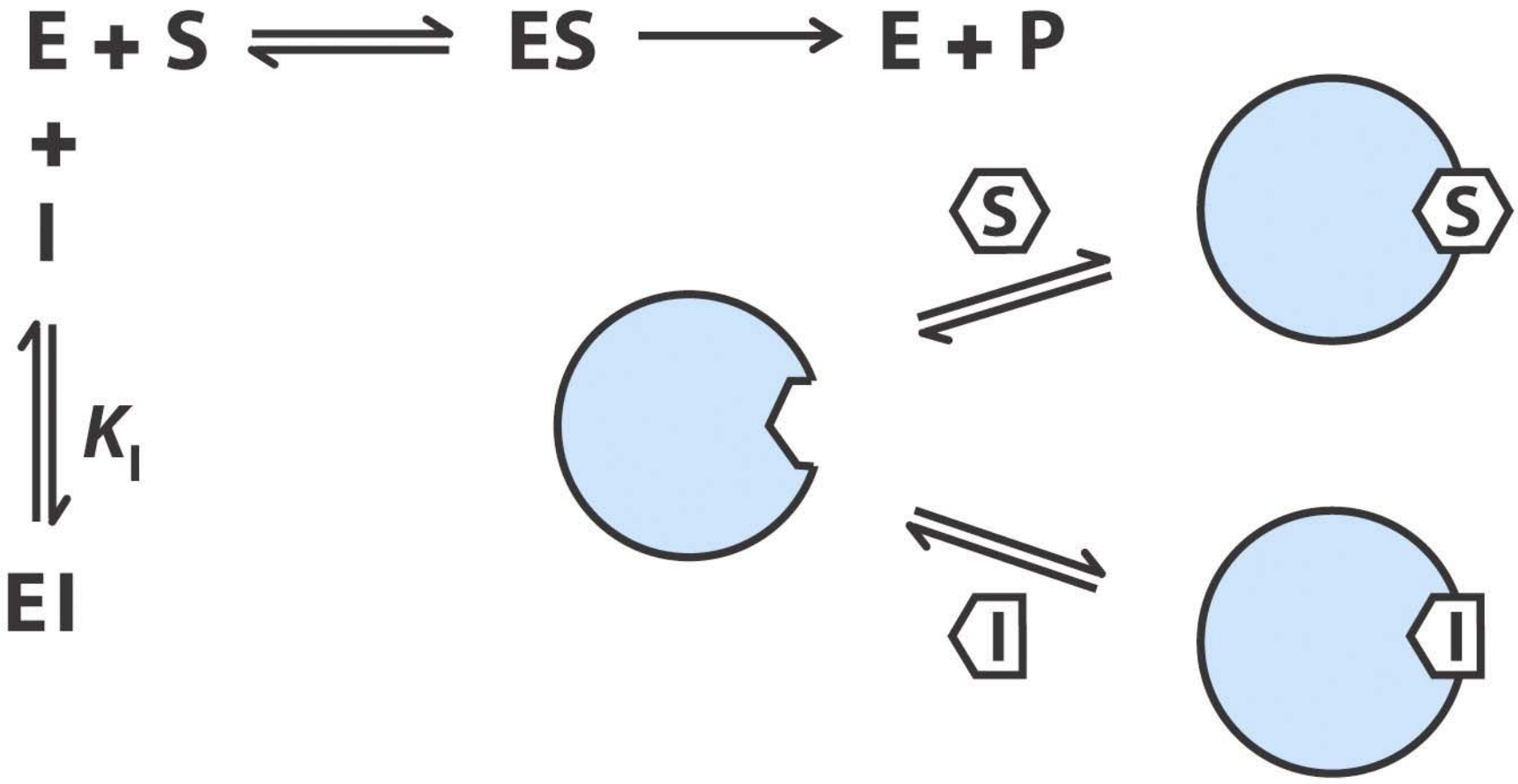

enzyme active site
Ser 195
His 57
Asp 102


enzyme active site
Ser 195
His 57
Asp 102

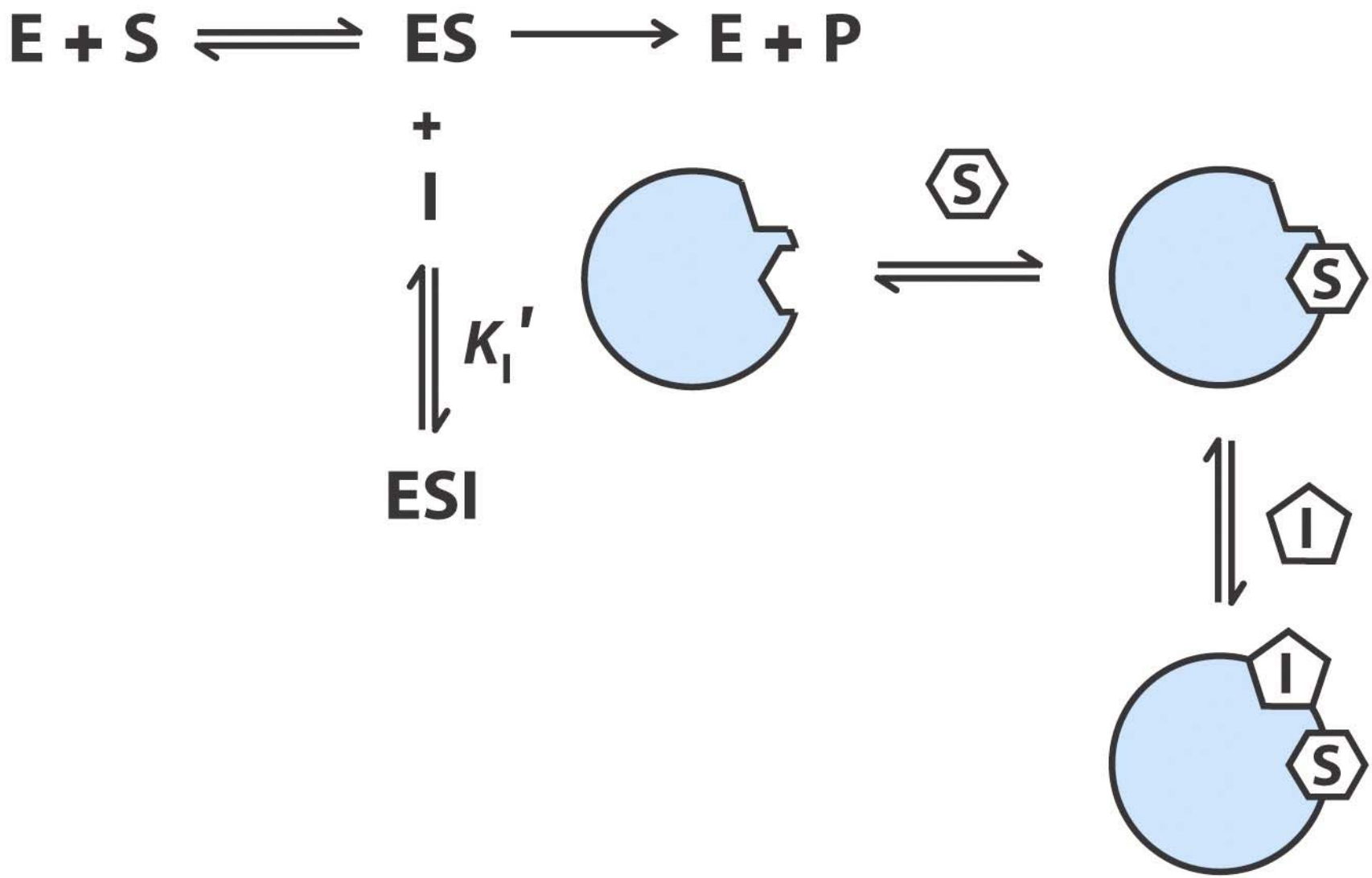

enzyme active site
Ser 195
His 57
Asp 102

Efecto de Inhibidores

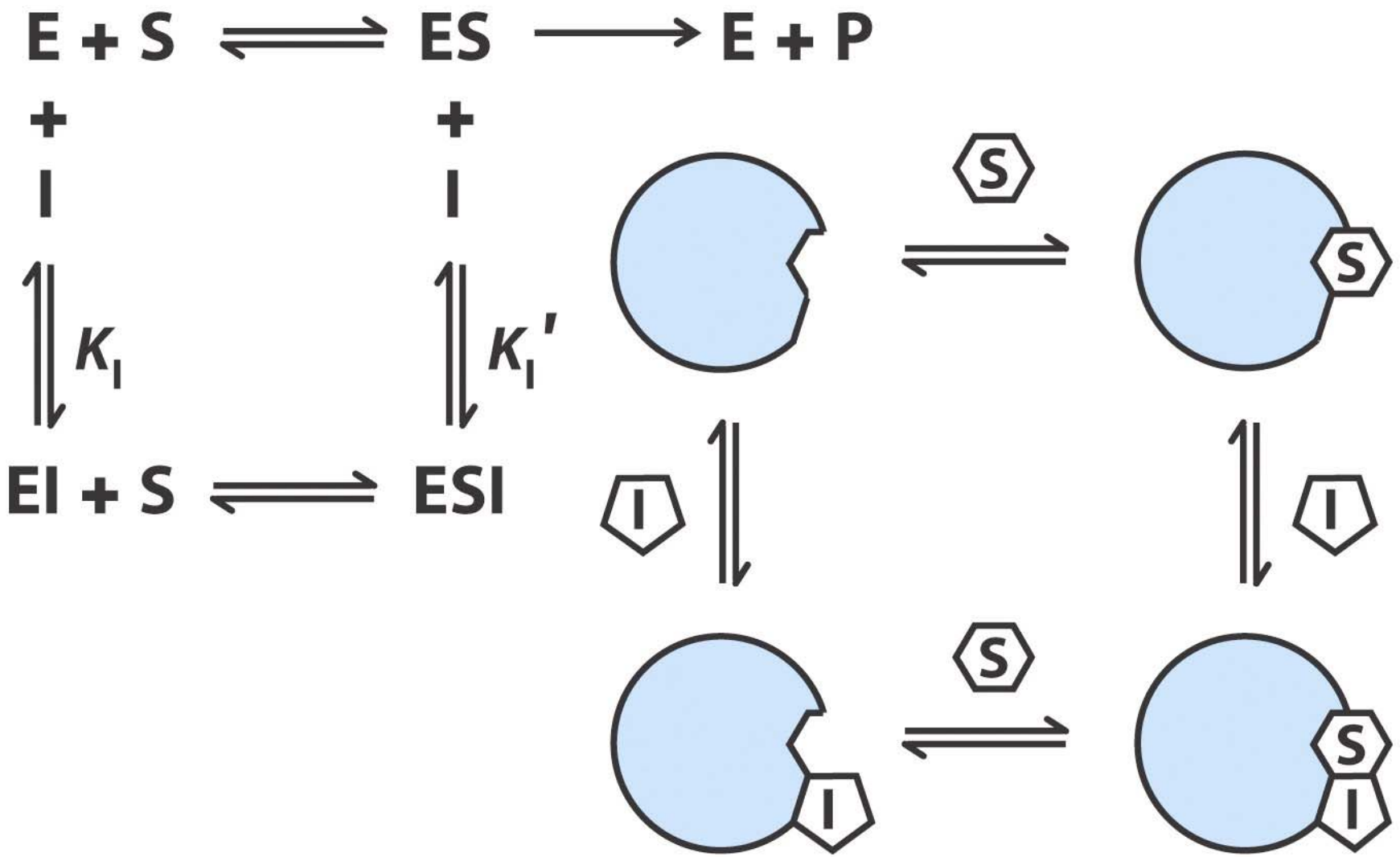
(a) Competitive inhibition

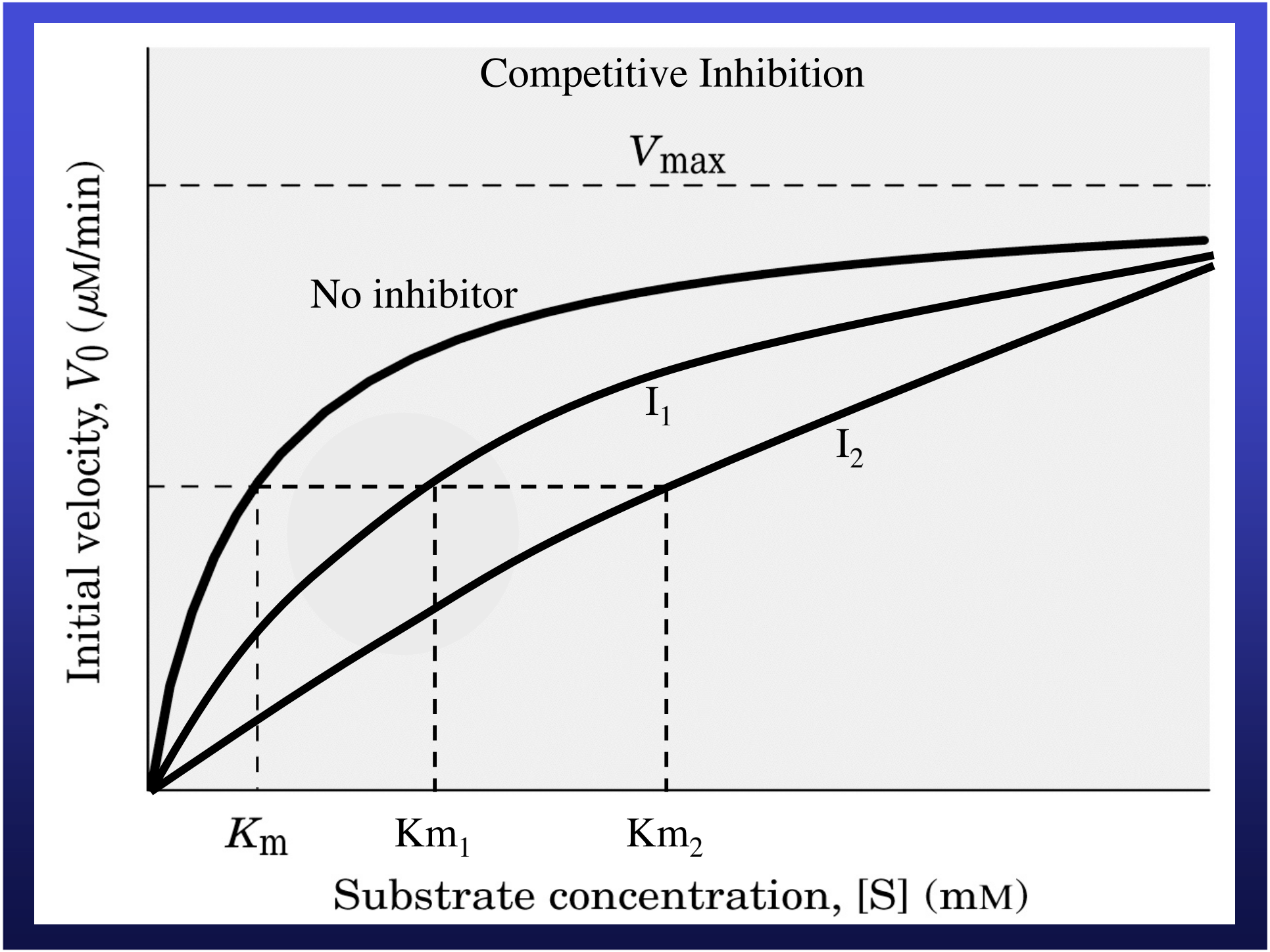


