Introducción a la Cinética Enzimática

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Orden de la reacción

$$v = -\frac{d[S]}{dt} = \frac{d[P]}{dt}$$

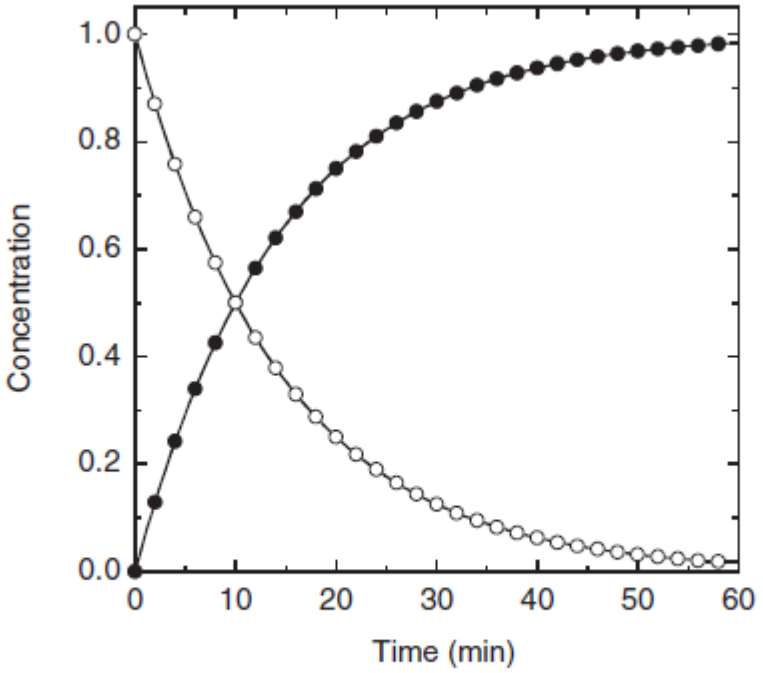


Figure A1.1 Progress curves for the first-order formation of product (*closed circles*) and the corresponding disappearance of reactant (*open circles*).

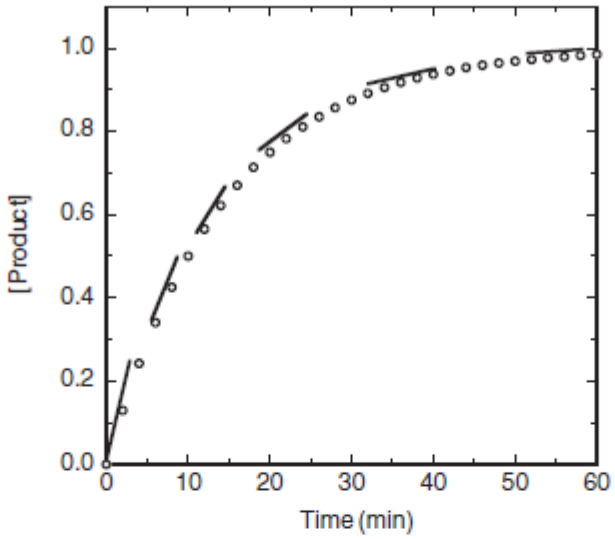


Figure A1.2 Determination of instantaneous velocity at various points in a reaction progress curve, from the slope of a tangent line drawn to a specific time point.

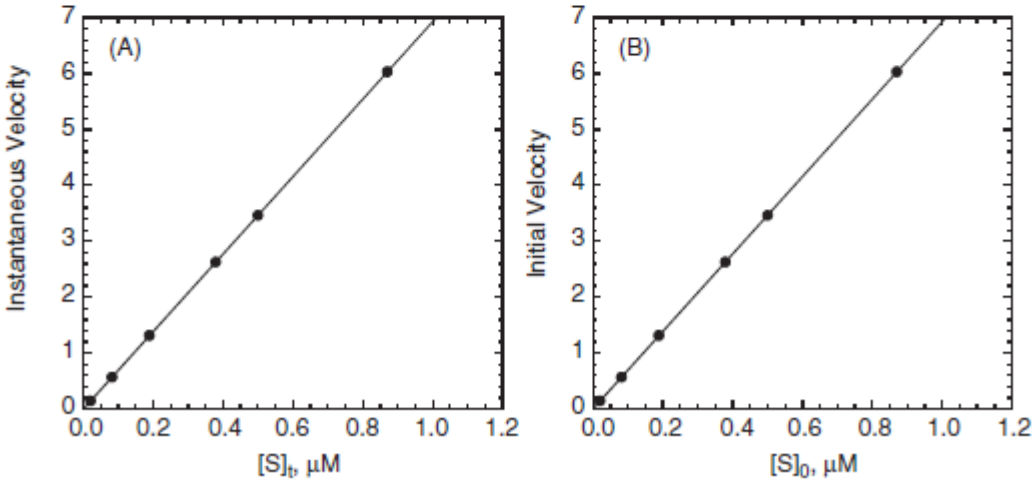


Figure A1.3 Linear relationship between (**A**) instantaneous velocity and $[S]_t$ and between (**B**) initial velocity and $[S]_0$ for a first-order reaction.

Ley de velocidad

$$v = \frac{-d[S]}{dt} = \frac{d[P]}{dt} = k[S]$$

k= cte de proporcionalidad

La velocidad de reacción es directamente proporcional a la conc. de reactivo

Ley de acción de masas

[S]t=conc. remanente S

[S]0=conc. inicial S

Orden uno

Disociación simple

$$EI \xrightarrow{k_{off}} E + I$$

k_{off}=cte. de disociación

$$\frac{d[EI]}{dt} = -k_{off}[EI]$$

Por la ley de acción de masas

Unidades:
k de primer orden
s^{-1}

$$\int \frac{d[EI]}{[EI]} = -k_{off} \int dt$$

$$\int \frac{d[EI]}{[EI]} = -k_{off} \int dt$$

$$[EI]_t = [EI]_0 e^{-k_{off}t}$$

t= ∞
[EI]→0

$$\ln\left(\frac{[EI]_t}{[EI]_0}\right) = -k_{off}t$$

t= 0
[P]=0
t= ∞
[P]→ $[EI]_0$

$$[P]_t = [EI]_0 - [EI]_t$$

$$[P]_t = [EI]_0 - [EI]_0 e^{-k_{off}t}$$

$$[P]_t = [EI]_0(1 - e^{-k_{off}t})$$

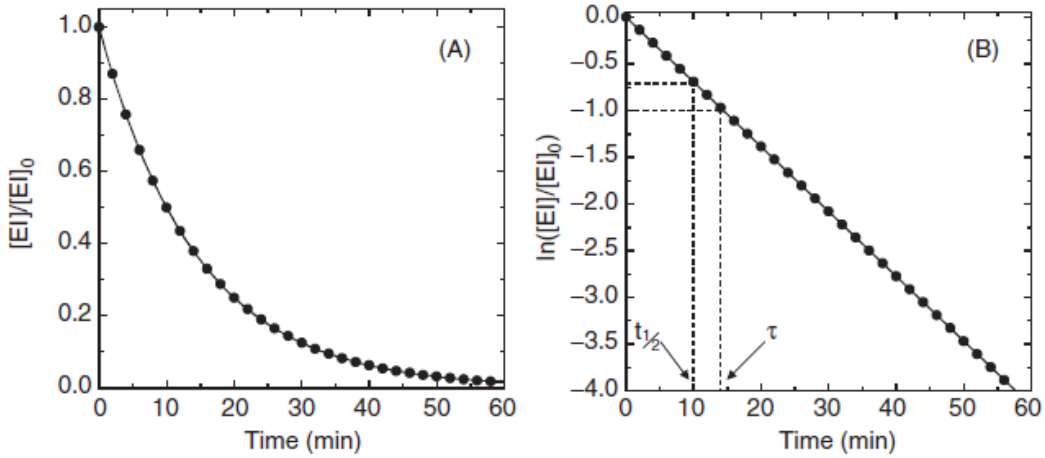


Figure A1.4 (A) Plot of the ratio $[EI]_t/[EI]_0$ as a function of time for a first-order dissociation reaction. (B) Plot of $\ln([EI]_t/[EI]_0)$ as a function of time for a first-order dissociation reaction. The time points corresponding to the relaxation time (τ) and $t_{1/2}$ are indicated on the semilog plot.

