Product Inhibition.
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Mechanism. Enzyme combines with a substrate molecule for form a complex, which leads to product. The product can also combine with an enzyme in a reversible manner. This is described schematically as follows.

$$S + E \underset{k_{1r}}{\overset{k_1}{\longleftrightarrow}} ES \underset{k_{2r}}{\overset{k_2}{\longleftrightarrow}} P + E$$

Derivation of Reaction Rate Expression with Equilibrium & Quasi-Steady State Assumption.

Given

1. dp/dt=rate=v $\quad v = k_2 \cdot ES - k_{2r} \cdot P \cdot E$
2. Conservation of enzyme species $\quad E_0 = E + ES$
3. Equilibrium and Quasi-Steady State Assumption for ES: $\quad k_1 \cdot S \cdot E + k_{2r} \cdot P \cdot E - k_{1r} \cdot ES - k_2 \cdot ES = 0$

We have three equations, and we can choose to solve for any of the three unknowns -- E, ES, and v. Find the analytical expression (via |Math|SmartMath|)

$$\text{Find}(E, ES, v) \rightarrow \begin{bmatrix} E_0 \cdot \dfrac{(k_{1r} + k_2)}{(k_1 \cdot S + k_{2r} \cdot P + k_{1r} + k_2)} \\ E_0 \cdot \dfrac{(k_1 \cdot S + k_{2r} \cdot P)}{(k_1 \cdot S + k_{2r} \cdot P + k_{1r} + k_2)} \\ E_0 \cdot \dfrac{(k_1 \cdot S \cdot k_2 - k_{2r} \cdot P \cdot k_{1r})}{(k_1 \cdot S + k_{2r} \cdot P + k_{1r} + k_2)} \end{bmatrix}$$

Thus, the last row is the analytical expression for v.

$$v = \frac{E_0 \cdot (k_1 \cdot S \cdot k_2 - k_{2r} \cdot P \cdot k_{1r})}{k_1 \cdot S + k_{2r} \cdot P + k_{1r} + k_2} = \frac{k_2 \cdot E_0 \cdot S - \dfrac{k_{1r} \cdot k_{2r}}{k_1} \cdot E_0 \cdot P}{\dfrac{k_{1r} + k_2}{k_1} + S + \dfrac{k_{2r}}{k_1} \cdot P}$$

Thus, the above form is transformed into the Michaelis-Menten form by defining:

$$v_{ms} = k_2 \cdot E_0 \qquad v_{mp} = \frac{k_{1r} \cdot k_{2r}}{k_1} \cdot E_0 \qquad K_m = \frac{k_{1r} + k_2}{k_1} \qquad K_p = \frac{k_{2.r}}{k_1}$$

$$v = \frac{v_{ms} \cdot s - v_{mp} \cdot p}{K_m + s + K_p \cdot p}$$

The reaction rate expression shows **product inhibition**. More specifically, this is **competitive** inhibition, as the apparent value of K_m varies without a change in v_{max}. Physically, this is competitive inhibition because product P competes with substrate S for active sites.

Note #1: As $P \to 0$, $v \to v_{ms} \cdot s/(K_m + s)$, we get back classical Michaelis-Menten kinetics.

Note #2: For $v \geq 0$, $\frac{v_{ms}}{v_{mp}} \cdot s > p$

Note #3: At equilibrium, v=0.

$$0 = \frac{v_{ms} \cdot s_{eq} - v_{mp} \cdot p_{.eq}}{K_m + s_{eq} + K_p \cdot p_{eq}} \longrightarrow \frac{p_{eq}}{s_{eq}} = \frac{v_{ms}}{v_{mp}} = \frac{k_1 \cdot k_2}{k_{1r} \cdot k_{2r}}$$

At equilibrium, we have the equilibrium constant K_{eq}. $\frac{p_{eq}}{s_{eq}} = K_{eq}$

Thus, $\frac{v_{ms}}{v_{mp}} = K_{eq} = e^{-\frac{\Delta G^0}{RT}}$ ∴ Use thermodynamics to obtain some of the kinetic data.