(b) Uncompetitive inhibition

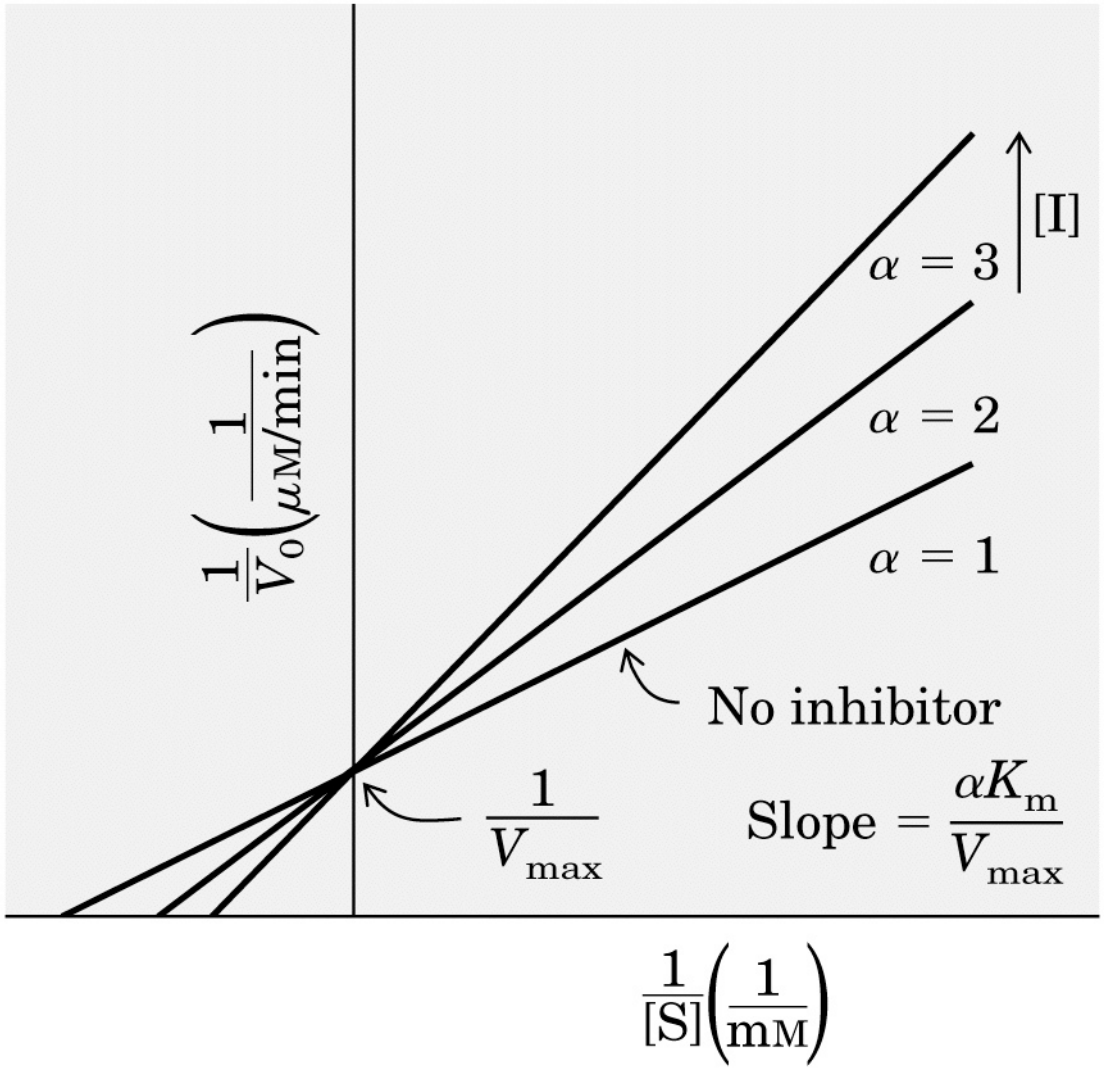


(c) Mixed inhibition

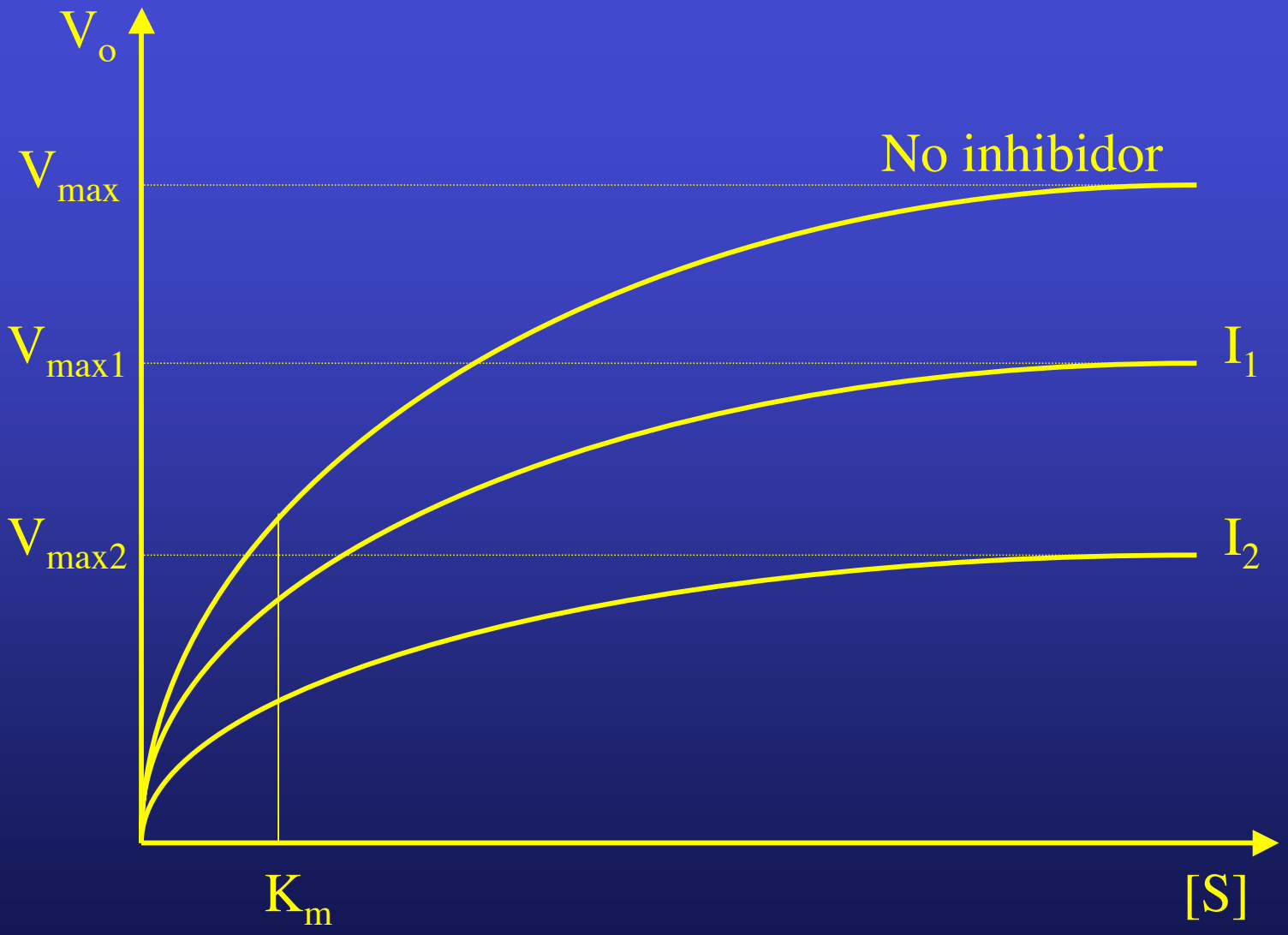


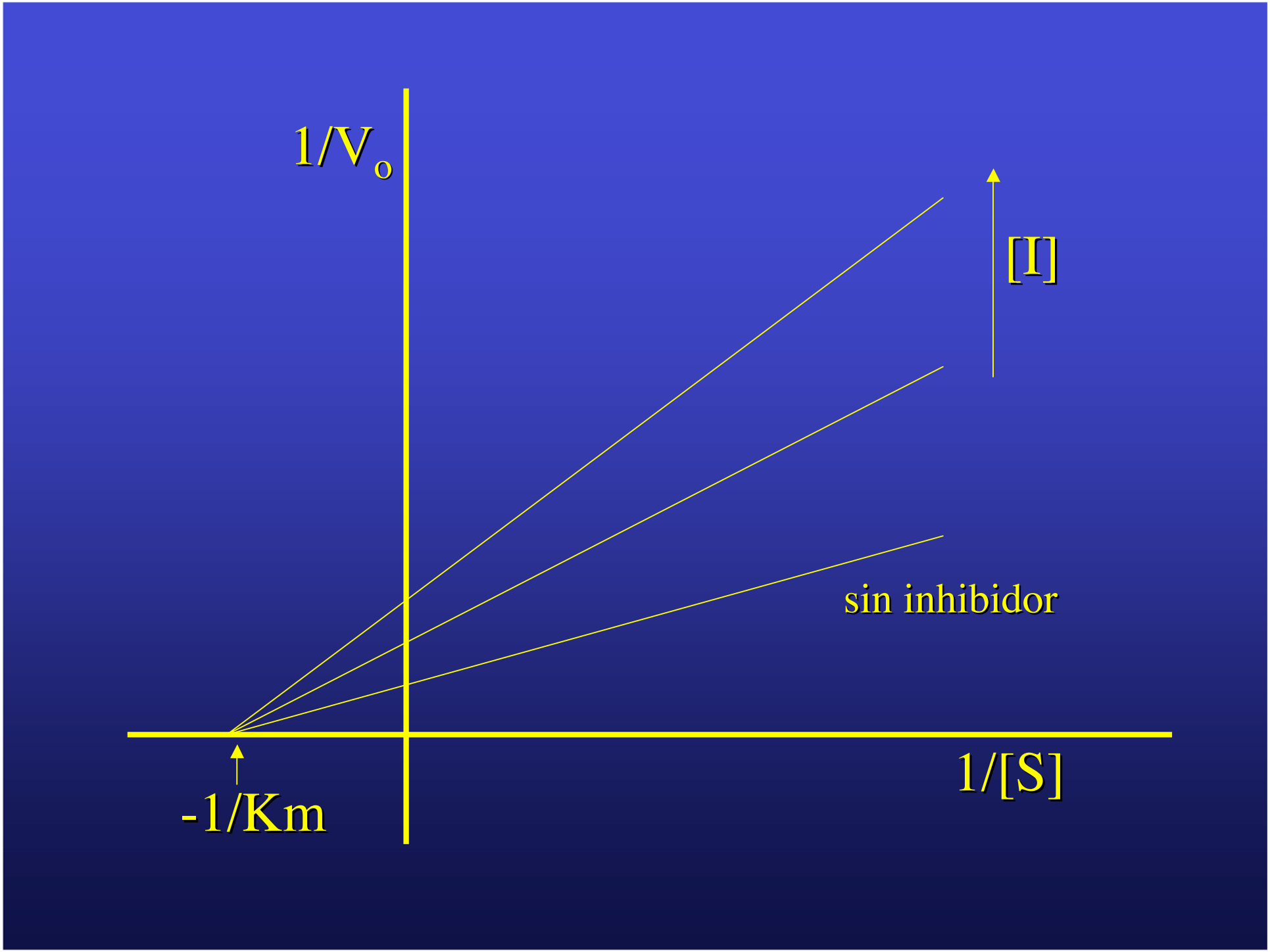

Competitive Inhibition
V_{max}
No inhibitor
I_1
I_2
Initial velocity, V_0 (μM/min)
K_m
Km_1
Km_2
Substrate concentration, [S] (mM)