Orden dos

Asociación

$$E + I \xrightarrow{k_{on}} EI$$

$$\frac{d[EI]}{dt} = k_{on}[E][I]$$

$$\frac{d[EI]}{dt} = k_{on}([E]_0 - [EI])([I]_0 - [EI])$$

$$\frac{[E]_0([I]_0 - [EI])}{[I]_0([E]_0 - [EI])} = e^{([I]_0 - [E]_0)k_{on}t}$$

Difícil de aplicar en situaciones prácticas

Pseudo primer orden

Si [I]>>[E]

$$\frac{d[EI]}{dt} = k'[E] \qquad k' = k_{on}[I]$$

Reacción de orden uno en esas condiciones experimentales

k´= cte. de pseudo primer orden

$$[EI]_t = [E]_0(1 - e^{-k't}) = [E]_0(1 - e^{-k_{on}[I]t})$$

Unidades:
k de segundo orden
$M^{-1}s^{-1}$

Reacción trimolecular

$$\mathbf{A + B + C \rightarrow P}$$

$$\mathbf{A + B} \xrightarrow{k_1} \mathbf{X}$$

$$\mathbf{X + C \rightarrow P}$$

El orden de la reacción depende de la relación entre k_1 y k_2 y es igual al de la etapa más lenta determinante de la velocidad de reacción. Si no hay etapa determinante de la velocidad de reacción el orden es complejo

$$\mathbf{A + B} \leftrightarrow \mathbf{X}$$

$$\mathbf{X + C} \xrightarrow{k_2} \mathbf{P}$$

$$v = k_2[X][C]$$

$$[X] = K[A][B]$$

$$v = k_2 K[A][B][C]$$

K=cte. de equilibrio

k_2=cte. de vel de orden dos del segundo paso

La cte. observada de tercer orden es el producto de una cte. de equilibrio y una cte. de segundo orden

Unidades: $M^{-2}s^{-1}$

Orden cero

- La v de reacción es independiente de la conc. de reactivo
- El reactivo está presente en tal exceso que satura al catalizador

$$\frac{d[P]}{dt}=k$$

$$v=k$$

Unidades:
$M\,s^{-1}$

Aproximación al equilibrio: cinética de reacciones reversibles

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} EI$$

Cte. de asociación

$$K_a = \frac{[EI]}{[E][I]} = \frac{k_{on}}{k_{off}}$$

Cte. de disociación

$$K_d = \frac{[E][I]}{[EI]} = \frac{k_{off}}{k_{on}}$$

k_{on}=cte. de segundo orden ($M^{-1}s^{-1}$)
k_{off}= cte. de primer orden (s^{-1})

Unidades:
$K_a = M^{-1}$
$K_d = M$

$$v = \frac{d[EI]}{dt} = k_{on}[E][I] - k_{off}[EI]$$

$[I] >> [E]$ Condiciones de pseudo primer orden

$[EI] = 0 \quad t = 0$ $\qquad$ $[EI] = [EI]_{eq} \quad t = \infty$

La conc. de [EI] en cualquier punto:

$$[EI]_t = [EI]_{eq}(1 - e^{-(k_{on}[I] + k_{off})t})$$

$$[EI]_t = [EI]_{eq}(1 - e^{-k_{obs}t})$$

La conc. de [EI] en el equilibrio:

$$[EI]_{eq} = [E]_0\left(\frac{k'}{k' + k_{off}}\right) = [E]_0\left(\frac{k_{on}[I]}{k_{on}[I] + k_{off}}\right)$$

$$k_{obs} = k_{on}[I] + k_{off}$$

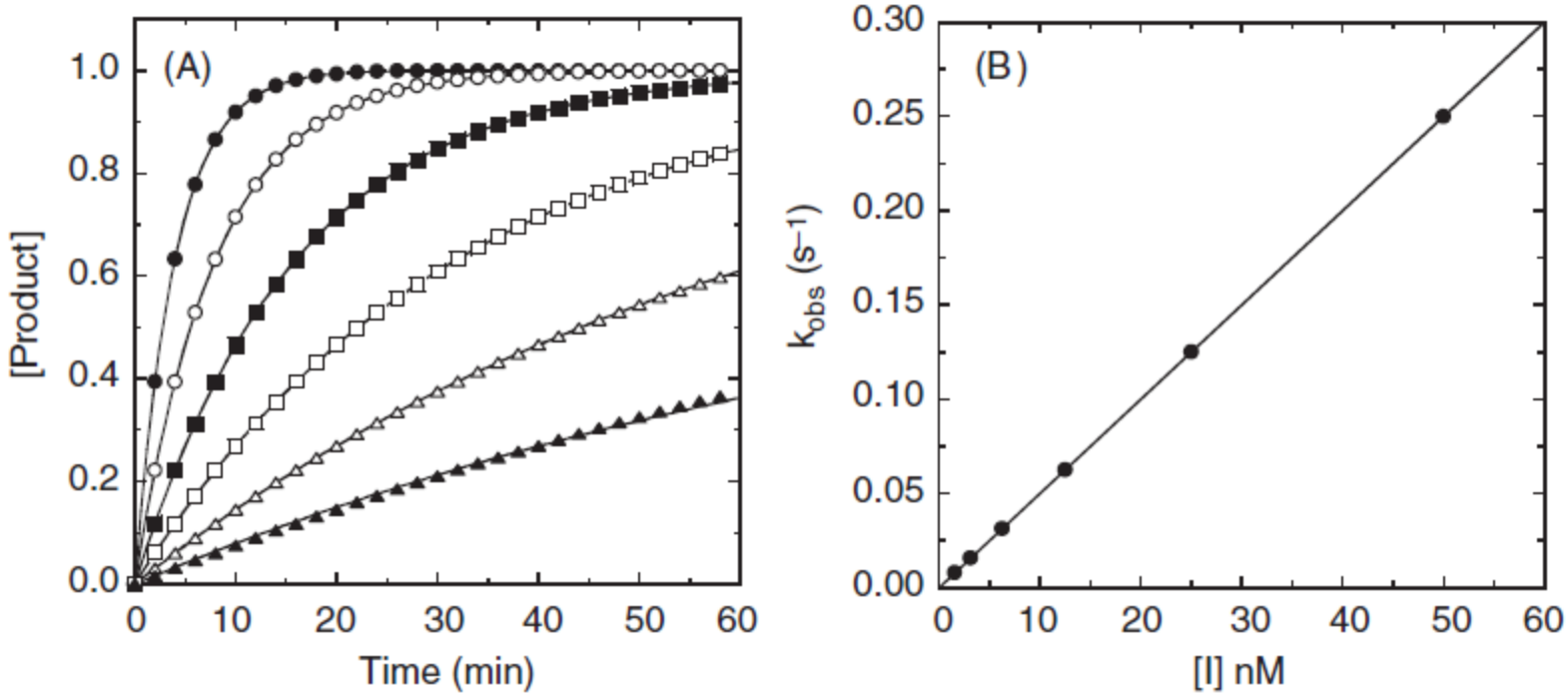
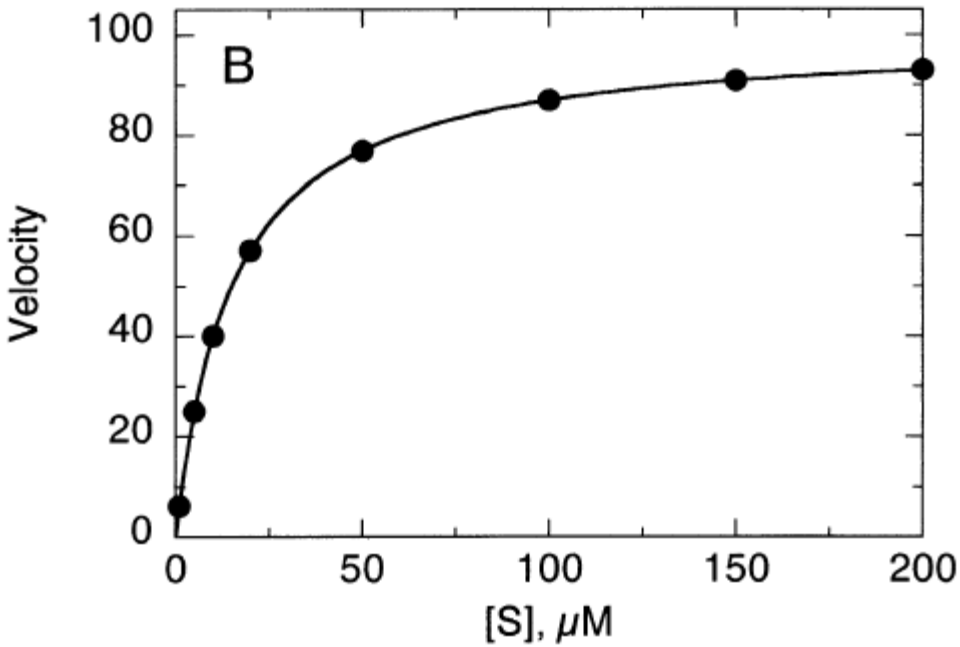


Figure A1.6 (**A**) Product (*EI*) formation as a function of time for a binding reaction run under pseudo–first-order conditions at varying concentrations of ligand ([*I*]). (**B**) Dependence of k_{obs} (from the fits of the curves in panel **A**) on inhibitor concentration ([*I*]) for a binding reaction run under pseudo–first-order conditions.