Alternate form: $v = \frac{v_{ms} \cdot \left(s - \frac{p}{K_{eq}}\right)}{K_m + s + K_p \cdot p}$

Plot of Product Inhibition Rate Expression

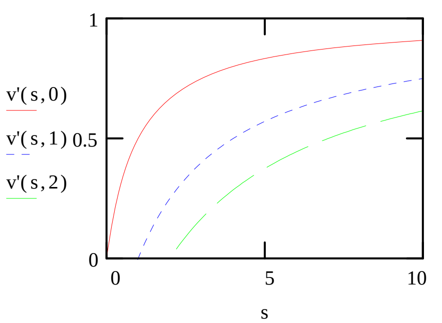
Model parameters

$v_{ms} := 1$ $v_{mp} := 1$ $K_m := 1$ $K_p := 1$

Product inhibition rate expression

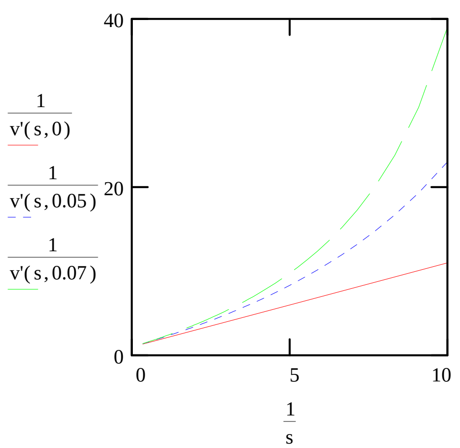
$$v'(s,p) := \frac{v_{ms} \cdot s - v_{mp} \cdot p}{K_m + s + K_p \cdot p}$$

Normal Rate Plot $s := 0, 0.1 .. 10$



The entire curve is depressed with increasing p.

Lineweaver-Burk Plot $s := 0.1, 0.11 .. 3$



As we increase p, the Lineweaver-Burk plot has the same intercept with the coordinate axis but an increasing slope.

Solve with an equilibrium assumption for the first step only. -- Incorrect.

Given

1. dp/dt=rate=v $v = k_2 \cdot ES - k_{2r} \cdot P \cdot E$
2. Conservation of enzyme species $E_0 = E + ES$
3. **Equilibrium Assumption for the first step**: $\frac{k_{1r}}{k_1} = \frac{E \cdot S}{ES}$

$$\mathrm{Find}(E, ES, v) \rightarrow \begin{bmatrix} k_{1r} \cdot \frac{E_0}{(k_{1r} + k_1 \cdot S)} \\ E_0 \cdot k_1 \cdot \frac{S}{(k_{1r} + k_1 \cdot S)} \\ E_0 \cdot \frac{(k_1 \cdot S \cdot k_2 - k_{2r} \cdot P \cdot k_{1r})}{(k_{1r} + k_1 \cdot S)} \end{bmatrix}$$

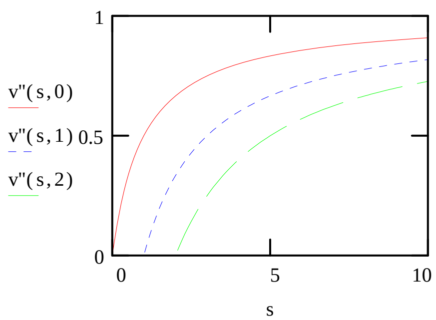
$$v = \frac{E_0 \cdot (k_2 \cdot k_1 \cdot S - k_{2r} \cdot P \cdot k_{1r})}{k_{1r} + k_1 \cdot S} = \frac{E_0 \cdot \left(k_2 \cdot S - \frac{k_{1r} \cdot k_{2r}}{k_1} \cdot P\right)}{\frac{k_{1r}}{k_1} + S}$$

Thus, the above form is transformed into the Michaelis-Menten form by defining:

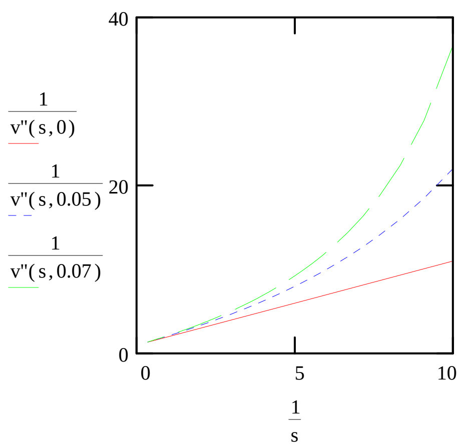
$v_{ms} = k_2 \cdot E_0$ $v_{mp} = \frac{k_{1r} \cdot k_{2r}}{k_1} \cdot E_0$ $K_m = \frac{k_{1r}}{k_1}$

$v''(s,p) := \frac{v_{ms} \cdot s - v_{mp} \cdot p}{K_m + s}$ ← This equation, capturing only part of the product dependence, is not the same for as before! Thus, it is not as general.

Normal Rate Plot $s := 0, 0.1 .. 10$



Lineweaver-Burk Plot $s := 0.1, 0.11 .. 3$



The general behavior of the plots are similar, but not quite identical, to that derived from the quasi-steady state assumption.