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$



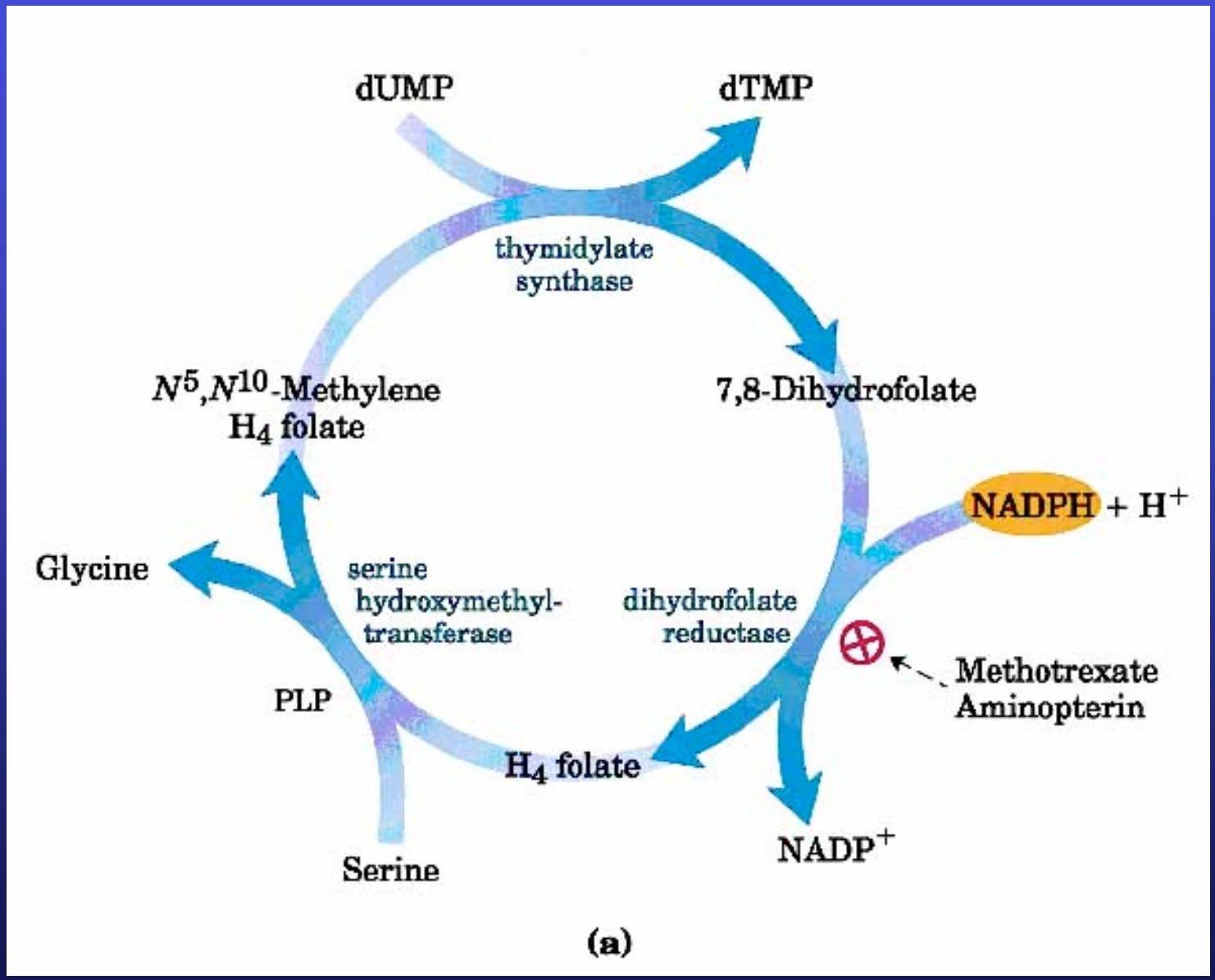
Inhibición no competitiva



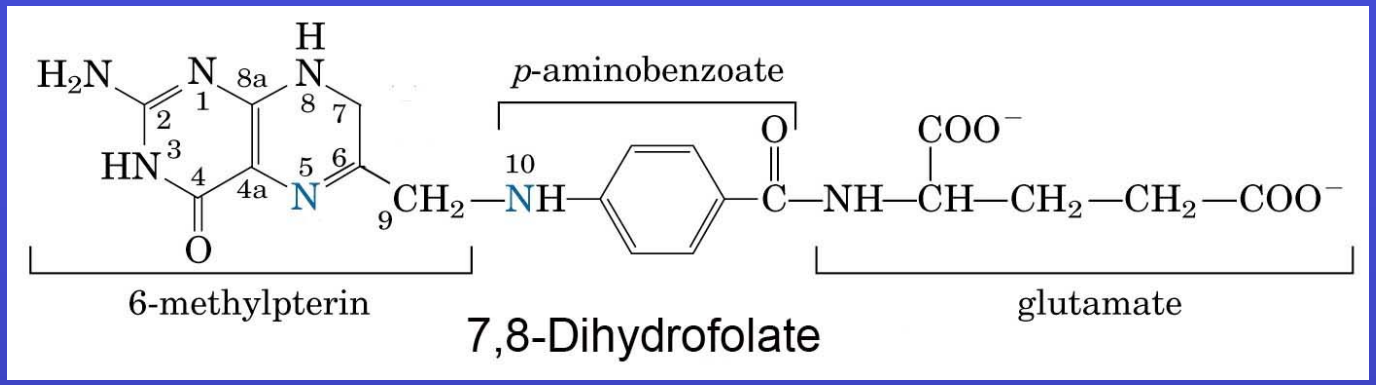

1/V o
[I]
sin inhibidor
1/[S]
-1/Km

Los inhibidores pueden ser utilizados como agentes farmacológicos.

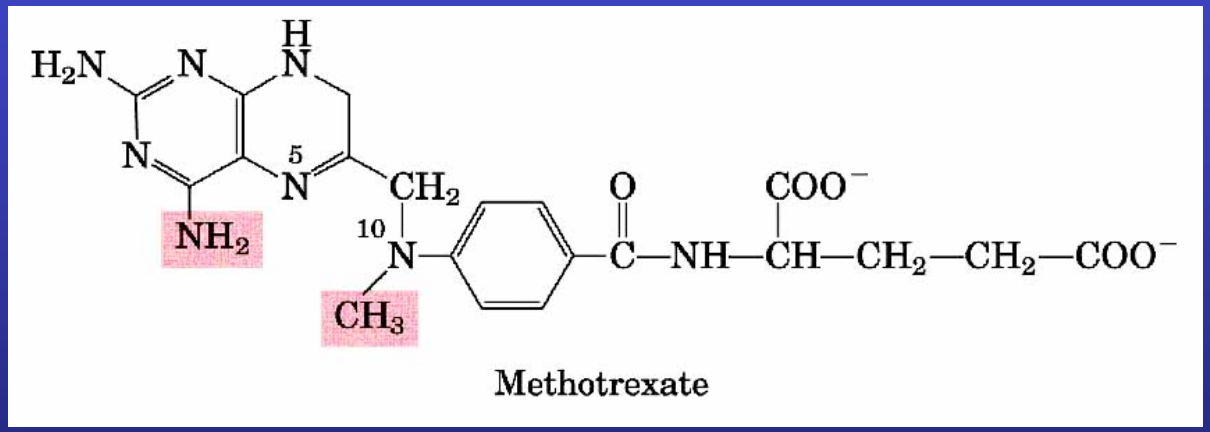
Drogas anticancerígenas: quimioterapia.


dUMP
dTMP
thymidylate synthase
7,8-Dihydrofolate
NADPH + H+
Methotrexate
Aminopterin
dihydrofolate reductase
NADP+
H4 folate
Serine
PLP
serine hydroxymethyl-transferase
Glycine
N5,N10-Methylene H4 folate

(a)

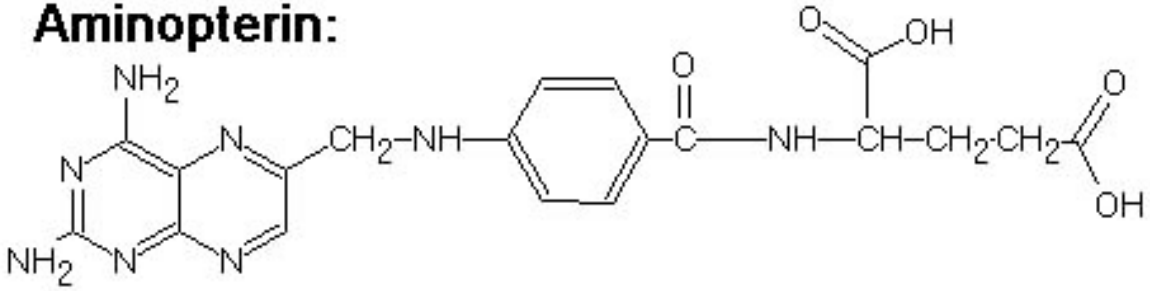


7,8-Dihydrofolate



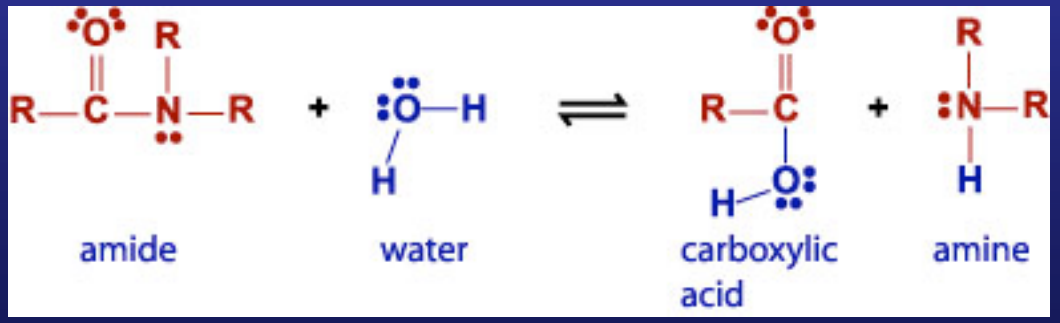
Methotrexate

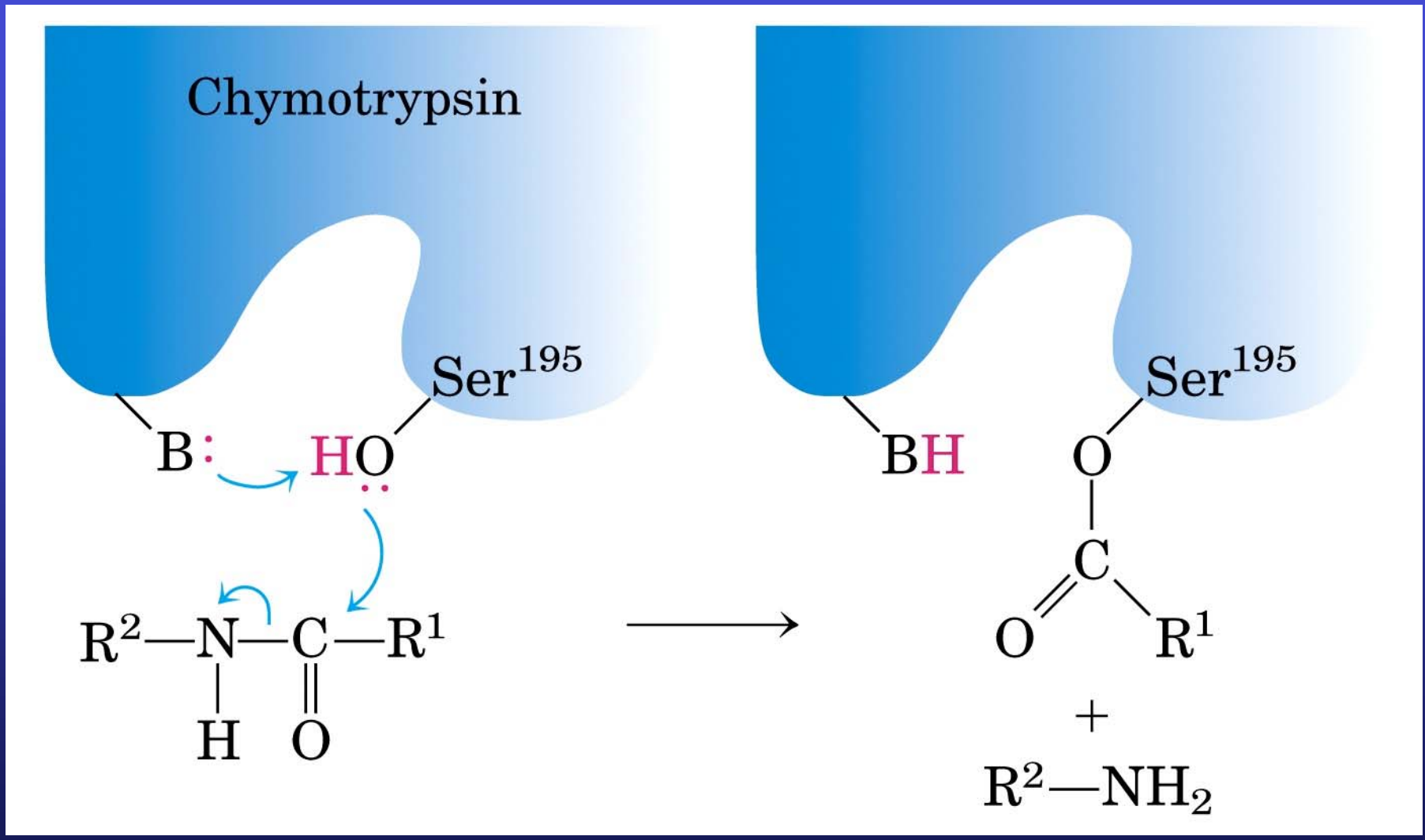
Aminopterin:



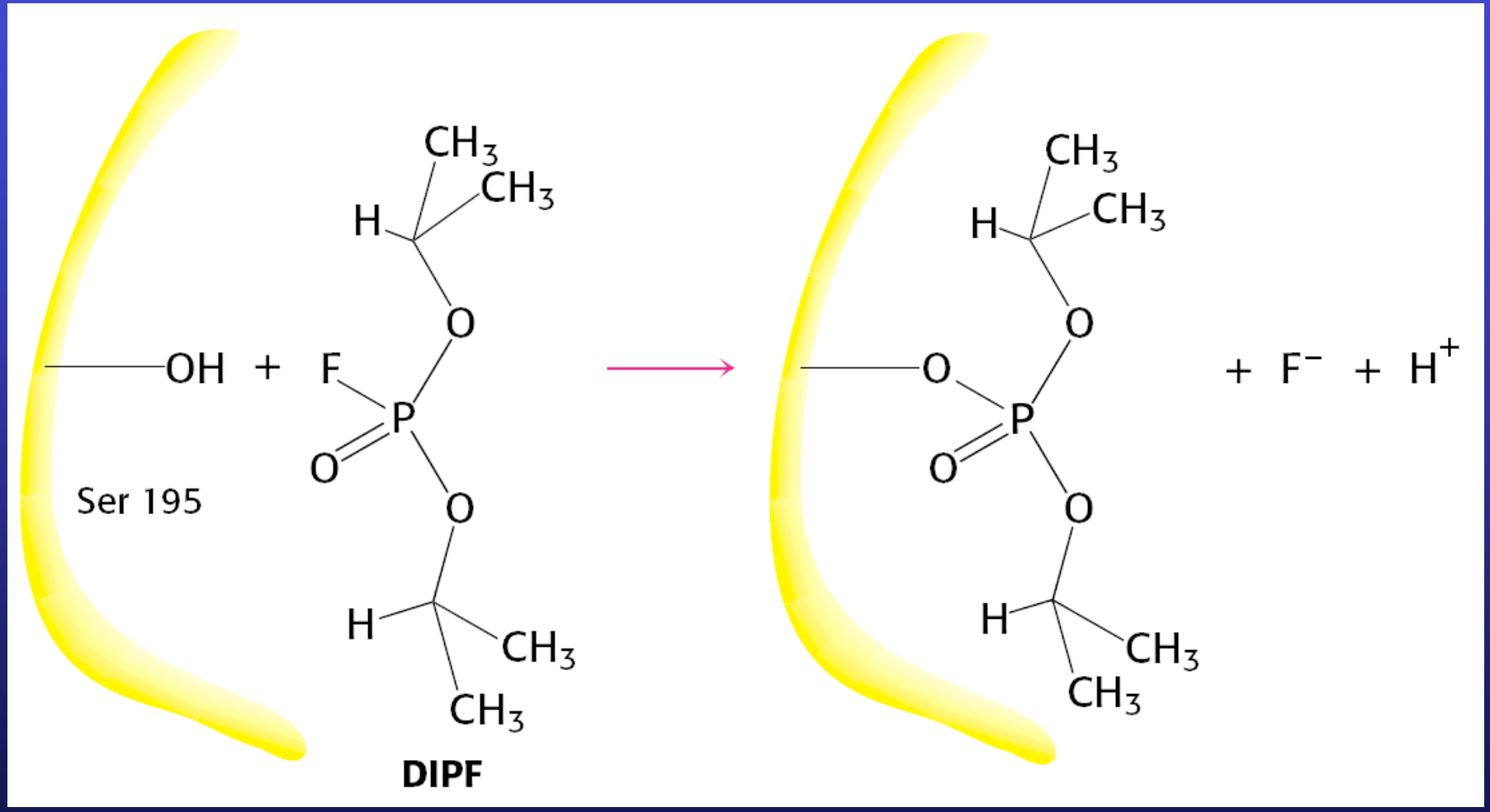
Inhibición irreversible o Inactivación

Quimotripsina

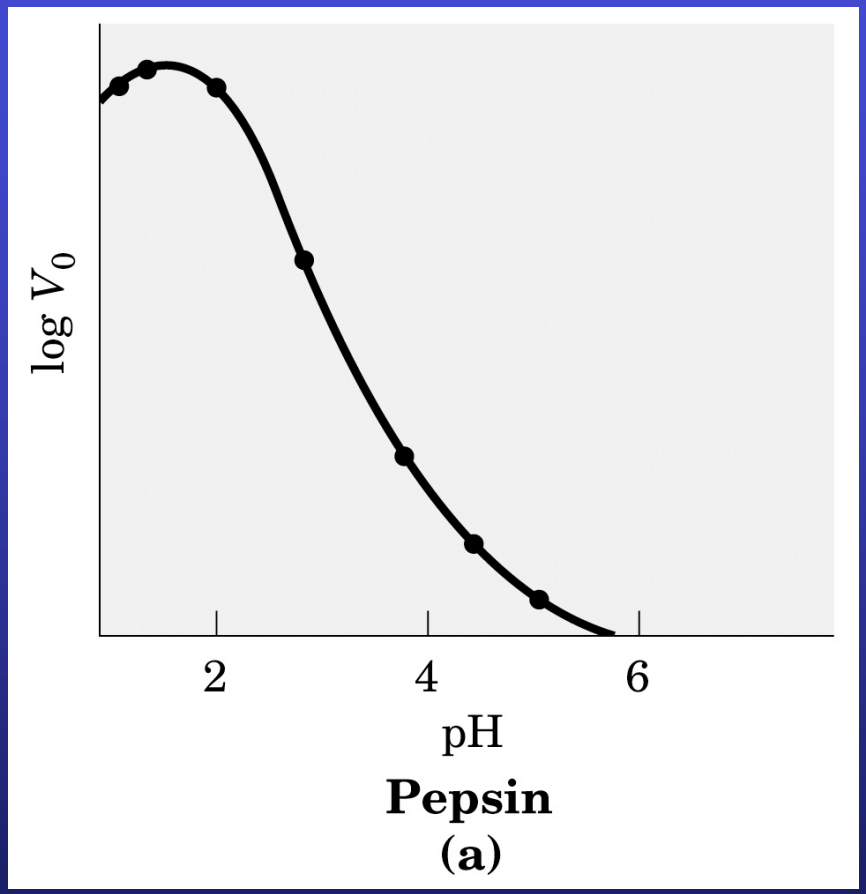



Chymotrypsin
Ser195
B:
HO
R2—N—C—R1
H O
BH
O
C
O
R1
+
R2—NH2

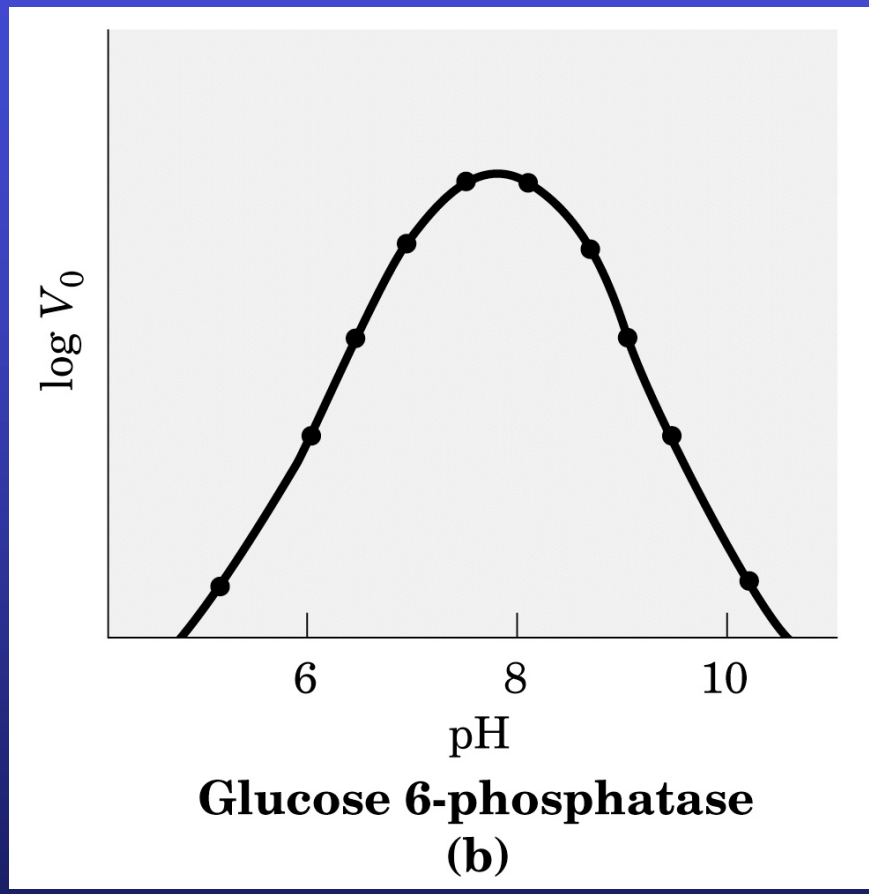
di-isopropil-fluorfosfato



Efecto del pH y la temperatura sobre la actividad enzimática

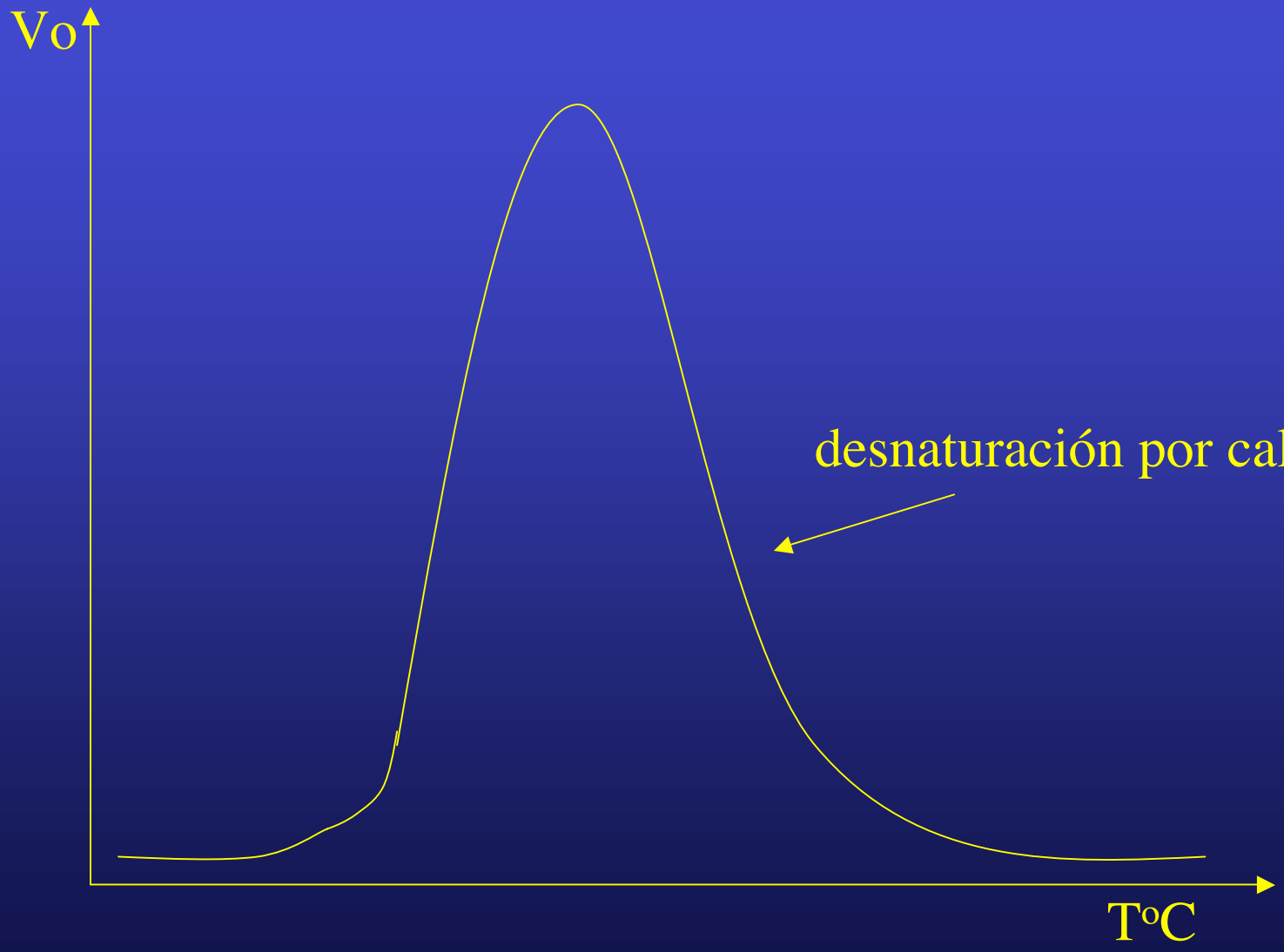


Pepsin
(a)



Glucose 6-phosphatase
(b)

Efecto de la Temperatura sobre la actividad enzimática



Regulación de la actividad enzimática

Alosterismo

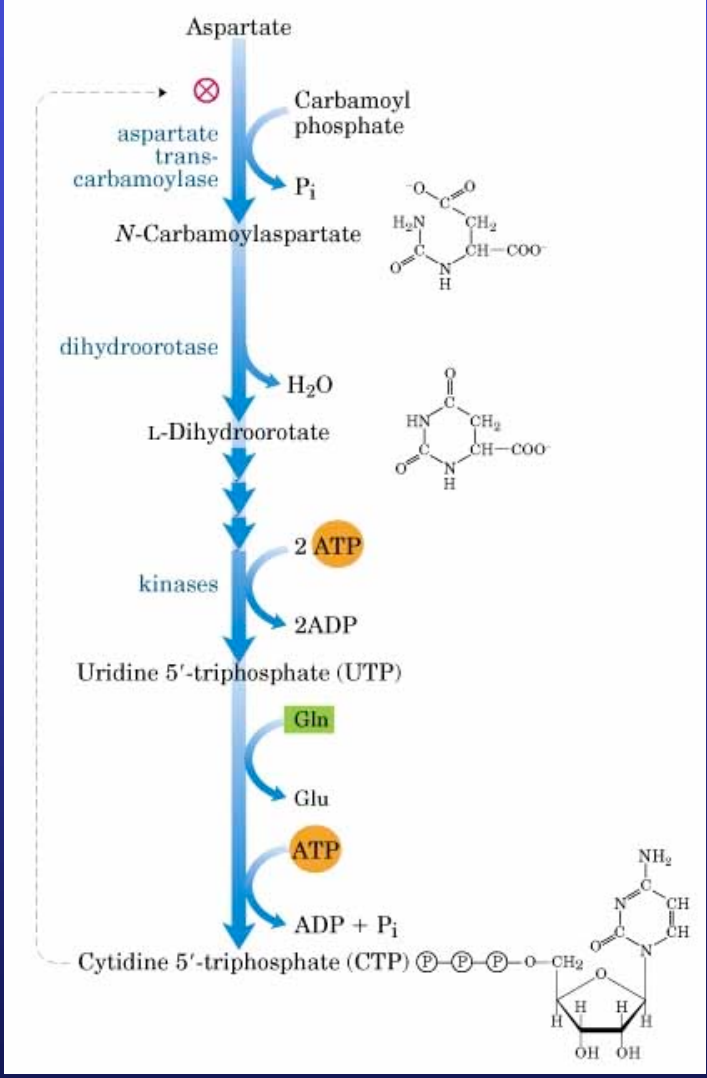
Enzimas alostéricas

Enzimas con sitios de unión para moléculas reguladoras de la actividad enzimática en sitios distintos al del sustrato