Velocidades iniciales.
Efecto de la [S] en la velocidad



Tres regiones
Bajo S: primer orden
Alto S: orden cero
Intermedio: dependencia curvilínea con [S]

Brown (1902):

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$v = k_2[ES]$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Modelo de equilibrio rápido

- Michaelis y Menten (1913): descripción matemática del modelo

Supuesto: Equilibrio rápido entre (E+S) y ES, seguido por una lenta conversión de ES a (E+P)

$$k_2 \ll k_{-1}$$

[S]>>[E]→ $[S]_f \approx [S]$

$$K_S = \frac{[E]_f[S]}{[ES]}$$ K_s=cte de disociación

$$[E]_f = [E] - [ES]$$

$$K_S = \frac{([E] - [ES])[S]}{[ES]}$$

$$[ES] = \frac{[E][S]}{K_S + [S]}$$

$$E + S \underset{K_S}{\rightleftharpoons} ES \xrightarrow{k_{cat}} E + P$$

k_{cat}: cte de primer orden

(tiene en cta. todos los eventos químicos para obtener E+P)

$$v = k_{cat}[ES] \qquad v = \frac{k_{cat}[E][S]}{K_S + [S]}$$

$$[S] \to \infty$$

$$V_{max} = k_{cat}[E]$$

Ecuación de Henri- Michaelis -Menten

$$v = \frac{V_{max}[S]}{K_S + [S]} = \frac{V_{max}}{1 + \frac{K_S}{[S]}}$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Modelo del estado estacionario

- En la mayoría de las condiciones experimentales [ES] es constante
- Briggs y Haldane (1925): aproximación al estado estacionario, no requiere $k_2 << k_{-1}$
- Estado estacionario: la velocidad de formación de ES es igual a la velocidad de decaimiento a E+P
- Asunciones:

1) Sólo se forma el complejo ES $[E] = [E]_f + [ES]$
2) $[S] \gg [E] \rightarrow [S]_f \sim [S]$
3) En la primera fase de la reacción: $[P] \sim 0$

ES cte $\frac{d[ES]}{dt} = 0$

Ejemplo

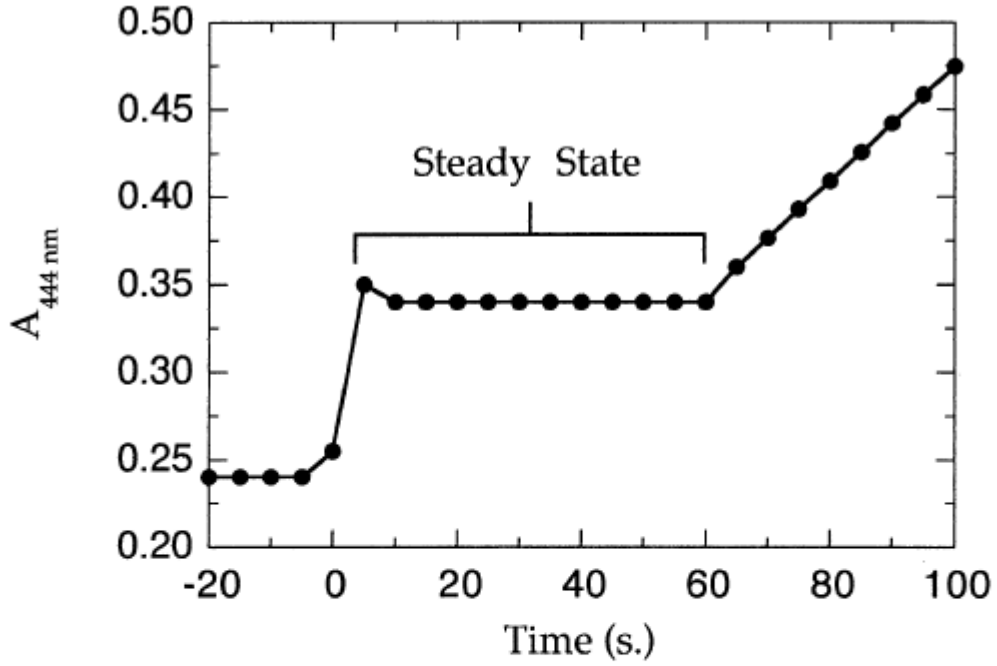


Figure 5.4 Development of the steady state for the reaction of cytochrome *c* oxidase with its substrates, cytochrome *c* and molecular oxygen. The absorbance at 444 nm reflects the ligation state of the active site heme cofactor of the enzyme. Prior to substrate addition (time < 0) the heme group is in the Fe^{3+} oxidation state and is ligated by a histidine group from the enzyme. Upon substrate addition, the active site heme iron is reduced to the Fe^{2+} state and rapidly reaches a steady state phase of substrate utilization in which the iron is ligated by some oxygen species. The steady state phase ends when a significant portion of the molecular oxygen in solution has been used up. At this point the heme iron remains reduced (Fe^{2+}) but is no longer bound to a ligand at its sixth coordination site; this heme species has a much larger extinction coefficient at 444 nm; hence the rapid increase in absorbance at this wavelength following the steady state phase. [Data adapted and redrawn from Copeland (1991).]

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad v = k_2[ES]$$

v de formación del complejo

$$\frac{d[ES]}{dt} = k_1[E]_f[S]_f$$

v de pérdida del complejo

$$\frac{-d[ES]}{dt} = (k_{-1} + k_2)[ES]$$

En condiciones de estado estacionario $\longrightarrow$ $k_1[E]_f[S]_f = (k_{-1} + k_2)[ES]$

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

$$[ES] = \frac{[E]_f[S]_f}{\frac{k_{-1} + k_2}{k_1}}$$

$$[S]_f \sim [S]$$

$$[E] = [E]_f + [ES]$$

$$[ES] = \frac{[E]_f[S]_f}{K_m}$$

$$[ES] = [E]\frac{[S]}{[S] + K_m}$$

$$v = k_2[E]\frac{[S]}{[S] + K_m}$$

$$v = k_{cat}[E]\frac{[S]}{[S] + K_m}$$

Caso general

$$\lim_{[S]\to\infty} \frac{[S]}{[S]+K_m} \cong \frac{[S]}{[S]} = 1 \qquad V_{max} = k_{cat}[E]$$

$$v = \frac{V_{max}[S]}{K_m + [S]} = \frac{V_{max}}{1 + \frac{K_m}{[S]}}$$

Ecuación diferente a la de equilibrio rápido

Igualmente llamada: Michaelis-Menten

$$K_m = \frac{k_{-1} + k_2}{k_1}$$ $=(s^{-1}+s^{-1})/s^{-1}M^{-1}$

Unidades: $K_m = M$

Si trabajamos a [S]=K_m

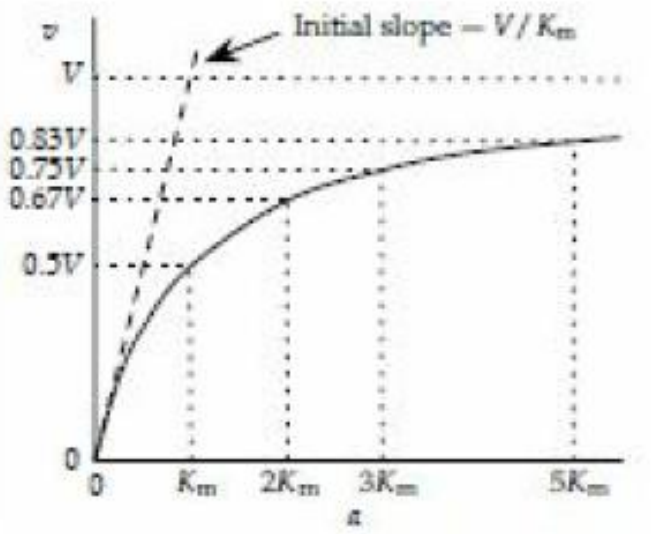
$$v = \frac{V_{max}[S]}{[S] + [S]} = \frac{V_{max}}{2}$$

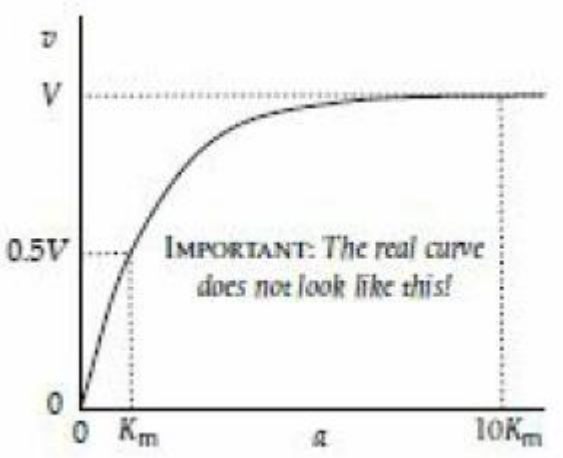
K_m= Constante de Michaelis

K_m: Conc. de S que provee una *v* que es la mitad de V_{max} obtenida a conc. saturantes de S

$$K_S = \frac{k_{-1}}{k_1} \qquad k_2 \ll k_{-1} \rightarrow$$ *K_m y K_s son equivalentes*

K_m= cte. cinética, no termodinámica


v
Initial slope = V / Km
V
0.83V
0.75V
0.67V
0.5V
0
0
Km
2Km
3Km
5Km
s


v
V
0.5V
0
0
Km
s
10Km
IMPORTANT: The real curve
does not look like this!