Solve with an equilibrium assumption for the second step only. -- Incorrect.

Given

1. dp/dt=rate=v $v = k_2 \cdot ES - k_{2r} \cdot P \cdot E$
2. Conservation of enzyme species $E_0 = E + ES$
3. Equilibrium Assumption for the second step: $\frac{k_{2r}}{k_2} = \frac{ES}{E \cdot P}$

$$Find(E, ES, v) \rightarrow \begin{bmatrix} k_2 \cdot \frac{E_0}{(k_{2r} \cdot P + k_2)} \\ E_0 \cdot k_{2r} \cdot \frac{P}{(k_{2r} \cdot P + k_2)} \\ 0 \end{bmatrix}$$

$v = 0$ Hmm... This result is expected, if the product formation step is in equilibrium, v=dp/dt=0 automatially. Thus, an equilibrium assumption for the product formation step leads to nonsense.

Now, we change the definition of v to be v=-ds/dt

Given

1. -ds/dt=rate=v $v = k_1 \cdot E \cdot S - k_2 \cdot ES$
2. Conservation of enzyme species $E_0 = E + ES$
3. Equilibrium Assumption for the second step: $\frac{k_{2r}}{k_2} = \frac{ES}{E \cdot P}$

$$Find(E, ES, v) \rightarrow \begin{bmatrix} k_2 \cdot \frac{E_0}{(k_{2r} \cdot P + k_2)} \\ E_0 \cdot k_{2r} \cdot \frac{P}{(k_{2r} \cdot P + k_2)} \\ k_2 \cdot E_0 \cdot \frac{(k_1 \cdot S - k_{2r} \cdot P)}{(k_{2r} \cdot P + k_2)} \end{bmatrix}$$

$$v = \frac{k_2 \cdot E_0 \cdot (k_1 \cdot S - k_{2r} \cdot P)}{k_2 + k_{2r} \cdot P} = \frac{E_0 \cdot \left(k_2 \cdot S - \frac{k_{2r}}{k_1} \cdot P \right)}{\frac{k_2}{k_1} + \frac{k_{2r}}{k_1} \cdot P}$$

Thus, the above form is transformed into the Michaelis-Menten form by defining:

$v_{ms} = k_2 \cdot E_0$ $v_{mp} = \frac{k_{2r}}{k_1} \cdot E_0$ $K_m = \frac{k_2}{k_1}$ $K_p = \frac{k_{2.r}}{k_1}$

$$v'''(s, p) := \frac{v_{ms} \cdot s - v_{mp} \cdot p}{K_m + K_p \cdot p}$$

← This equation, capturing only part of the product dependence, is not the same for as before! Furthermore, at equilibrium, v=0 leads to p/s=$v_{ms}/v_{mp} = k_1 \cdot k_2 / k_{2r}$, which is incorrect.

Mechanism: A Series of Reversible Reactions.

$$S + E \underset{k_{1r}}{\overset{k_1}{\longleftrightarrow}} ES \underset{k_{2r}}{\overset{k_2}{\longleftrightarrow}} EP \underset{k_{3r}}{\overset{k_3}{\longleftrightarrow}} P + E$$

Given

1. dp/dt=rate=v $v = k_2 \cdot ES - k_{2r} \cdot EP$
2. Conservation of enzyme species $E_0 = E + ES + EP$
3. Equilibrium and Quasi-Steady State Assumption for ES: $k_1 \cdot S \cdot E + k_{2r} \cdot EP - k_{1r} \cdot ES - k_2 \cdot ES = 0$

   Equilibrium and Quasi-Steady State Assumption for EP: $k_2 \cdot ES + k_{3r} \cdot P \cdot E - k_{2r} \cdot EP - k_3 \cdot EP = 0$

We have four equations, and we can choose to solve for four unknowns -- E, ES, EP, and v.