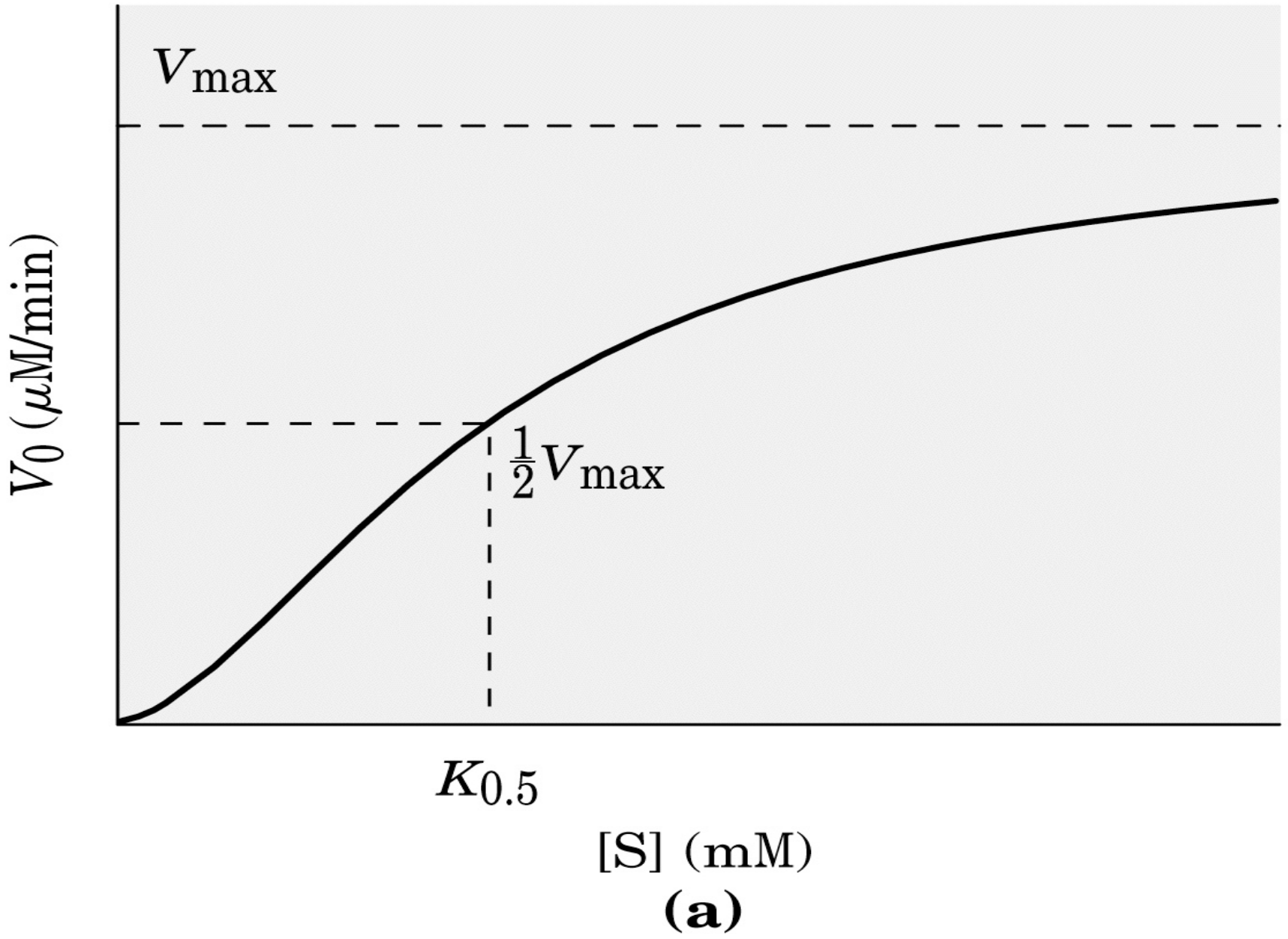
Ejemplo: Biosíntesis de nucleótidos de pirimidina

Aspartato transcarbamilasa

presenta un sitio alosterico para la unión del CTP o producto final de la vía metabólica

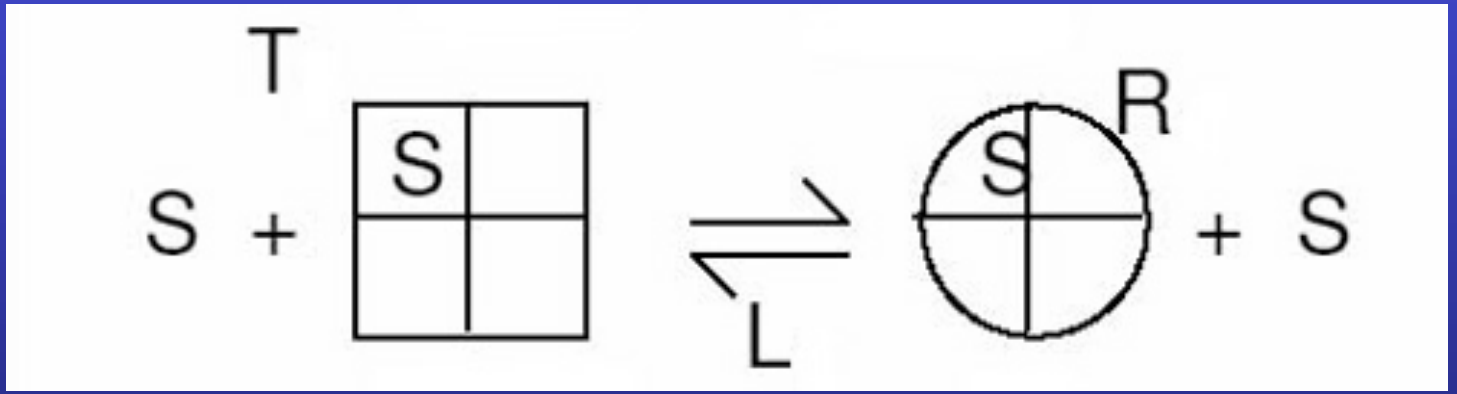


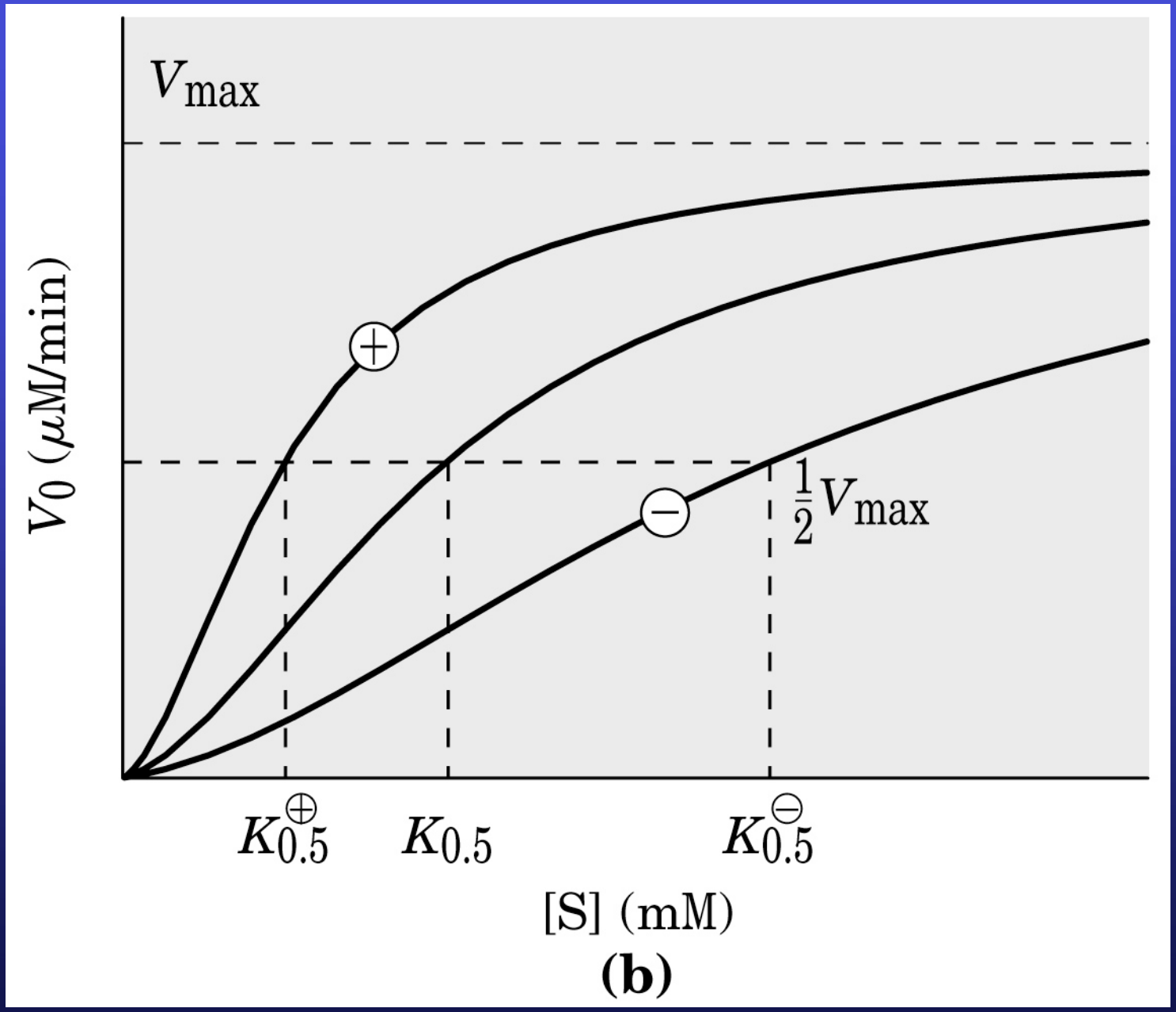
Estas enzimas presentan cinéticas diferentes a la Michaeliana:
Cinética Sigmoide



(a)

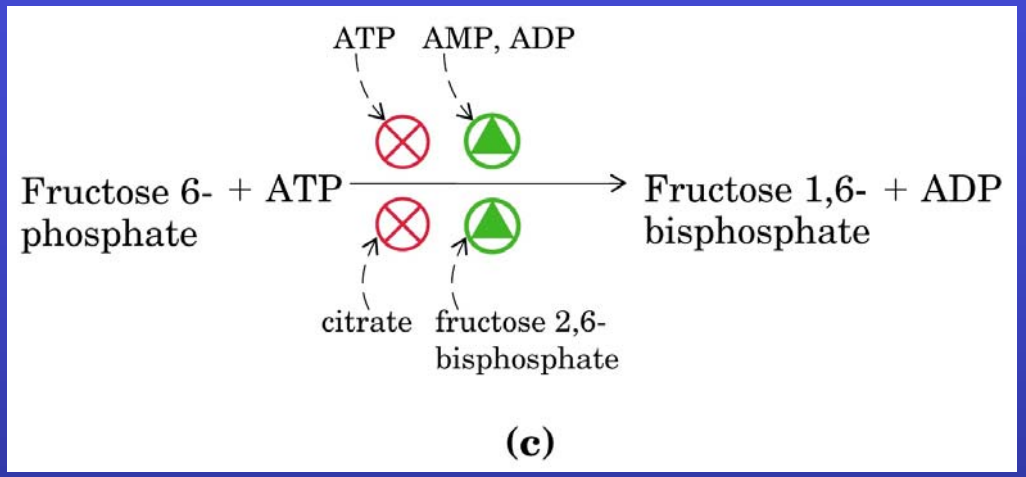
Modelo Concertado

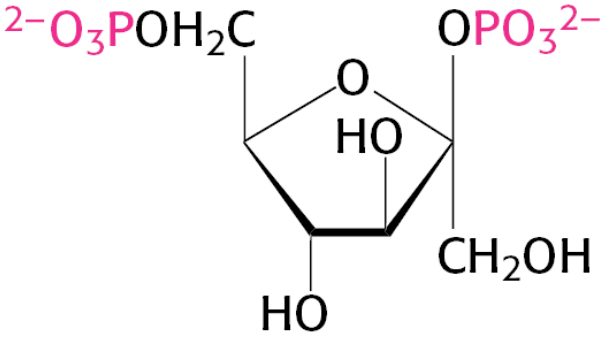




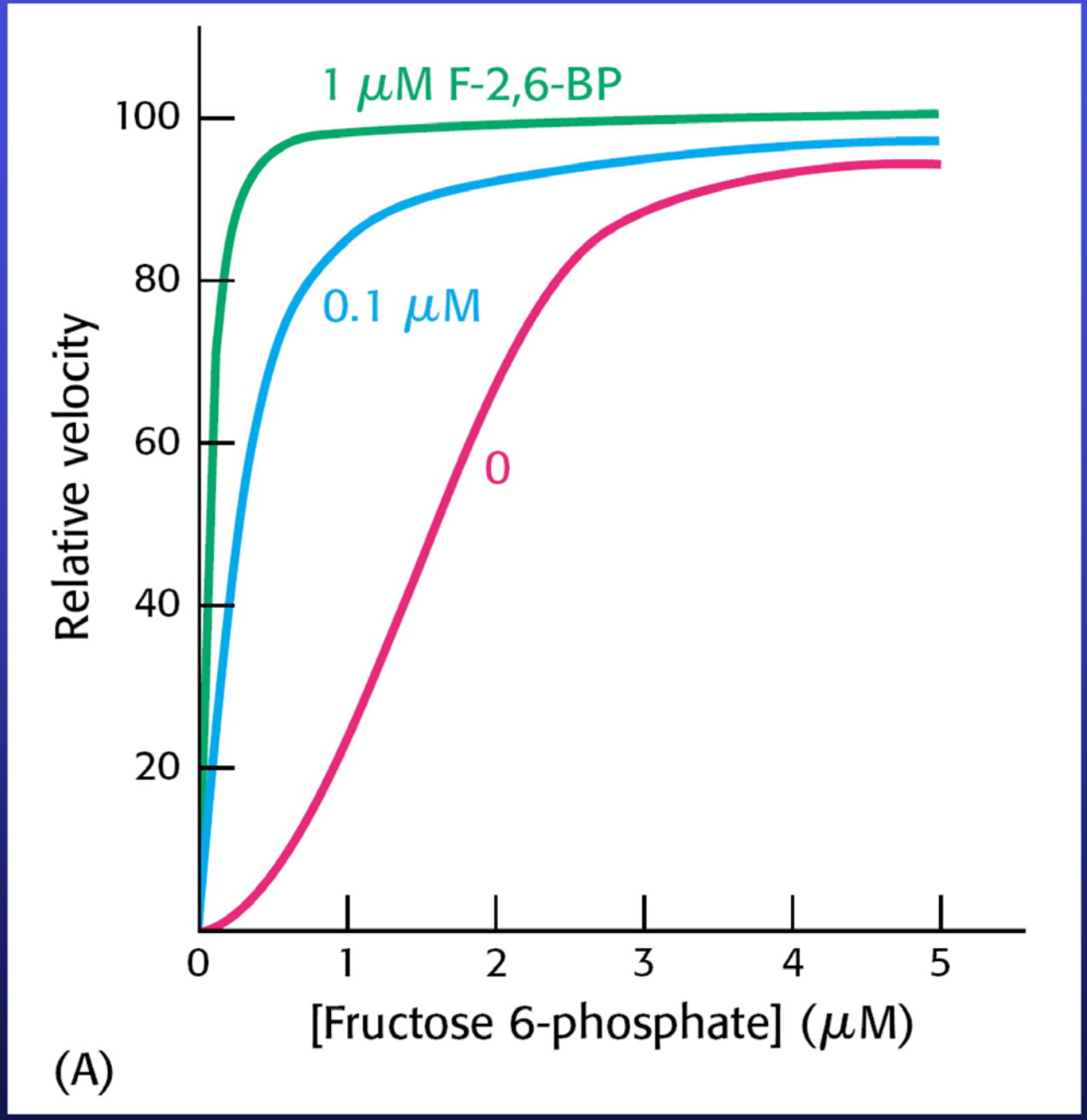
(b)