Relación entre K_m y K_s

K_s= afinidad real
K_m= afinidad aparente

1) Michaelis y Menten (un intermediario): $k_2 <<< k_{-1}$ $K_m \cong K_s$

$$K_m = \frac{k_{-1} + k_2}{k_1} \cong \frac{k_{-1}}{k_1} = K_S$$

2) Briggs y Haldane (1 intermediario) ; $K_S < K_m$

$$K_m = \frac{k_{-1} + k_2}{k_1} \cong \frac{k_{-1}}{k_1} + \frac{k_2}{k_1} = K_S + \frac{k_2}{k_1} > K_S$$

Subestima afinidad

3) Michaelis Menten (más de un intermediario): $K_S > K_M$

$$E + A \underset{K_S}{\leftrightarrow} EA \underset{K}{\leftrightarrow} EA' \xrightarrow{k_3} E + P$$

$$K_m = \frac{K_s K}{1+K} = \frac{K_s}{\frac{1}{K}+1} < K_s$$

Sobreestima afinidad

4) Briggs y Haldane (más de un intermediario)

$$E + A \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} EA \underset{k_{-2}}{\overset{k_2}{\leftrightarrow}} EA' \xrightarrow{k_3} E + P$$

$$v \cong \frac{[E]_0[A]\dfrac{k_2k_3}{k_{-2}+k_3+k_2}}{\dfrac{k_{-1}k_{-2}+k_{-1}k_3+k_2k_3}{k_1(k_{-2}+k_3+k_2)}+[A]}$$

Según los valores relativos de las constantes de velocidad de disociación de sustrato y de producto tendremos tres casos posibles:

$$K_m = \frac{k_{-1}k_{-2}+k_{-1}k_3+k_2k_3}{k_1(k_{-2}+k_3+k_2)} = K_s \frac{k_{-2}+k_3+\dfrac{k_2k_3}{k_{-1}}}{k_{-2}+k_3+k_2}$$

CASOS POSIBLES

a) $\dfrac{k_3}{k_{-1}} > 1 \quad K_m > K_s$

b) $\dfrac{k_3}{k_{-1}} = 1 \quad K_m = K_s$

c) $\dfrac{k_3}{k_{-1}} < 1 \quad K_m < K_s$

Significado de k_{cat} y K_m

- ***K_m***

Representa la [S] en la cual la mitad de los sitios activos de la E están saturados de S en el estado estacionario

$$E + A \underset{K_S}{\leftrightarrow} EA \underset{K}{\leftrightarrow} EA' \xrightarrow{k_3} E$$

$$v = [EA']k_3$$

$$\frac{v}{[E]_0} = \frac{[EA']k_3}{[E]+[EA]+[EA']}$$

$$\frac{v}{[E]_0} = \frac{\frac{[E][A]}{K_s K}k_3}{[E]+\frac{[E][A]}{K_s}+\frac{[E][A]}{K_s K}} = \frac{\frac{[A]}{K_S K}k_3}{1+\frac{[A]}{K_s}+\frac{[A]}{K_s K}} = \frac{[A]k_3}{K_s K+[A](1+K)}$$

$$K_m = \frac{K_s K}{1+K} = \frac{K_s}{\frac{1}{K}+1}$$

$$K_m = K_s \frac{K}{(1+K)} = \frac{[E][A]}{[EA]} \frac{1}{\frac{1}{K}+1} = \frac{[E][A]}{[EA]} \frac{1}{\frac{[EA']}{[EA]}+1} = \frac{[E][A]}{[EA]} \frac{[EA]}{[EA']+[EA]}$$

$$K_m = \frac{[E][A]}{]EA]} \frac{[EA]}{[EA']+[EA]} = \frac{[E][A]}{\sum [EA]}$$

K_m es una constante de seudoequilibrio que relaciona el producto de las concentraciones de sustrato y de enzima libre (capaz de unir sustrato) con la suma de las concentraciones de todas las formas de enzima que tienen unido sustrato o que son incapaces de unirlo de manera cinéticamente competente

- k_{cat}

- A pesar de que k_{cat} puede referirse a mas de un paso en el mecanismo, tiene las propiedades de una constante de velocidad de primer orden, definiendo la capacidad del complejo ES de formar P.

- También llamada "número de recambio", define el número de ciclos catalíticos ("turnovers") por unidad de tiempo, o el número de moléculas de S que una molécula de E puede convertir en P en una unidad de tiempo

- En el laboratorio se puede determinar k_{cat} midiendo velocidad bajo condiciones que [S]>>K_m, entonces v se aproxima a V_{max}

- Sin embargo, *in vivo*, [S]≈0.1-1.0K_m $\longrightarrow$[S]<<K_m ($[E]_f \approx [E]$) $\longrightarrow$ $v = \frac{k_{cat}}{K_m}[E][S]_f$

La v global está limitada por la difusión entre las moléculas de E y S (10^8-10^9 M^{-1} s^{-1})

k_{cat}: máxima velocidad a la cual una reacción enzimática puede proceder a una concentración fija de E e infinita disponibilidad de S

Cambios en k_{cat} reflejan pertubaciones que afectan los pasos químicos en la catálisis enzimática (mutaciones en la E, condiciones de la solución, identidad del S)

k_{cat} es la menor de todas las constantes de primer orden (determinante de la v)

k_{cat}/K_m

$$v = \frac{k_{cat}[E]_0[A]}{K_m + [A]} \quad (96)$$

$$K_m = \frac{[E][A]}{\sum[EA]} = \frac{[E][A]}{[E]_0 - [E]} \quad (97)$$

$$[E]_0 = [E] + \sum[EA] = [E] + \frac{[E][A]}{K_m} = [E]\left(1 + \frac{[A]}{K_m}\right) \quad (98)$$

Remplazando en **(96)**

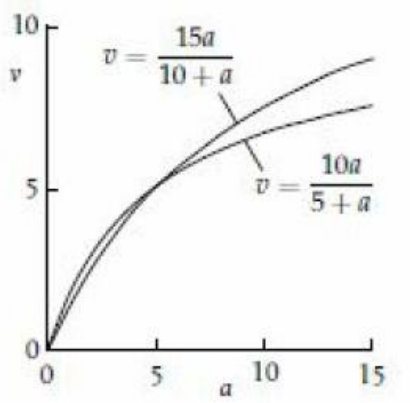
$$v = \frac{k_{cat}[E]\left(1 + \frac{[A]}{K_m}\right)[A]}{K_m + [A]} = \frac{k_{cat}[E]\left(1 + \frac{[A]}{K_m}\right)[A]}{\left(1 + \frac{[A]}{K_m}\right)K_m} = \frac{k_{cat}[E][A]}{K_m} = \frac{k_{cat}}{K_m}[E][A] \quad (99)$$

k_{cat}/K_M es una constante aparente de velocidad de segundo orden referida a las propiedades y a las reacciones de la enzima libre y del sustrato libre

constante de especificidad
eficiencia catalítica de la enzima libre

Si se comparan diferentes enzimas como catalizadores de un mismo S (ej. E y E mutada) pueden llegarse a conclusiones incorrectas. En el mismo valor de k_{cat}/K_m puede tener diferente proporción de velocidad a distintas [S] y la E con el mayor valor de k_{cat}/K_m podría tener v menores en algún rango de concentraciones

Figure 2.9. Inappropriateness of using k_{cat}/K_m alone to compare two enzymes as catalysts of the same reaction. The reaction catalyzed by the enzyme with the higher k_{cat}/K_m is faster at low substrate concentrations, but the reaction catalyzed by the enzyme with the higher k_{cat} is faster at high substrate concentrations.



k_{cat}/K_m para determinar **especificidad** de distintos S con una E

Algunas E muestran valores de k_{cat}/K_m de 10^8-10^9 $M^{-1}s^{-1}$ (límite de la difusión)

