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

The binding is fast: \( E + S \rightleftharpoons ES \)
The conversion to product and its release is slow: \( ES \rightarrow E + P \)
The rate of formation of product is the rate of the second, slow step.
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, \([S]_0\).

Enzymes catalyze biochemical reactions

Carbonic anhydrase, \(E\), catalyzes hydration of \(\text{CO}_2(\text{aq})\), \(S\), to \(\text{HCO}_3^-\)(aq), \(P\).
Sketch how you imagine initial rate, \(V_0\), depends on the initial substrate concentration, \([S]_0\).

Enzymes catalyze biochemical reactions

Plot initial rate, \(V_0\), versus initial substrate concentration, \([S]_0\).

<table>
<thead>
<tr>
<th>([S]_0) mmol/L</th>
<th>(V_0) mmol/(L s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.100</td>
<td>0.00248</td>
</tr>
<tr>
<td>0.250</td>
<td>0.00610</td>
</tr>
<tr>
<td>0.500</td>
<td>0.0119</td>
</tr>
<tr>
<td>1.000</td>
<td>0.0227</td>
</tr>
<tr>
<td>2.500</td>
<td>0.0500</td>
</tr>
</tbody>
</table>

Plot initial rate, \(V_0\), versus initial substrate concentration, \([S]_0\).

The red dots are the initial rate, \(V_0\), of formation of bicarbonate, in mmol/L/s, versus initial concentration, \([S]_0\), of \(\text{CO}_2\), in mmol/L, at pH = 7.1, 273.5 K, and an enzyme concentration of 2.3 nmol/L.
Enzymes catalyze biochemical reactions

The green line shows that for very small [CO₂], the rate of formation of bicarbonate is first order in [CO₂].

As [CO₂] becomes larger, however, the rate of formation of bicarbonate progressively slows.

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

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

Enzyme catalysis shows these characteristic features:

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.

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.

\[
E + S \rightleftharpoons ES
\]

rate\text{1,for} = k_1 [E] [S]_0
rate\text{1,rev} = k_{-1} [ES]

Typical enzyme concentrations are \text{nmol/L} and typical substrate concentrations are \text{mmol/L}. For initial rates this means …

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

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

\[
ES \rightarrow E + P
\]

rate\text{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),

\[V_0 = k_2 [ES]\]
### Michaelis-Menten mechanism of enzyme action

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

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

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

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

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

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

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

In terms of the Michaelis constant

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

### Determining the units of the Michaelis constant $K_m$

**Hint:**

$E + S ⇄ ES$, rate$_{1,\text{for}} = k_1 [E] [S]_0$, rate$_{1,\text{rev}} = k_{-1} [ES]$

$ES → E + P$, rate$_{2,\text{for}} = k_2 [ES]$

Using our expression of $[ES]$, $[ES] = [E] [S]_0 / ([S]_0 + K_m)$ we can write the rate of formation of product as the Michaelis-Menten equation

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

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

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

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

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

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

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 $V_{max}/K_m$.

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 $1/[S]_0$.
Getting $V_{\text{max}}$ and $K_m$ from kinetic data

The equation for the Lineweaver-Burk plot is

$$\frac{1}{V_0} = \left( \frac{K_m}{V_{\text{max}}} \right) \frac{1}{[S]_0} + \frac{1}{V_{\text{max}}}$$

The plateau rate, $V_{\text{max}}$, is the reciprocal the $y$ intercept. The Michaelis constant, $K_m$, is $V_{\text{max}}$ times the slope.

getting $V_{\text{max}}$ 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

<table>
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<th>$V_0$ (nmol/L s)</th>
</tr>
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<tr>
<td>1.25</td>
<td>$2.78 \times 10^{-2}$</td>
</tr>
<tr>
<td>2.5</td>
<td>$5.00 \times 10^{-2}$</td>
</tr>
<tr>
<td>5</td>
<td>$8.33 \times 10^{-2}$</td>
</tr>
<tr>
<td>20</td>
<td>$1.67 \times 10^{-1}$</td>
</tr>
</tbody>
</table>

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

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

$K_m = 10.0$ nmol/L

As a check, plot the Michaelis-Menten equation,

$$V_0 = \frac{V_{\text{max}} [S]_0}{[S]_0 + K_m}$$

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

Finally, add to your plot the line

$$V_0 = \left( \frac{V_{\text{max}}}{K_m} \right) [S]_0$$

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

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Here is what your results should look like.