Ejemplo: Fosfofuctoquinasa

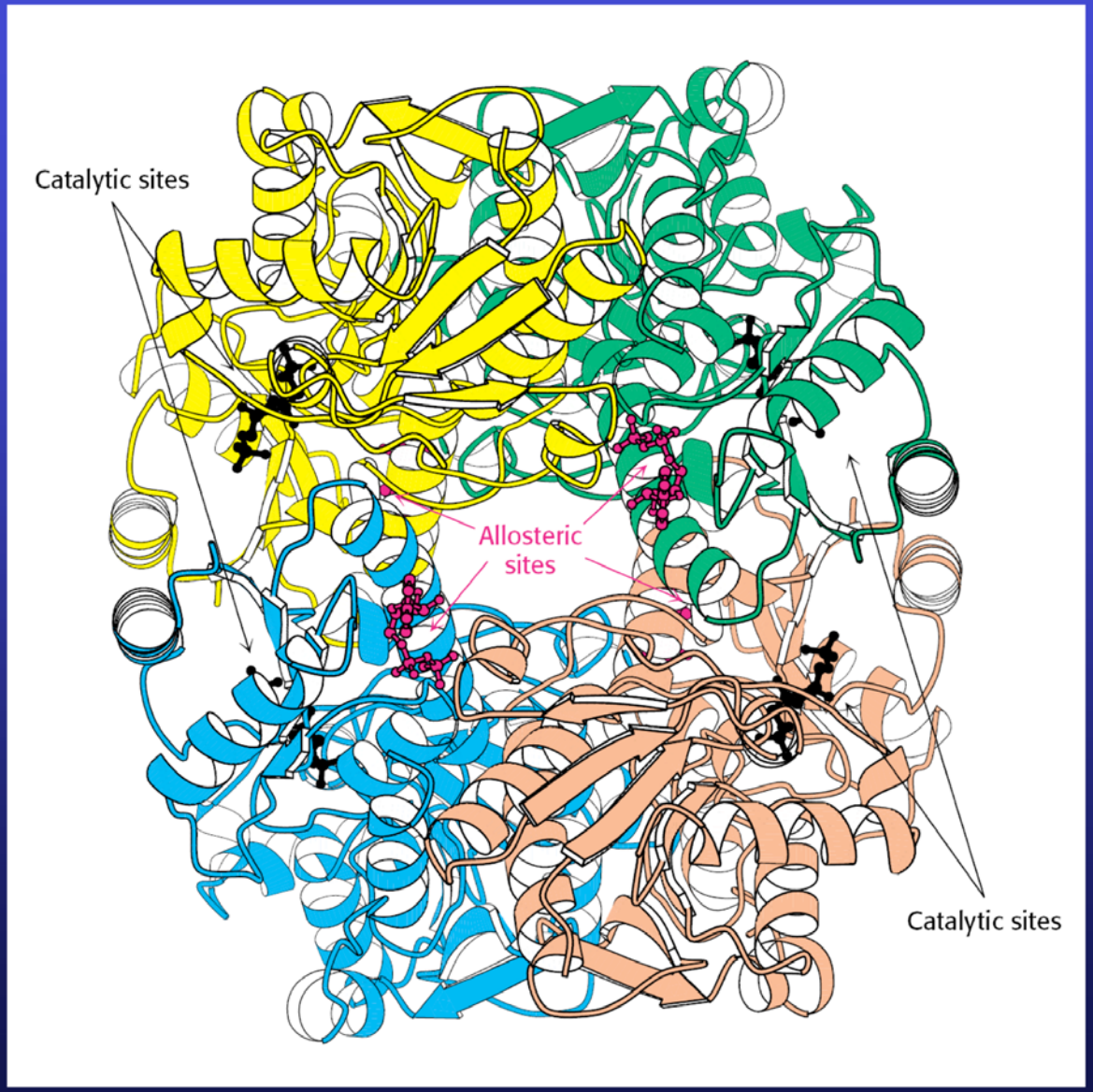




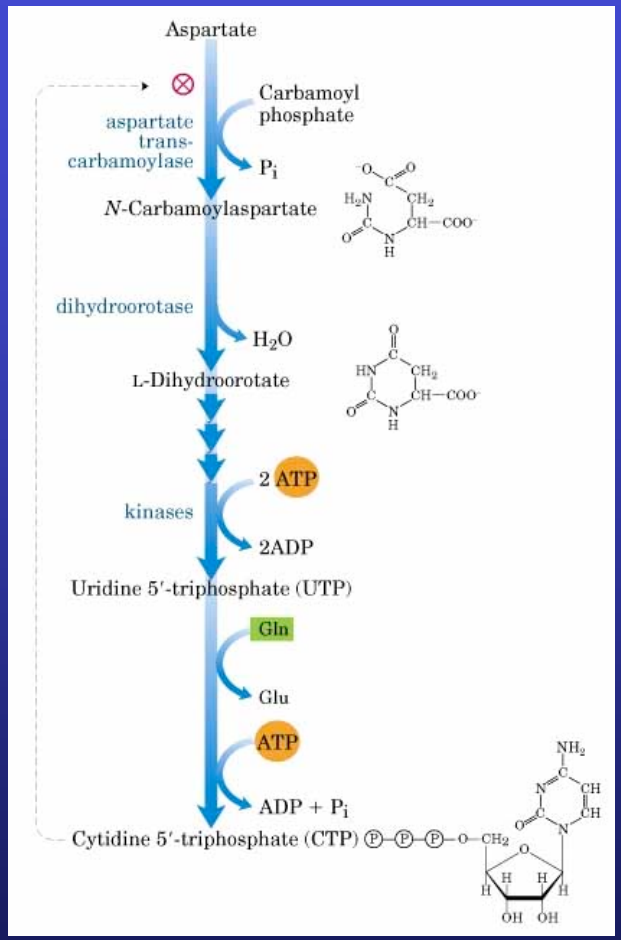
Regulador alostérico: activador

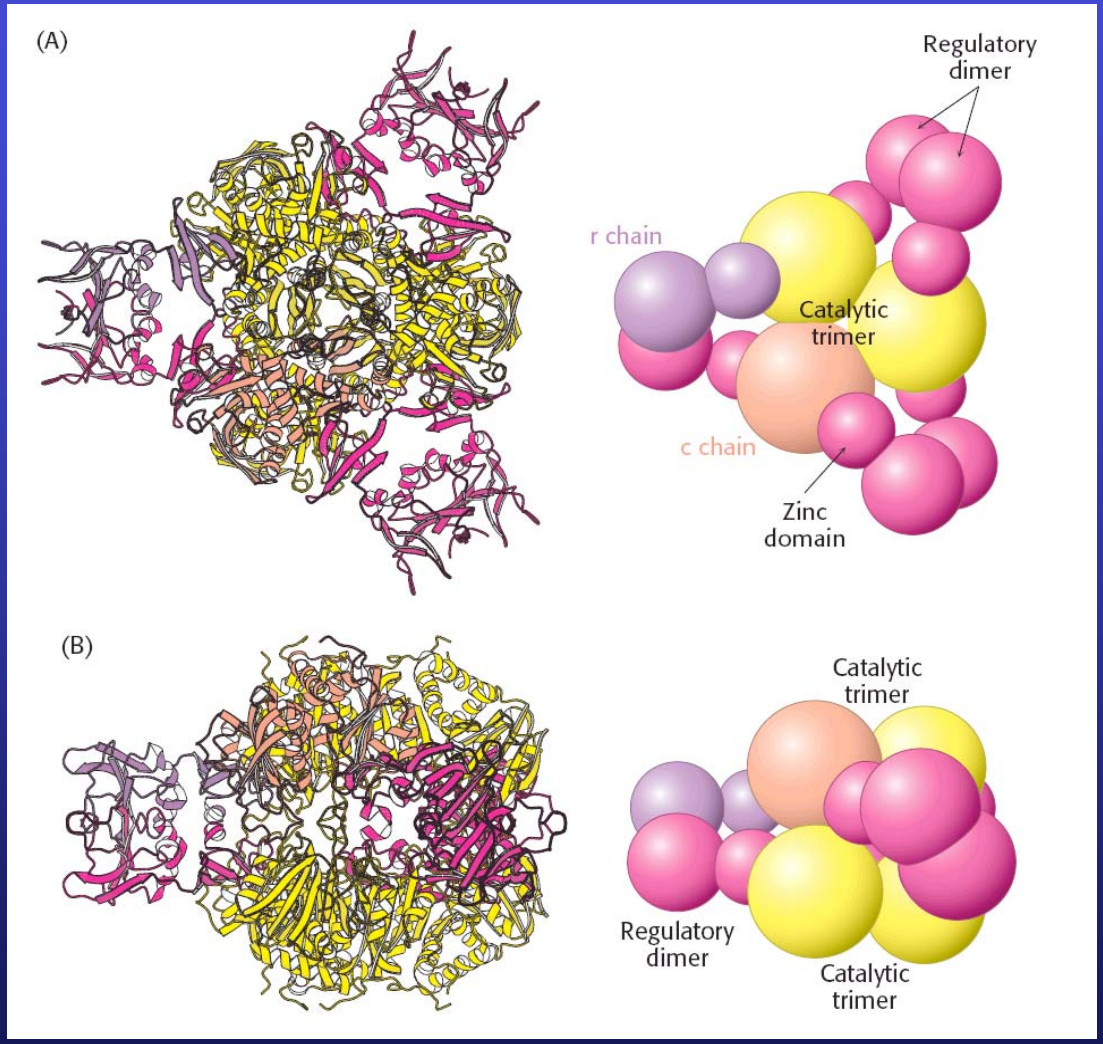

1 μM F-2,6-BP
0.1 μM
0
Relative velocity
100
80
60
40
20
0
1
2
3
4
5
[Fructose 6-phosphate] (μM)
(A)