Perfección cinética: convierten S a P tan rápido como el S llega al sitio activo

El límite de la difusión es el límite superior de k_{cat}/K_m

Determinación experimental de K_m y V_{max}

- Se obtienen gráficamente las v_i a distintas concentraciones de S

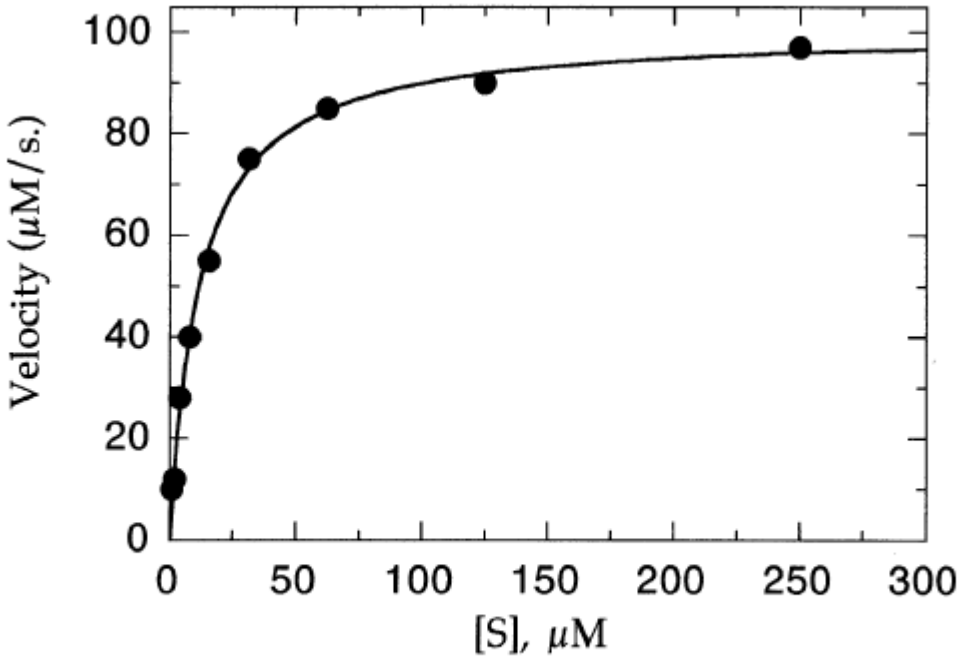
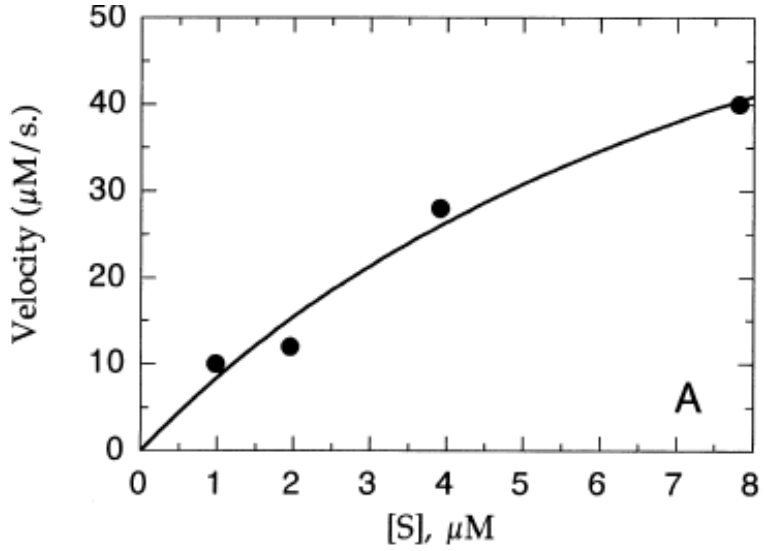
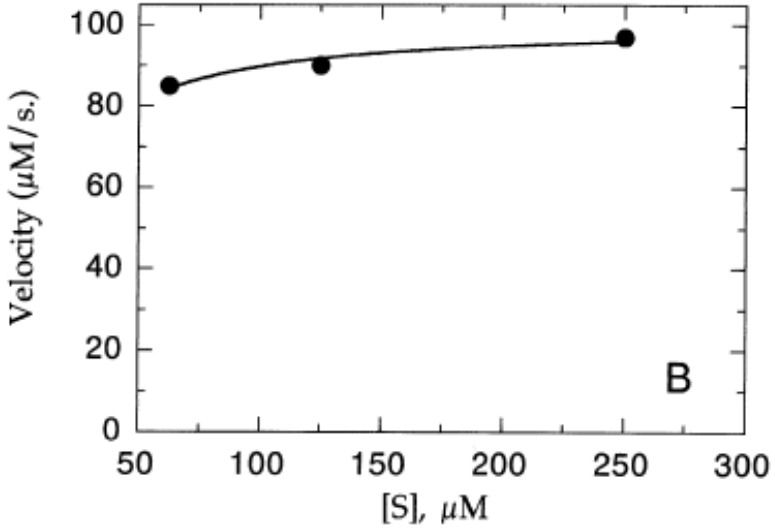


Figure 5.5 Michaelis–Menten plot for the velocity data in Table 5.1. The solid line through the data points represents the nonlinear least-squares best fit to Equation 5.24.



[S] muy baja



[S] muy elevada

Figure 5.6 Michaelis–Menten plots for restricted data from Table 5.1. (A) The range of [S] values is inappropriately low ($\leqslant 0.33K_m$), hence K_m and V_{max} appear to be infinite. (B) The range of [S] values is inappropriately high, with the result that every data point represents near-saturating conditions; one may be able to approximate V_{max}, but K_m cannot be determined.

Para comenzar a realizar estudios cinéticos…

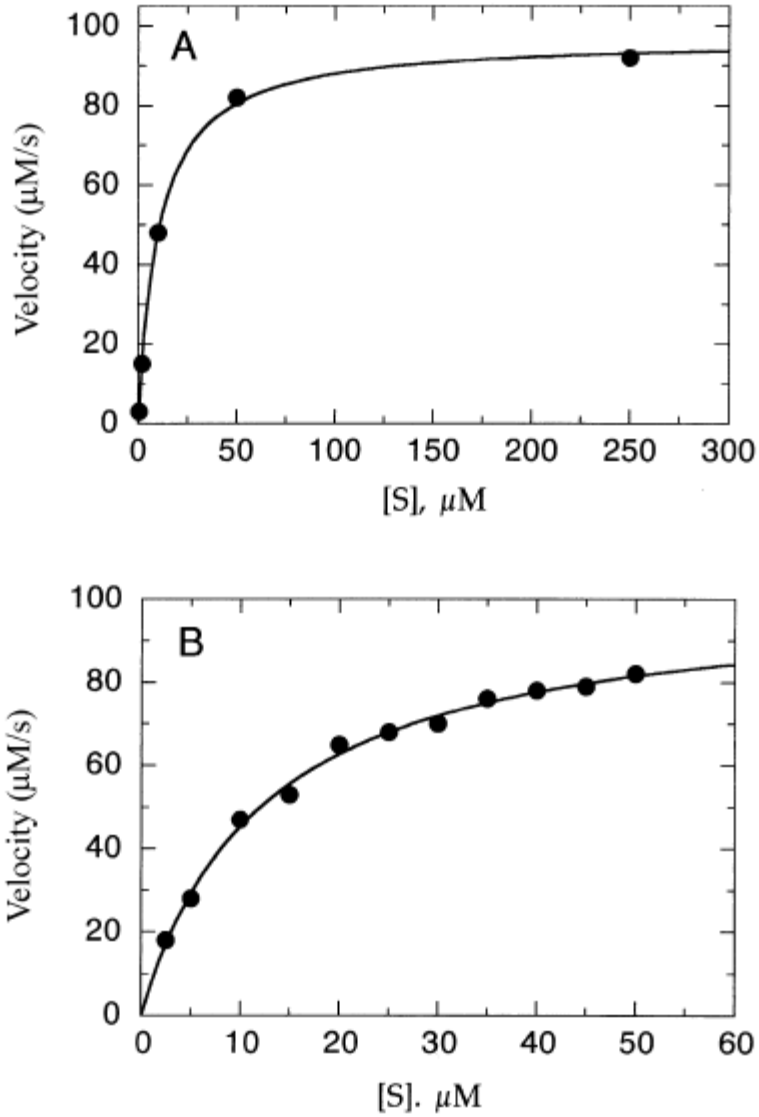


Figure 5.7 Experimental strategy for estimating K_m and V_{max}. (A) A limited data set is collected over a broad range of [S] to get a rough estimate of the kinetic constants. (B) Once a rough estimate of K_m has been determined, a second set of experiments is performed with more data within the range of 0.25–5.0K_m to obtain more precise estimates of the kinetic constants.

Plots de Lineaweaver-Burk

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

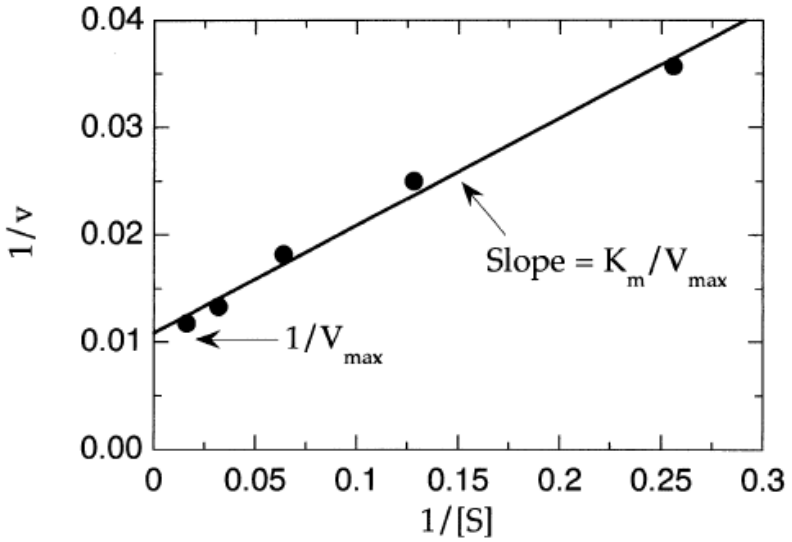


Figure 5.8 Lineweaver–Burk double-reciprocal plot for selected data from Table 5.1 within the range of [S] = 0.25–5.0K_m.

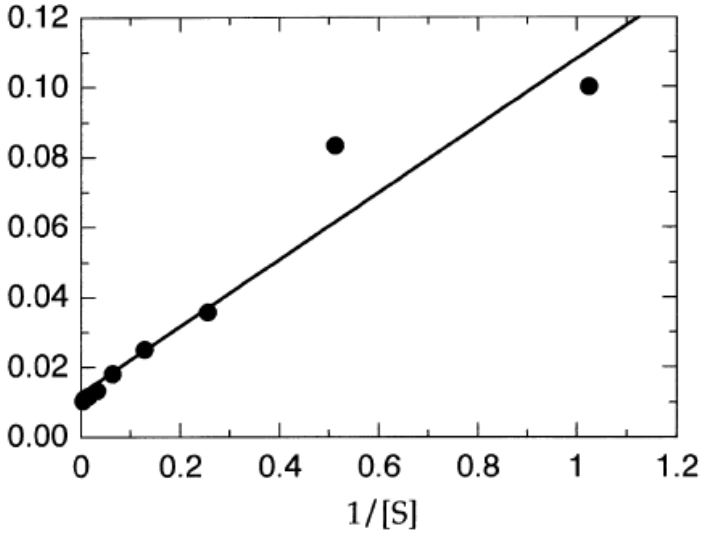


Figure 5.9 Lineweaver–Burk double-reciprocal plot for the full data set from Table 5.1. Note the strong influence of the data points at low [S] (high 1/[S] values) on the best fit line from linear regression.