$$\text{Find}(E, ES, EP, v) \rightarrow \begin{bmatrix} E_0 \cdot \dfrac{(k_{2r} \cdot k_{1r} + k_3 \cdot k_{1r} + k_3 \cdot k_2)}{(k_1 \cdot S \cdot k_2 + k_{2r} \cdot k_{3r} \cdot P + k_{3r} \cdot P \cdot k_{1r} + k_{3r} \cdot P \cdot k_2 + k_{2r} \cdot k_1 \cdot S + k_{2r} \cdot k_{1r} + k_3 \cdot k_1 \cdot S} \\ E_0 \cdot \dfrac{(k_3 \cdot k_1 \cdot S + k_{2r} \cdot k_{3r} \cdot P + k_{2r} \cdot k_1 \cdot S)}{(k_1 \cdot S \cdot k_2 + k_{2r} \cdot k_{3r} \cdot P + k_{3r} \cdot P \cdot k_{1r} + k_{3r} \cdot P \cdot k_2 + k_{2r} \cdot k_1 \cdot S + k_{2r} \cdot k_{1r} + k_3 \cdot k_1 \cdot S} \\ (k_1 \cdot S \cdot k_2 + k_{3r} \cdot P \cdot k_{1r} + k_{3r} \cdot P \cdot k_2) \cdot \dfrac{E_0}{(k_1 \cdot S \cdot k_2 + k_{2r} \cdot k_{3r} \cdot P + k_{3r} \cdot P \cdot k_{1r} + k_{3r} \cdot P \cdot k_2 + k_{2r} \cdot k_1 \cdot S + k_{2r}} \\ E_0 \cdot \dfrac{(k_2 \cdot k_3 \cdot k_1 \cdot S - k_{1r} \cdot k_{2r} \cdot k_{3r} \cdot P)}{(k_1 \cdot S \cdot k_2 + k_{2r} \cdot k_{3r} \cdot P + k_{3r} \cdot P \cdot k_{1r} + k_{3r} \cdot P \cdot k_2 + k_{2r} \cdot k_1 \cdot S + k_{2r} \cdot k_{1r} + k_3 \cdot k_1 \cdot S} \end{bmatrix}$$

Thus, the last row is the analytical expression for v.

$$v = -E_0 \cdot \frac{(-k_2 \cdot k_3 \cdot k_1 \cdot S + k_{1r} \cdot k_{2r} \cdot k_{3r} \cdot P)}{(k_2 \cdot k_1 \cdot S + k_{2r} \cdot k_{3r} \cdot P + k_{3r} \cdot P \cdot k_{1r} + k_{3r} \cdot P \cdot k_2 + k_{2r} \cdot k_1 \cdot S + k_{2r} \cdot k_{1r} + k_3 \cdot k_1 \cdot S + k_3 \cdot k_{1r} + k_3 \cdot k_2)}$$

After collecting like-terms and some rearrangement, we have:

$$v = \frac{\dfrac{k_1 \cdot k_2 \cdot k_3}{k_1 \cdot (k_2 + k_3 + k_{2r})} \cdot E_0 \cdot S - \dfrac{k_{1r} \cdot k_{2r} \cdot k_{3r}}{k_1 \cdot (k_2 + k_3 + k_{2r})} \cdot E_0 \cdot P}{\dfrac{(k_{1r} \cdot k_{2r} + k_{1r} \cdot k_3 + k_2 \cdot k_3)}{k_1 \cdot (k_2 + k_3 + k_{2r})} + S + \dfrac{k_{3r} \cdot (k_{1r} + k_2 + k_{2r})}{k_1 \cdot (k_2 + k_3 + k_{2r})} \cdot P}$$

Thus, the above form is transformed into the Michaelis-Menten form by defining:

$$v_{ms} = \frac{k_1 \cdot k_2 \cdot k_3}{k_1 \cdot (k_2 + k_3 + k_{2r})} \cdot E_0 \qquad v_{mp} = \frac{k_{1r} \cdot k_{2r} \cdot k_{3r}}{k_1 \cdot (k_2 + k_3 + k_{2r})} \cdot E_0$$

$$K_m = \frac{(k_{1r} \cdot k_{2r} + k_{1r} \cdot k_3 + k_2 \cdot k_3)}{k_1 \cdot (k_2 + k_3 + k_{2r})} \qquad K_p = \frac{k_{3r} \cdot (k_{1r} + k_2 + k_{2r})}{k_1 \cdot (k_2 + k_3 + k_{2r})} \qquad K_{eq} = \frac{k_1 \cdot k_2 \cdot k_3}{k_{1r} \cdot k_{2r} \cdot k_{3r}}$$

$$v = \frac{v_{ms} \cdot s - v_{mp} \cdot p}{K_m + s + K_p \cdot p} \quad \text{or} \quad v = \frac{v_{ms} \cdot \left(s - \dfrac{p}{K_{eq}}\right)}{K_m + s + K_p \cdot p}$$

← Exactly the same form as before.

$$+ k_3 \cdot k_{1r} + k_3 \cdot k_2)$$

$$+ k_3 \cdot k_{1r} + k_3 \cdot k_2)$$

$$\cdot k_{1r} + k_3 \cdot k_1 \cdot S + k_3 \cdot k_{1r} + k_3 \cdot k_2)$$

$$+ k_3 \cdot k_{1r} + k_3 \cdot k_2)$$