Fosfofructoquinasa: tetramero

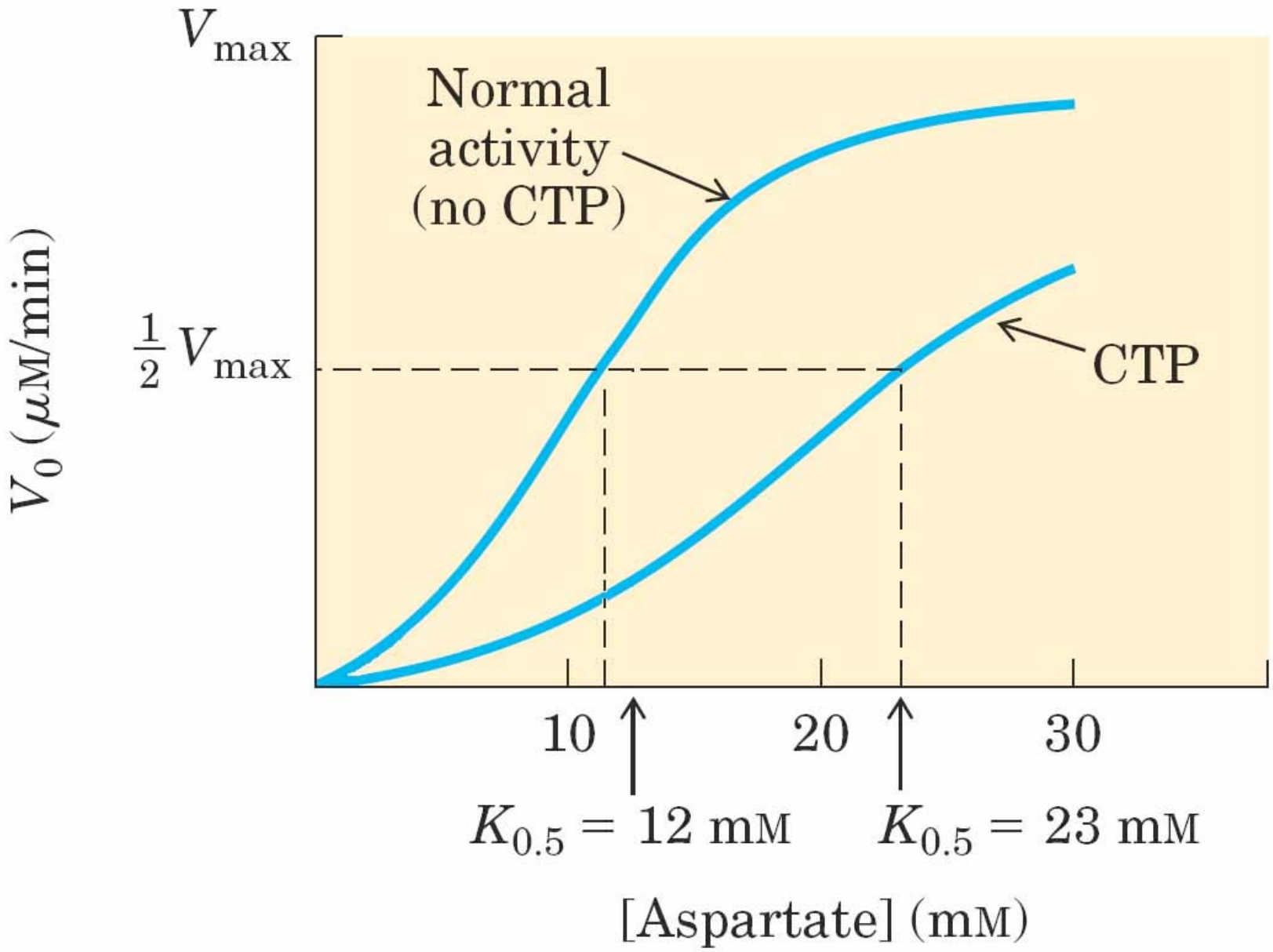


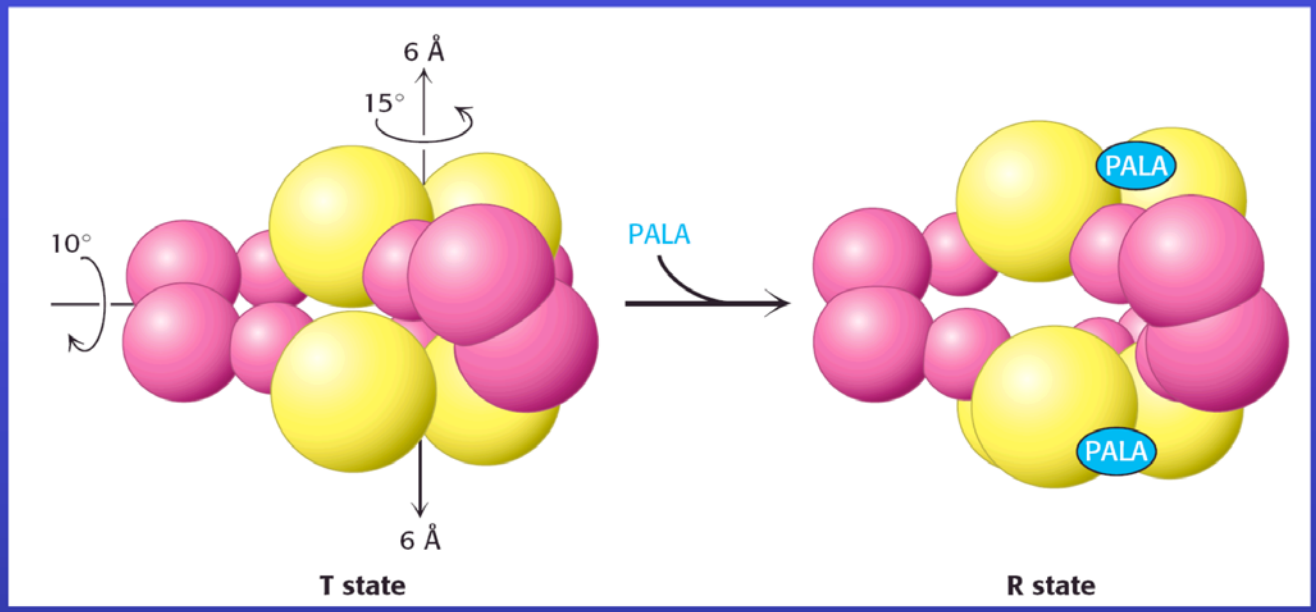
Aspartato transcarbamilasa: seis subunidades catalíticas y seis reguladoras



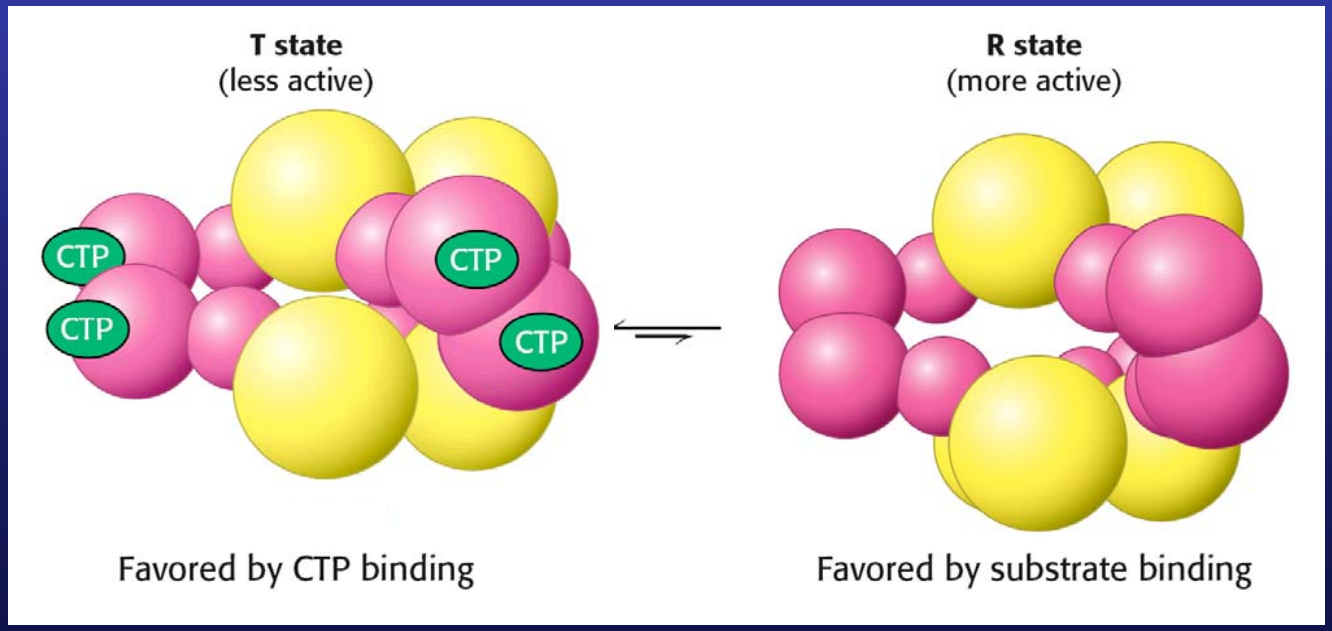


CTP:inhibidor alostérico


V_{max}
Normal activity (no CTP)
$\frac{1}{2} V_{max}$
CTP
V_0 (μM/min)
10
20
30
$K_{0.5}$ = 12 mM
$K_{0.5}$ = 23 mM
[Aspartate] (mM)

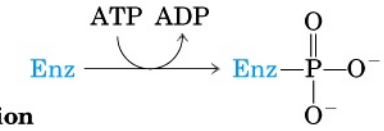
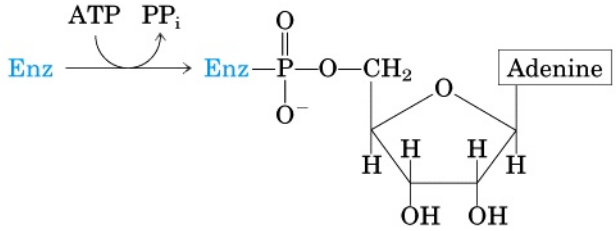
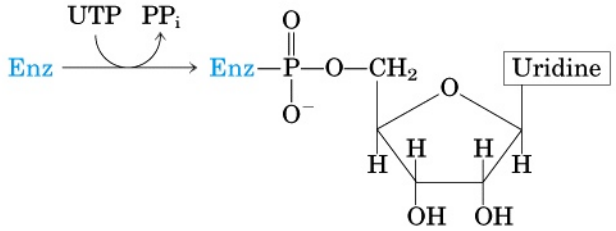
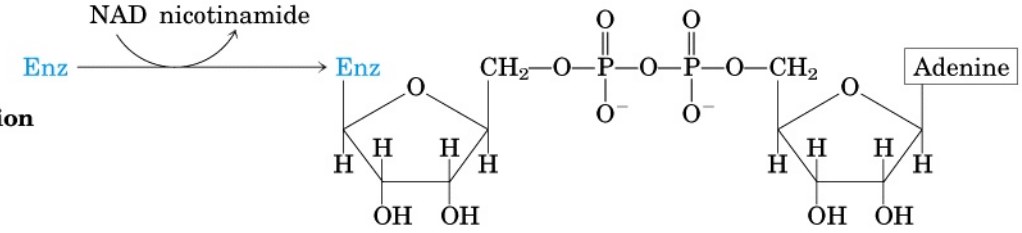
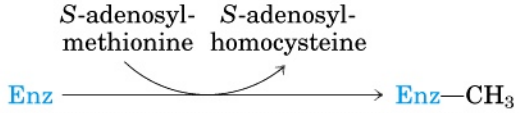