Eadie-Hofstee plots

$$v = \frac{V_{max}[S]}{K_m + [S]} = \frac{V_{max}}{1 + \frac{K_m}{[S]}}$$

Multiplicando por $K_m + [S]$ y dividiendo por [S]

$$v = V_{max} - K_m\left(\frac{v}{[S]}\right)$$

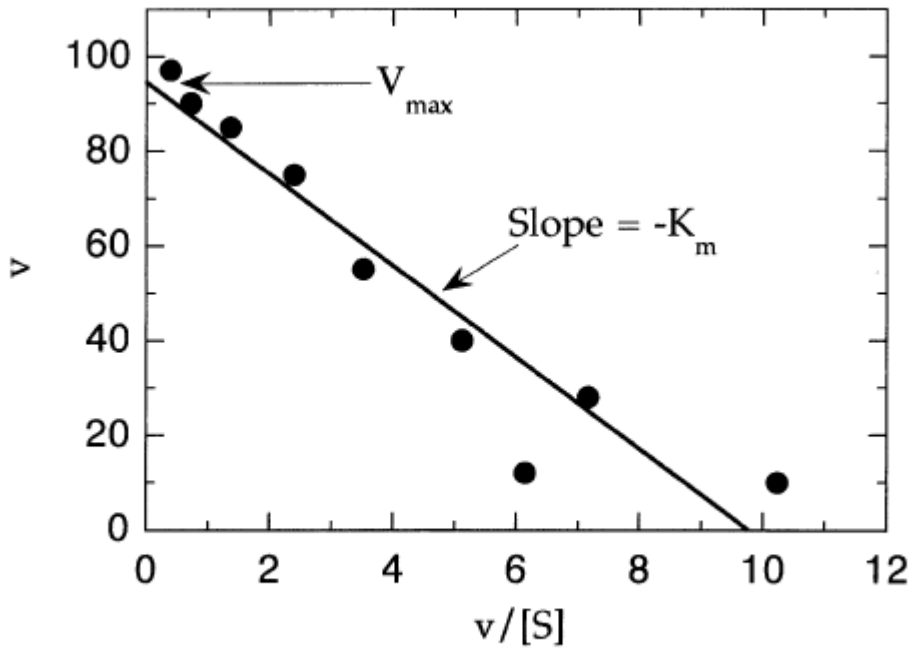


Figure 5.10 Eadie–Hofstee plot of enzyme kinetic data. Data taken from Table 5.1.

Hanes-Wolff plots

Multiplicando ambos miembros de la ecuación de Lineaweaver – Burk por [S]

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

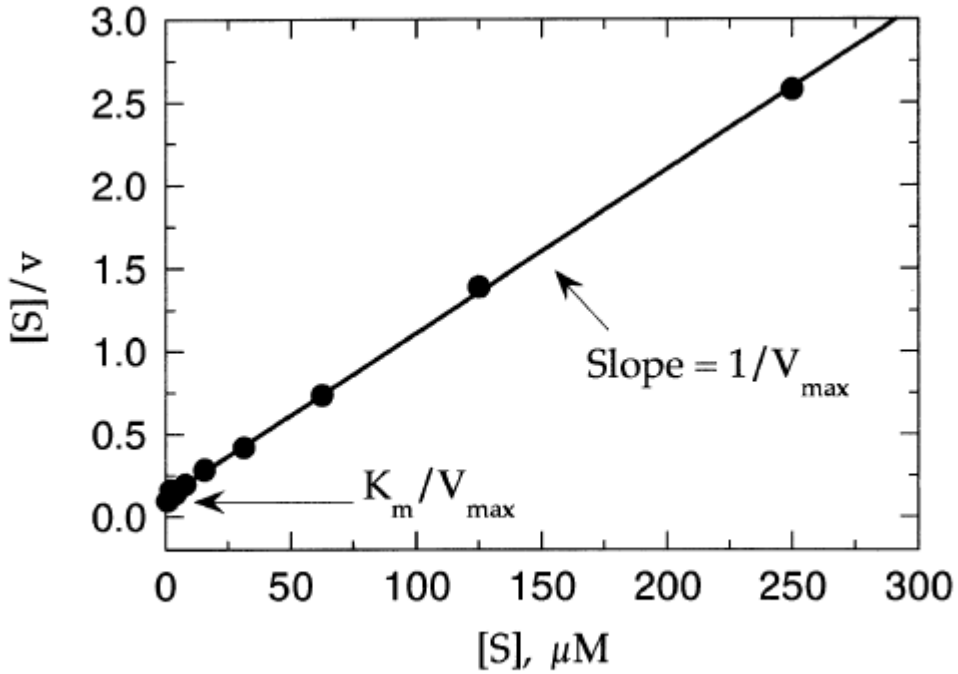
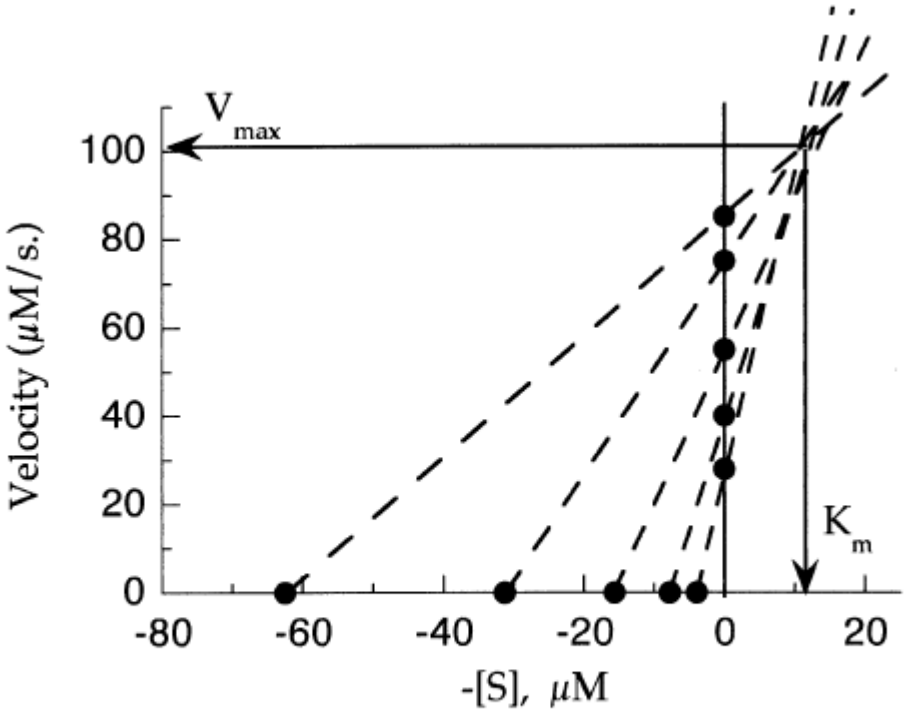


Figure 5.11 Hanes–Wolff plot of enzyme kinetic data. Data taken from Table 5.1.

Eisenthal–Cornish-Bowden Direct Plots



Se grafican los valores de ***v*** a lo largo del eje ***y*** y los valores de negativos de [S] a lo largo del eje ***x.***
Para cada par se unen los puntos de los dos ejes y se extrapolan las rectas hasta la intersección. Trazando Líneas rectas a los ejes se determinan K_m y V_{max}

Figure 5.12 Eisenthal–Cornish-Bowden direct plot of enzyme kinetic data. Selected data taken from Table 5.1.

Table 5.2 Estimates of the kinetic constants V_{max} and K_m from various graphical treatments of the data from Table 5.1

Graphical Method	$K_m(\mu M)$	Deviation from True $K_m(\%)$	$V_{max}(\mu M/s)$	Deviation from True $V_{max}(\%)$
True values	12.00		100.00	
Michaelis–Menten	11.63	3.08	100.36	0.36
Lineweaver–Burk (full data set)	7.57	36.92	79.28	20.72
Lineweaver–Burk ([S] = 0.25–5.0K_m only)	9.17	23.58	91.84	8.16
Eadie–Hofstee	9.66	19.50	94.45	5.55
Hanes–Wolff	11.84	1.33	100.97	0.97
Eisenthal–Cornish-Bowden	11.53	3.92	100.64	0.64

