Michaelis-Menten theory of enzyme action

CH102 Spring 2014
Boston University

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Enzyme kinetics

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Enzyme action due to enzyme-substrate complex

An enzyme, E, catalyzes conversion of substrate, S, to product, P.

1. Substrate binds to the enzyme
2. Substrate is converted to product
3. Product is released from the enzyme

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Enzyme action due to enzyme-substrate complex

The binding is **fast**: **E + S ⇄ ES**

The conversion to product and its release is **slow**: **ES → E + P**

The rate of formation of product is the rate of the second, slow step

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Enzymes catalyze biochemical reactions

Typical enzyme concentrations, [E], are **nmol/L**

Typical substrate concentrations, [S], are **much larger**, **mmol/L**.

By limiting measurements to **initial rate, V_0**, of formation of product, substrate concentration nearly constant at its **initial value, $[S]_0$**.

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Enzymes catalyze biochemical reactions

Carbonic anhydrase, E, catalyzes hydration of $CO_2(aq)$, S, to $HCO_3^-(aq)$, P.

Sketch how you imagine **initial rate, V_0**, depends on the **initial substrate concentration, $[S]_0$**.

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Enzymes catalyze biochemical reactions

$[S]_0$ mmol/L	V_0 mmol/(L s)	$[S]_0$ mmol/L	V_0 mmol/(L s)
0.100	0.00248	5.00	0.083
0.250	0.00610	10.0	0.125
0.500	0.0119	15.0	0.150
1.00	0.0227	20.0	0.167
2.50	0.0500	25.0	0.179

Plot **initial rate, V_0**, versus **initial substrate concentration, $[S]_0$**.

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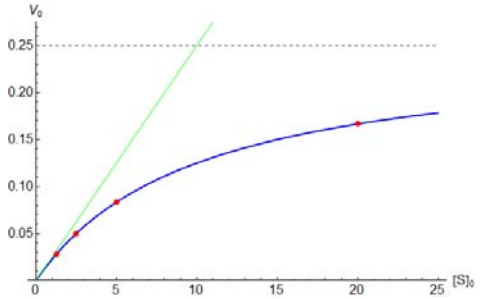
Enzymes catalyze biochemical reactions

The **red dots** are the initial rate, V_0, of formation of bicarbonate, in mmol/L/s, versus initial concentration, $[S]_0$, of CO_2, in mmol/L, at pH = 7.1, 273.5 K, and an enzyme concentration of 2.3 nmol/L.



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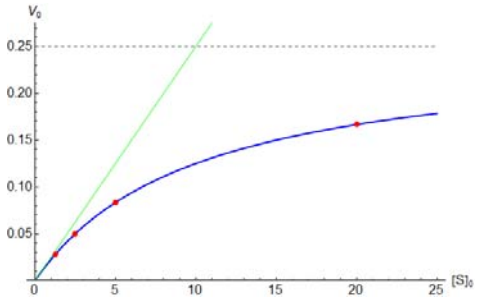
Enzymes catalyze biochemical reactions

The **green line** shows that for **very small [CO_2]** , the rate of formation of bicarbonate is **first order in [CO_2]**.



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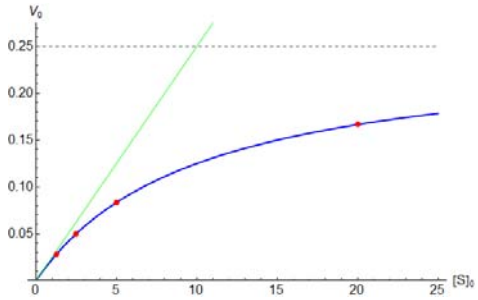
Enzymes catalyze biochemical reactions

As [CO_2] becomes larger, however, the rate of formation of bicarbonate **progressively slows**.



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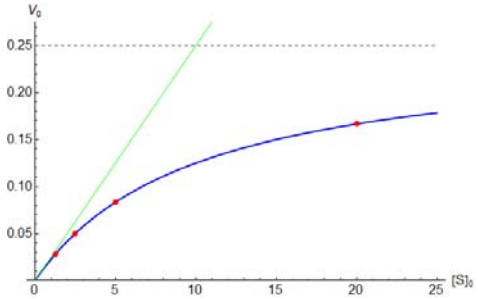
Enzymes catalyze biochemical reactions

The **blue curve** is a fit to the observed data. The dashed line is the asymptote of the fit. It is the maximum rate, V_{max}, possible rate of formation of bicarbonate for the given enzyme concentration.