L-aspartato favorece el estado R

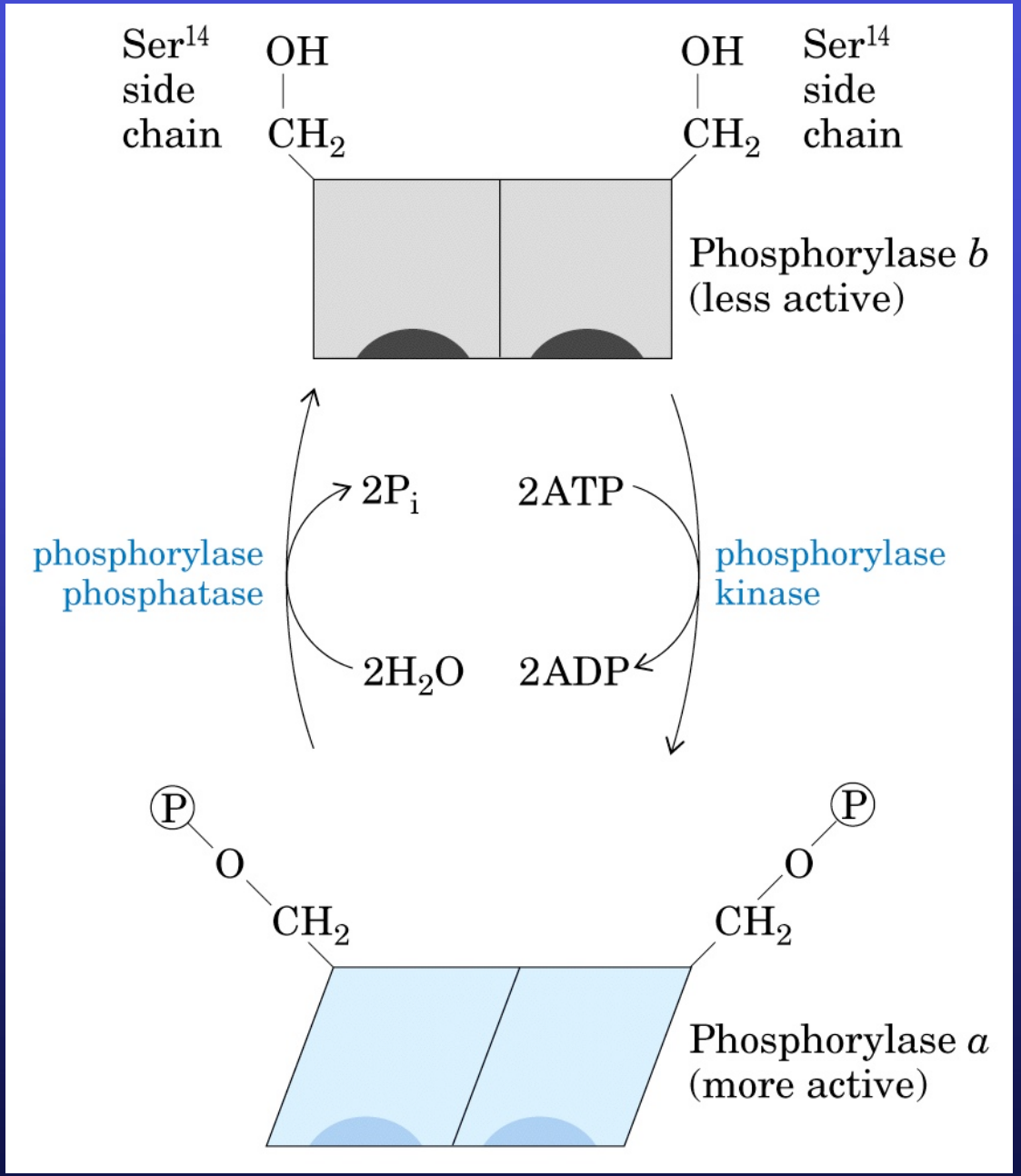


CTP favorece el estado T

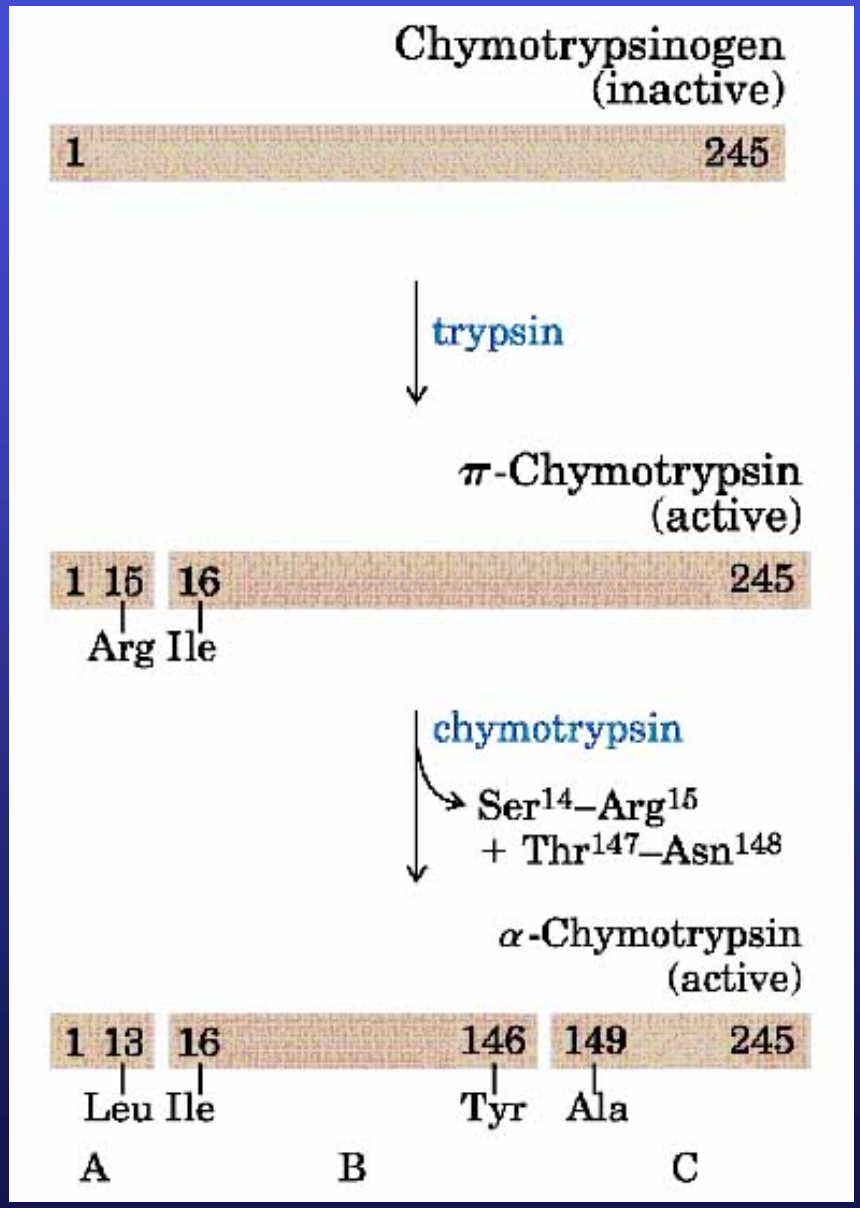
Regulación de la actividad de una enzima por modificación covalente

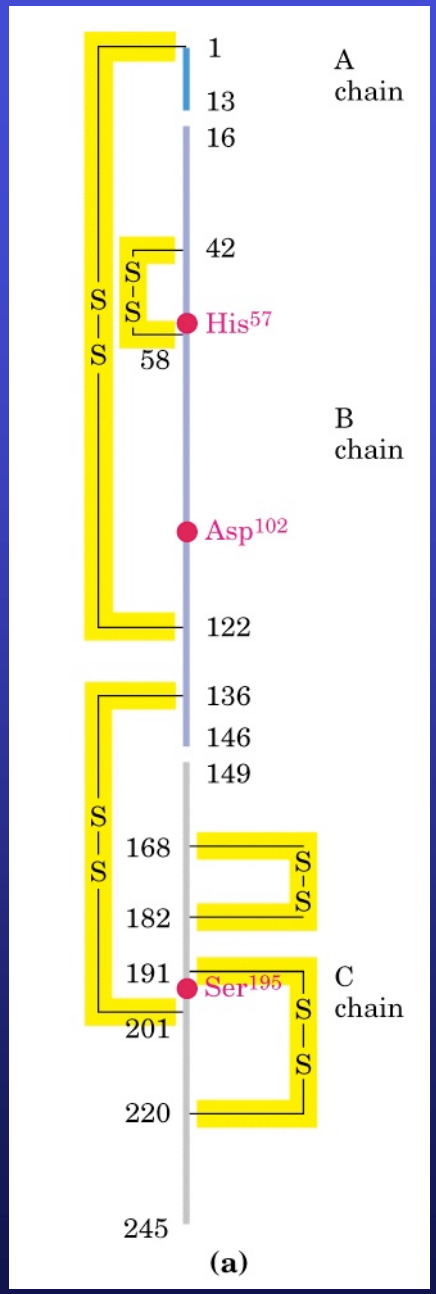
Covalent modification	Amino acid residues known to accept covalent modification
Phosphorylation 	Tyr, Ser, Thr, His
Adenylylation 	Tyr
Uridylylation 	Tyr
ADP-ribosylation 	Arg, Gln, Cys, diphthamide (a modified His)
Methylation 	Glu

fosforilación-desfosforilación

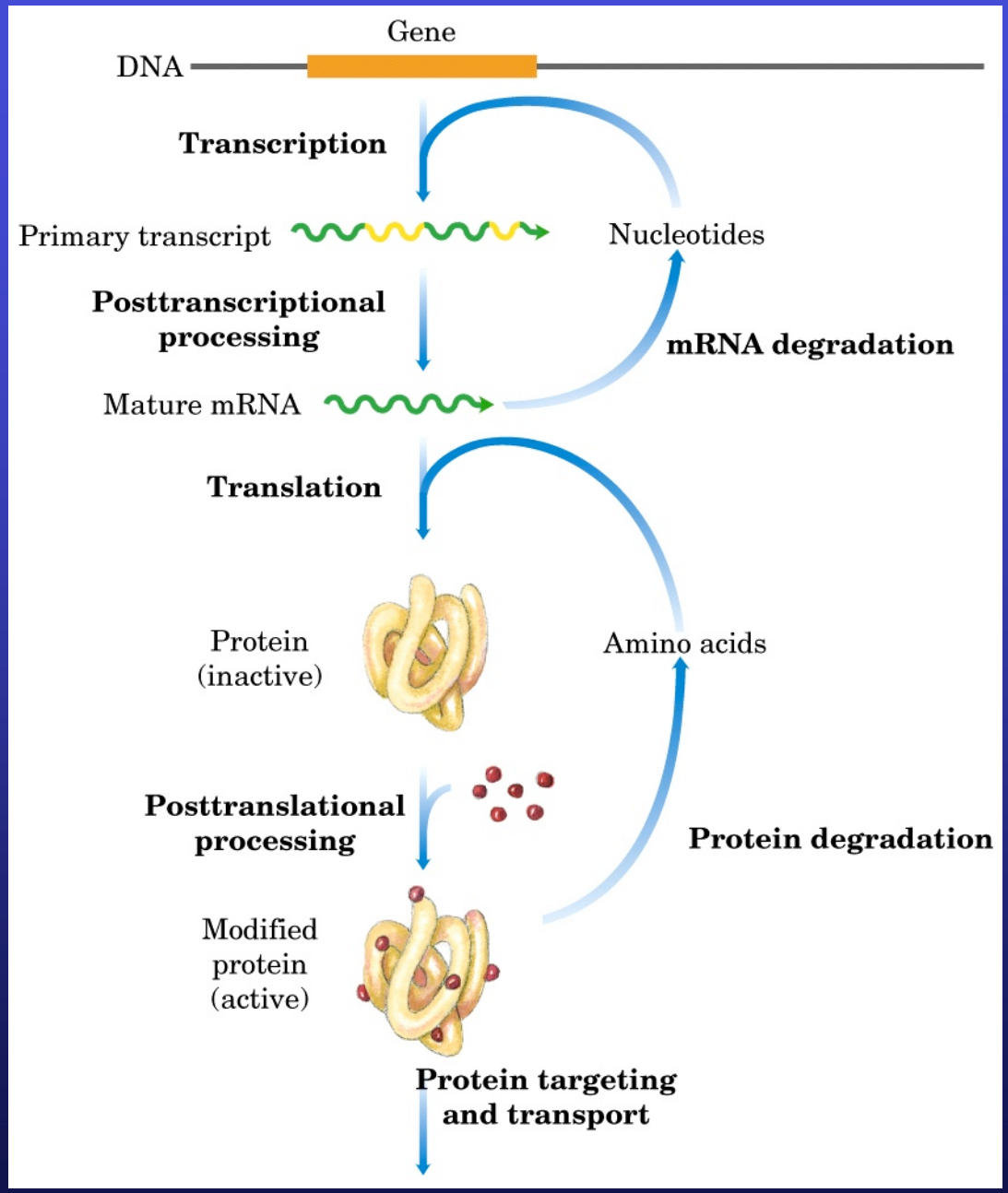


activación por digestión proteolítica

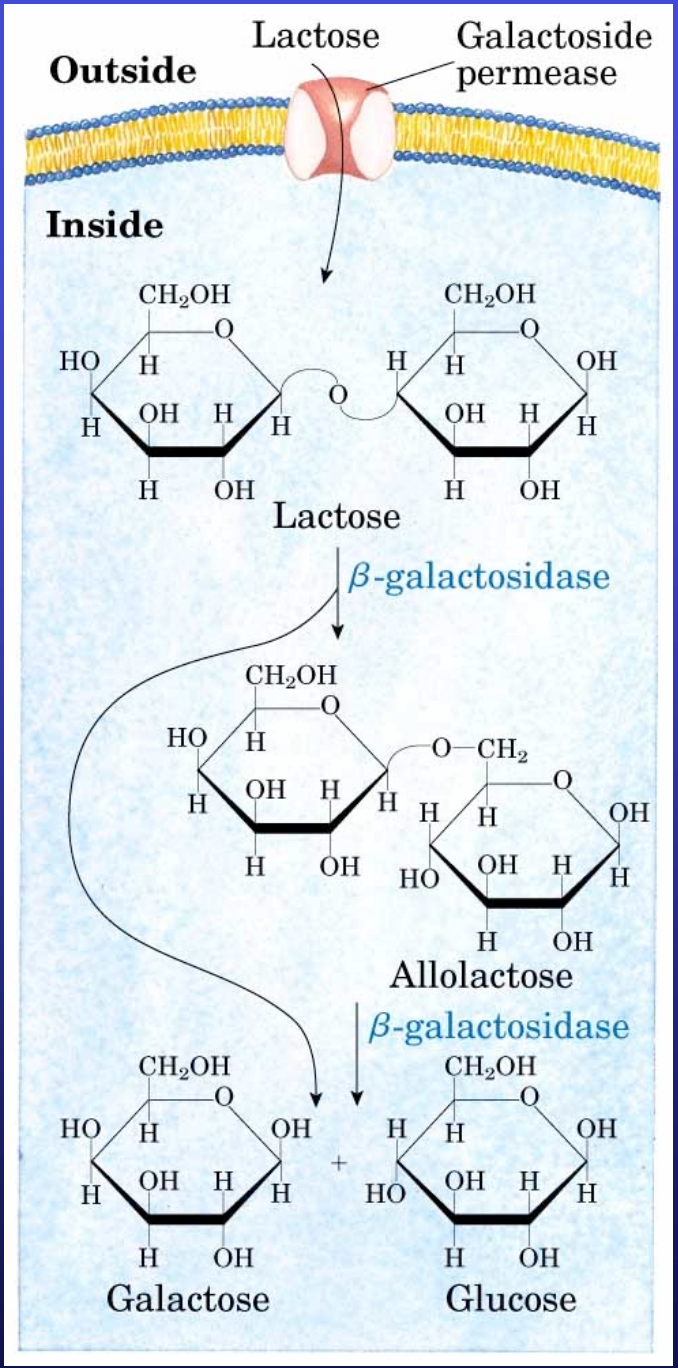


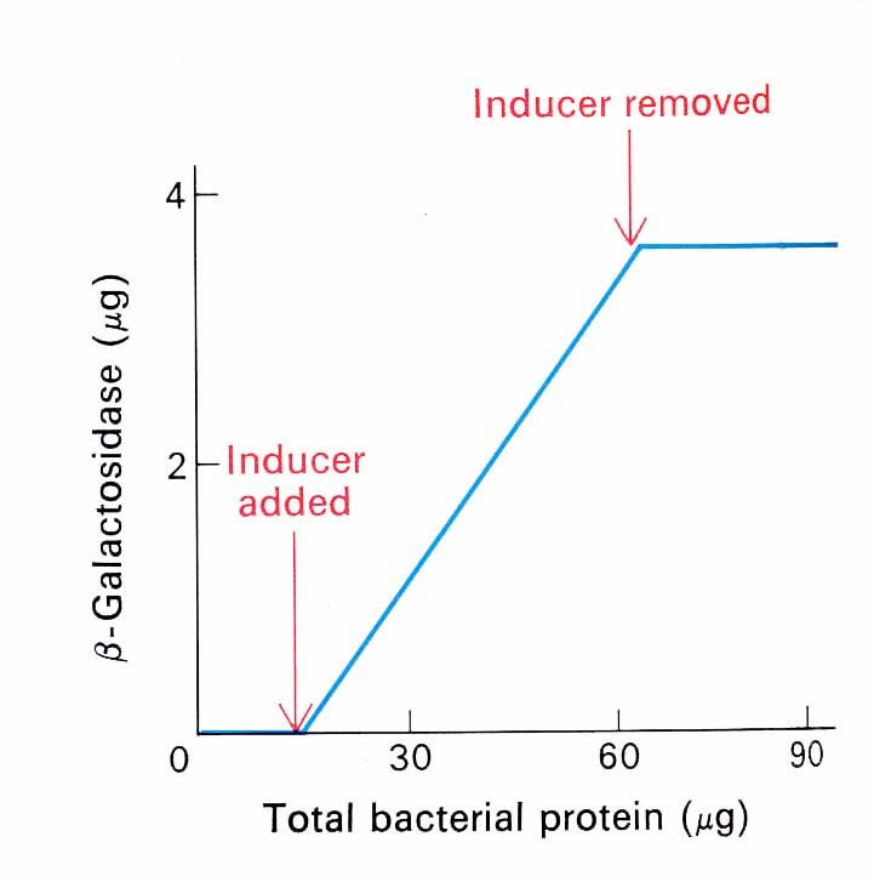


Regulación de la actividad enzimática por modificación de su concentración


Gene
DNA
Transcription
Primary transcript
Nucleotides
Posttranscriptional processing
mRNA degradation
Mature mRNA
Translation
Protein (inactive)
Amino acids
Posttranslational processing
Protein degradation
Modified protein (active)
Protein targeting and transport

Inducción Enzimática





FIN