REACCION REVERSIBLE E INHIBICION POR PRODUCTO

$$E + A \underset{K_A}{\overset{}{\longleftrightarrow}} EA \underset{k_{-2}}{\overset{k_2}{\longleftrightarrow}} EP \overset{K_P}{\longleftrightarrow} E + P$$

$$v = k_2[EA] - k_{-2}[EP]$$

: k_2 k_{-2} gobiernan la reacción

$$\frac{v}{[E]_0} = \frac{k_2[EA] - k_{-2}[EP]}{[E] + [EA] + [EP]}$$

$$\frac{v}{[E]_0} = \frac{k_2\frac{[E][A]}{K_A} - k_{-2}\frac{[E][P]}{K_P}}{[E] + \frac{[E][A]}{K_A} + \frac{[E][P]}{K_P}}$$

$$\frac{v}{[E]_0} = \frac{k_2\frac{[A]}{K_A} - k_{-2}\frac{[P]}{K_P}}{1 + \frac{[A]}{K_A} + \frac{[P]}{K_P}} = \frac{k_2[A] - k_{-2}\frac{K_A}{K_P}[P]}{K_A + [A] + \frac{K_A}{K_P}[P]}$$

$$\frac{v}{[E]_0} = \frac{k_2[A] - k_{-2}\frac{K_A}{K_P}[P]}{K_A\left(1 + \frac{[P]}{K_P}\right) + [A]}$$

Si la transformación química (segundo paso) es irreversible La acumulación de P hace que la E quede secuestrada en EP y P se comporta como inh. competitivo

$$\frac{v}{[E]_0} = \frac{k_2[A] - \frac{k_{-2}k_{-1}k_{-3}}{k_1k_3}[P]}{K_A\left(1 + \frac{[P]}{K_P}\right) + [A]} = \frac{k_2\left([A] - \frac{k_{-2}k_{-1}k_{-3}}{k_2k_1k_3}[P]\right)}{K_A\left(1 + \frac{[P]}{K_P}\right) + [A]}$$

$$\frac{v}{[E]_0} = \frac{k_2\left([A] - \frac{[P]}{K_{eq}}\right)}{K_A\left(1 + \frac{[P]}{K_P}\right) + [A]}$$

$K_A\left(1 + \frac{[P]}{K_P}\right)$ K_m^{app} aumenta con [P]

Reacciones de múltiples sustratos

Table 11.1 General nomenclature for enzymatic reactions

Reaction	Name
$A \longrightarrow P$	Uni uni
$A + B \longrightarrow P$	Bi uni
$A + B \longrightarrow P_1 + P_2$	Bi bi
$A + B + C \longrightarrow P_1 + P_2$	Ter bi
⋮	⋮

$$E + AX + B \rightleftharpoons E + A + BX$$

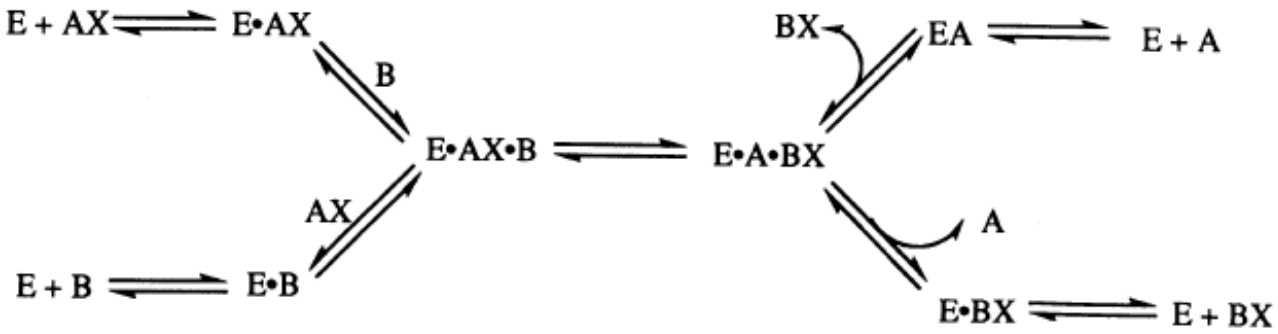
Bi Bi

Tres mecanismos: Random ordenado
Ordenado
Doble desplazamiento o Ping Pong

11.2 Bi Bi REACTION MECHANISMS

11.2.1 Random Ordered Bi Bi Reactions

In the random ordered bi bi mechanism, either substrate can bind first to the enzyme, and either product can leave first. Regardless of which substrate binds first, the reaction goes through an intermediate ternary complex (E · AX · B), as illustrated:



$$v = k_{cat}[E \cdot AX \cdot B] = \frac{k_{cat}[E_t][E \cdot AX \cdot B]}{[E] + [E \cdot AX] + [E \cdot B] + [E \cdot AX \cdot B]}$$

the binding of AX to the free enzyme (E) is described by the dissociation constant K^{AX}
binding of B to E is likewise described by K^{B}
the binding of AX to the preformed E · B complex is described by the constant αK^{AX}
binding of B to the preformed E · AX complex is described by αK^{B}
When B is saturating, the value of αK^{AX} is equal to K_m^{AX}
when AX is saturating, $\alpha K^{B} = K_m^{B}$

$$v = \frac{V_{max}[AX][B]}{\alpha K^{AX} K^{B} + \alpha K^{B}[AX] + \alpha K^{AX}[B] + [AX][B]}$$

when [B] is fixed and [AX] varies

$$v = \frac{V_{max}[AX]}{\alpha K^{AX}\left(1 + \frac{K^{B}}{[B]}\right) + [AX]\left(1 + \frac{\alpha K^{B}}{[B]}\right)}$$

At high, fixed concentrations of B, the terms $K^{B}/[B]$ and $\alpha K^{B}/[B]$ go to zero. Thus, at saturating concentrations of B we find:

$$v = \frac{V_{max}^{app}[AX]}{K_{m}^{AX,app} + [AX]} \tag{11.4}$$

and likewise, at fixed, saturating [AX]:

$$v = \frac{V_{max}^{app}[B]}{K_{m}^{B,app} + [B]} \tag{11.5}$$

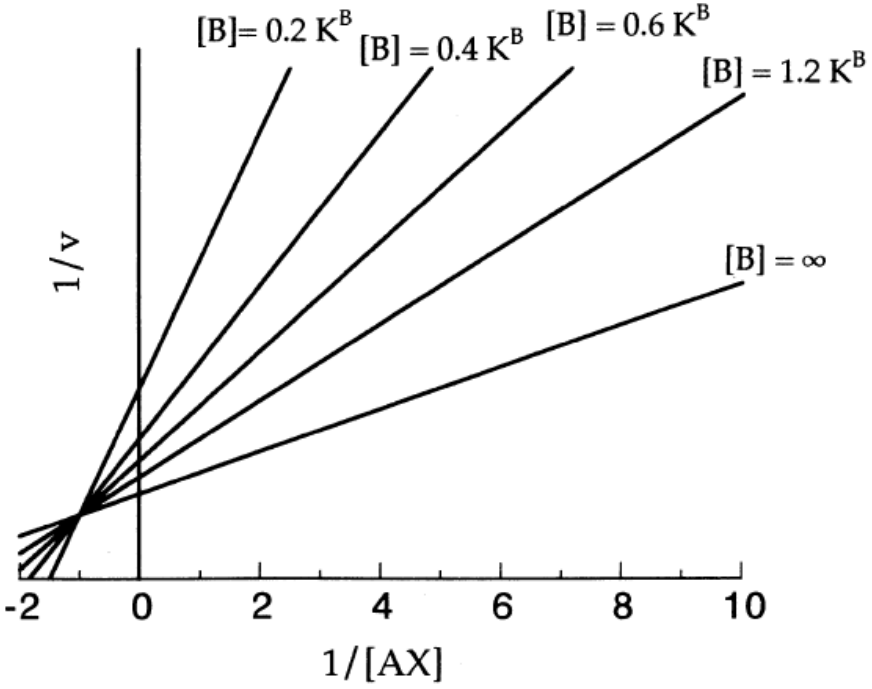


Figure 11.1 Double-reciprocal plot for a random ordered bi bi enzymatic reaction.

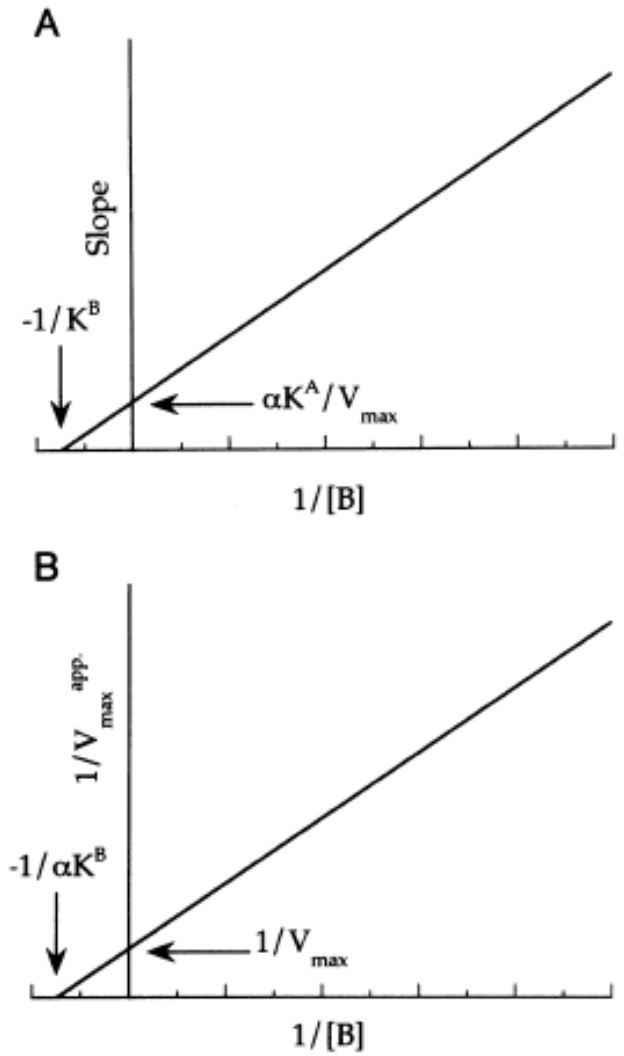


Figure 11.2 (A) Slope and (B) *y*-intercept replots of the data from Figure 11.1, illustrating the graphical determination of $K^{A:X}$, K^{B}, and V_{max} for a random ordered bi bi enzymatic reaction.