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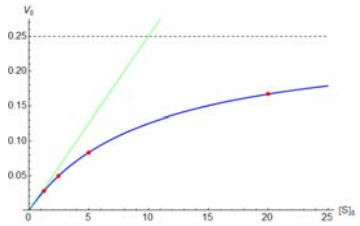
Enzymes catalyze biochemical reactions

Add a curve showing how you imagine **initial rate, V_0**, changes when the initial **enzyme concentration is lowered.**



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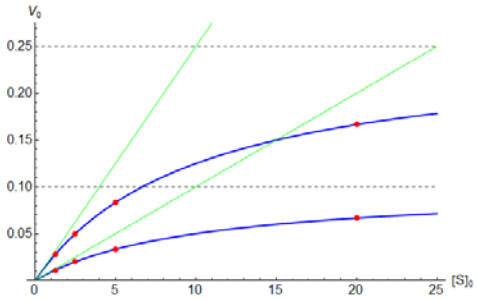
Enzymes catalyze biochemical reactions

Repeating the measurements for 40% of the original enzyme concentration shows that both the initial slope and the asymptote are proportional to the enzyme concentration.



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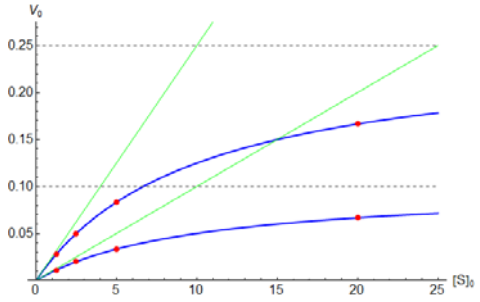
Michaelis-Menten mechanism of enzyme action

Enzyme catalysis shows these characteristic features:



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Leonor Michaelis and Maud Menten, ~ 1913

In 1913 Leonor **Michaelis** and Maud **Menten** provided the general theory of enzyme action that accounts for these features of enzyme catalysis

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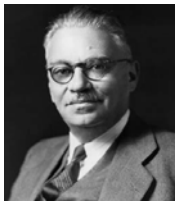
Leonor Michaelis and Maud Menten , ~ 1945



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Michaelis-Menten mechanism of enzyme action

The Michaelis-Menten (MM) mechanism accounts for enzyme action in two steps.

First, the enzyme, E, binds to the substrate, S, to form an enzyme-substrate complex, ES,

(1) $E + S \rightleftarrows ES$

$rate_{1,for} = k_1 [E] [S]_0$

$rate_{1,rev} = k_{-1} [ES]$

Typical enzyme concentrations are **nmol/L** and typical substrate concentrations are **mmol/L**. For **initial rates** this means …

[S] **changes only a little** from its initial value, $[S]_0$,

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Michaelis-Menten mechanism of enzyme action

Then, ES transforms the substrate into product much more slowly,

(2) $ES \rightarrow E + P$

$rate_{2,for} = k_2 [ES]$

Since we are concerned just with initial rates …

[P] remains so small that we can **ignore the reverse process**,

$E + P \rightarrow ES$

Therefore, the rate of formation of product is the rate of step (2),

$V_0 = k_2 [ES]$

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Michaelis-Menten mechanism of enzyme action

The initial rate $V_0 = k_2 [ES]$ depends on the concentration of the enzyme-substrate complex.

Because not all of the enzyme is bound in ES, let's instead express the total enzyme concentration as

$[E_t] = [E] + [ES]$

We can then express the unbound enzyme concentration as

$[E] = [E_t] - [ES]$

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Michaelis-Menten mechanism of enzyme action

ES is formed by forward reaction (1),

$(d[ES]/dt)_{formed} = k_1 [E] [S]_0$

$= k_1 ([E_t] - [ES]) [S]_0$

ES is depleted by the reverse of reaction (1) and by forward reaction (2).

$(d[ES]/dt)_{depleted} = k_{-1} [ES] + k_2 [ES]$

$= (k_{-1} + k_2) [ES]$

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Michaelis-Menten mechanism of enzyme action

Since reaction (2) is so slow, we assume a **steady state** [ES] is achieved,

$(d[ES]/dt)_{formed} = (d[ES]/dt)_{depleted}$

$k_1 ([E_t] - [ES]) [S]_0 = (k_{-1} + k_2) [ES]$

We can rearrange this steady-state equality to express [ES] as

$[ES] = k_1 [E_t] [S]_0 / (k_1 [S]_0 + (k_{-1} + k_2))$

$= [E_t] [S]_0 / ([S]_0 + (k_{-1} + k_2) / k_1)$

$= [E_t] [S]_0 / ([S]_0 + K_m)$

In terms of the **Michaelis constant**

$K_m = (k_{-1} + k_2) / k_1$

Michaelis-Menten mechanism of enzyme action

Determine the units of the Michaelis constant $K_m = (k_{-1} + k_2) / k_1$

Hint:

$E + S \rightleftarrows ES$,

$rate_{1,for} = k_1 [E] [S]_0$, $rate_{1,rev} = k_{-1} [ES]$

$ES \rightarrow E + P$,

$rate_{2,for} = k_2 [ES]$

Michaelis-Menten mechanism of enzyme action

Since

$rate_{1,for} = k_1 [E] [S]_0$, and so $k_1 \sim 1/(M\ s)$

$rate_{1,rev} = k_{-1} [ES]$, and so $k_{-1} \sim 1/s$

$rate_{2,for} = k_2 [ES]$, and so $k_2 \sim 1/s$

the units of the Michaelis constant are …

$K_m = (k_{-1} + k_2) / k_1$

$\sim (1/s + 1/s) / (1/(M\ s))$

$\sim M$

Michaelis-Menten mechanism of enzyme action

Using our expression of [ES],

$[ES] = [E_t] [S]_0 / ([S]_0 + K_m)$

we can write the rate of formation of product as the **Michaelis-Menten equation**

$V_0 = k_2 [E_t] [S]_0 / ([S]_0 + K_m)$

Michaelis-Menten mechanism of enzyme action

For values of $[S]_0$ **very large** relative to K_m,

$[S]_0 + K_m \approx [S]_0$

Therefore, the Michaelis-Menten equation simplifies to

$$V_0 = k_2 [E_t] = V_{max}$$

That is, V_{max} is the plateau, that the rate approaches in proportional to the total enzyme concentration.

This means we can express the **Michaelis-Menten equation** as

$$V_0 = V_{max} [S]_0 / ([S]_0 + K_m)$$

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Michaelis-Menten mechanism of enzyme action

For values of $[S]_0$ **very small** relative to K_m,

$[S]_0 + K_m \approx K_m$

Therefore, the Michaelis-Menten equation simplifies to

$$V_0 = k_2 [E_t] [S]_0 / K_m = (V_{max} / K_m) [S]_0$$

That is, for very small values of $[S]_0$, the rate of formation of product is **linear in the substrate concentration $[S]_0$**, with **slope V_{max}/K_m**.

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Getting V_{max} and K_m from kinetic data
Lineweaver-Burk plot

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Getting V_{max} and K_m from kinetic data

Rearrange the Michaelis-Menten equation

$V_0 = V_{max} [S]_0 / ([S]_0 + K_m)$

to sketch how $1/V_0$ depends on $1/[S]_0$

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Getting V_{max} and K_m from kinetic data

The equation for the **Lineweaver-Burk plot** is

$$1/V_0 = (K_m / V_{max})\ \ 1/[S]_0 + 1 / V_{max}$$

The plateau rate, V_{max}, is the reciprocal the **y intercept**.

The Michaelis constant, K_m, is V_{max} **times the slope**.

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Getting V_{max} and K_m from kinetic data

Here is data for catalysis of the hydrolysis of aqueous carbon dioxide at pH = 7.1, 273.5 K, and carbonic anhydrase concentration 2.3 nmol/L is

CO_2 / (nmol/L)	1.25	2.5	5	20
V_0 / (nmol/(L s))	2.78×10^{-2}	5.00×10^{-2}	8.33×10^{-2}	1.67×10^{-1}

Construct the Lineweaver-Burk plot for this catalysis reaction and use it to determine that

V_{max} = 0.250 nmol/(L s)

K_m = 10.0 nmol/L

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Getting V_{max} and K_m from kinetic data

As a check, plot the Michaelis-Menten equation,

$$V_0 = V_{max}\,[S]_0 / (\,[S]_0 + K_m\,)$$

using V_{max} = 0.250 nmol/(L s) and K_m = 10.0 nmol/L.

Do this by calculating V_0 for values of $[S]_0$ equal to 0.10, 0.50, 1, 1.25, 2.5, 5, 10, 15, 20, and 25. Note that your values for $[S]_0$ equal to 1.25, 2.5, 5, and 20 should reproduce the measured values V_0.

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Getting V_{max} and K_m from kinetic data

As a check, plot the Michaelis-Menten equation,

$$V_0 = V_{max}\,[S]_0 / (\,[S]_0 + K_m\,)$$

using V_{max} = 0.250 nmol/(L s) and K_m = 10.0 nmol/L.

Finally, add to your plot the line

$$V_0 = (\,V_{max} / K_m\,)\,[S]_0$$

To see that it is tangent to the Michaelis-Menten curve near $[S]_0 \approx 0$.

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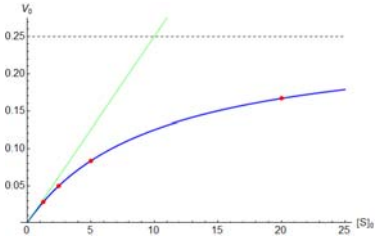
Getting V_{max} and K_m from kinetic data

As a check, plot the Michaelis-Menten equation,

$$V_0 = V_{max} [S]_0 / ([S]_0 + K_m)$$

using V_{max} = 0.250 nmol/(L s) and K_m = 10.0 nmol/L.

Here is what your results should look like.



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