11.2.2 Compulsory Ordered Bi Bi Reactions

In compulsory ordered bi bi reactions, one substrate, say AX, must bind to the enzyme before the other substrate (B) can bind. As with random ordered reactions, the mechanism proceeds through formation of a ternary intermedi-

$$E + AX \rightleftharpoons E \cdot AX \overset{B}{\rightleftharpoons} E \cdot AX \cdot B \rightleftharpoons E \cdot A \cdot BX \overset{-BX}{\rightleftharpoons} E \cdot A \rightleftharpoons E + A$$

If conversion of the $E \cdot AX \cdot B$ complex to $E \cdot A \cdot BX$ is the rate-limiting step in catalysis, then E, AX, B, and $E \cdot AX \cdot B$ are all in equilibrium, and the velocity of the reaction will be given by:

$$v = \frac{V_{max}[AX][B]}{K^{AX}K^{B} + K^{B}[AX] + [AX][B]} \tag{11.6}$$

If, however, the conversion of $E \cdot AX \cdot B$ to $E \cdot A \cdot BX$ is as rapid as the other steps in catalysis, steady state assumptions must be used in the derivation of the velocity equation. For a compulsory ordered bi bi reaction, the steady state treatment yields Equation 11.7:

$$v = \frac{V_{max}[AX][B]}{K^{AX}K^{B}_{m} + K^{B}_{m}[AX] + K^{AX}_{m}[B] + [AX][B]} \tag{11.7}$$

As we have described before, the term K^{AX} in Equation 11.7 is the dissocation constant for the $E \cdot AX$ complex, and K^{AX}_{m} is the concentration of AX that yields a velocity of half V_{max} at fixed, saturating [B].

one cannot distinguish between random and compulsory ordered bi bi mechanisms on the basis of reciprocal plots alone

11.2.3 Double Displacement or Ping-Pong Bi Bi Reactions

The double displacement, or Ping-Pong, reaction mechanism involves binding of AX to the enzyme and transfer of the group, X, to some site on the enzyme. The product, A, can then leave and the second substrate, B, binds to the E–X form of the enzyme (in this mechanism, B cannot bind to the free enzyme form). The group, X, is then transferred (i.e., the second displacement reaction) to the bound substrate, B, prior to the release from the enzyme of the final product, BX. This mechanism is diagrammed as follows:

$$\mathrm{E} + \mathrm{AX} \rightleftharpoons \mathrm{E}\cdot\mathrm{AX} \rightleftharpoons \mathrm{EX}\cdot\mathrm{A} \overset{-\mathrm{A}}{\rightleftharpoons} \mathrm{EX} \overset{\mathrm{B}}{\rightleftharpoons} \mathrm{EX}\cdot\mathrm{B} \rightleftharpoons \mathrm{E}\cdot\mathrm{BX} \rightleftharpoons \mathrm{E} + \mathrm{BX}$$

Using steady state assumptions, the velocity equation for a double-displacement reaction can be obtained:

$$v = \frac{V_{\max}[\mathrm{AX}][\mathrm{B}]}{K_{\mathrm{m}}^{\mathrm{B}}[\mathrm{AX}] + K_{\mathrm{m}}^{\mathrm{AX}}[\mathrm{B}] + [\mathrm{AX}][\mathrm{B}]} \tag{11.8}$$

If we fix the value of [B], then Equation 11.8 for variable [AX] becomes:

$$v = \frac{V_{\max}[\mathrm{AX}]}{K_{\mathrm{m}}^{\mathrm{AX}} + [\mathrm{AX}]\left(1 + \dfrac{K_{\mathrm{m}}^{\mathrm{B}}}{[\mathrm{B}]}\right)} \tag{11.9}$$

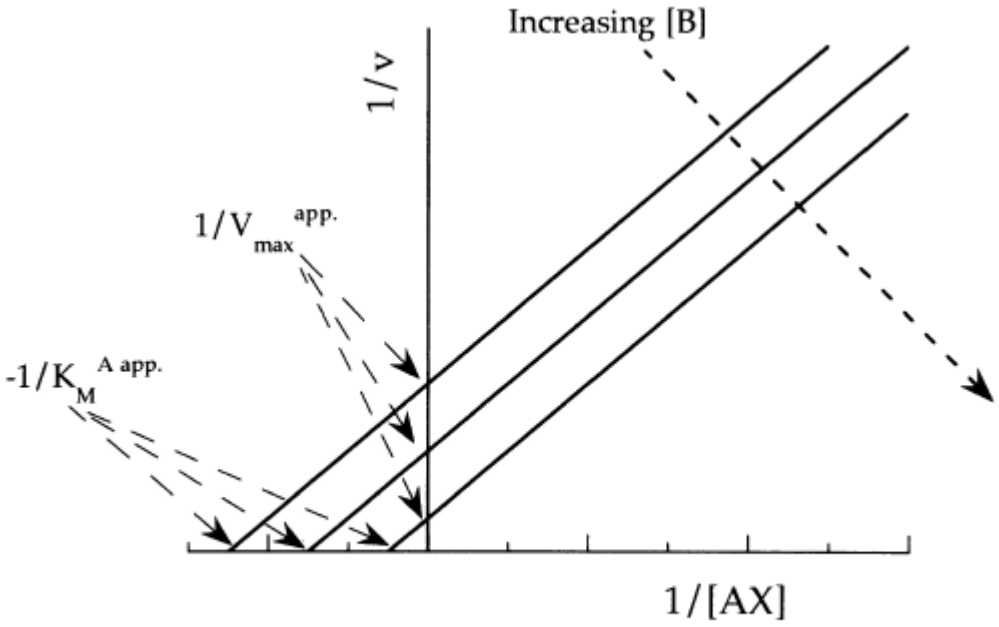


Figure 11.3 Double-reciprocal plot for a double-displacement (Ping-Pong) bi bi enzymatic reaction.

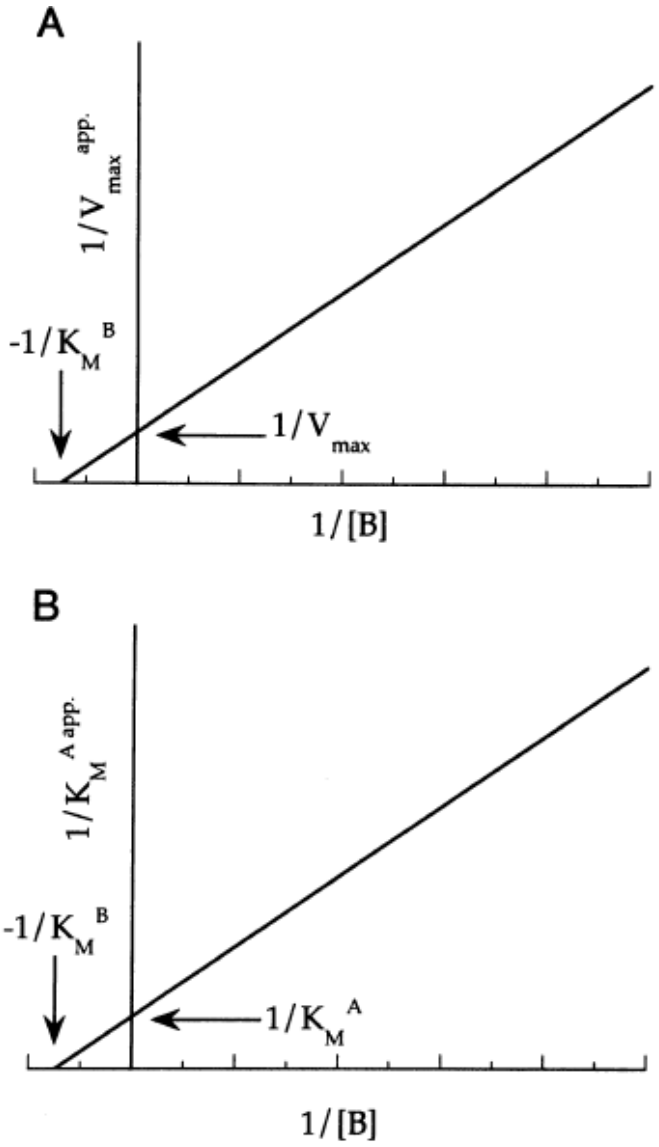


Figure 11.4 Replots of the data from Figure 11.3 as (A) $1/V_{max}^{app}$ versus 1/[B] and (B) $1/K_m^{AX,app}$ versus 1/[B], illustrating the graphical determination of K_m^{AX}, K_m^B, and V_{max} for a double-displacement (Ping-Pong) bi bi enzymatic reaction.

Bibliografía: