PHEN 612

SPRING 2008

WEEK 11

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Collection and Analysis of Rate Data

How to obtain Km and Vmax for Michaelis-Menten models

- Run series of batch runs with different levels of substrate concentration
- $-r_s$ (dC_s/dt) as a function of initial substrate concentration
  - Langmuir (Hanes-Woolf) plot
  - Eadie-Hofstee plot
  - Lineweaver-Burk plot

Rate data analysis can help to distinguish between competitive and non-competitive inhibitions

- A competitive inhibitor has a strong structural resemblance to the substrate.
- Both the inhibitor and the substrate compete for the active site of the enzyme.

$$E + S \underset{k2}{\overset{k1}{\rightleftarrows}} ES$$

$$E + I \underset{k4}{\overset{k3}{\rightleftarrows}} EI$$

$$ES \xrightarrow{k5} E + P$$

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- Slow step is the product formation step:

$$r_p = \frac{k_5 C_{E0} C_S}{C_S + K_S\left(1 + \frac{C_I}{K_I}\right)}$$

$$V_{\max} = k_5 C_{E0}; \qquad K_{MI} = K_S\left(1 + \frac{C_I}{K_I}\right)$$

- See handouts to study the effects of competitive inhibition

- Reaction rate decreases due to the presence of competitive inhibitor

- Maximum reaction rate does not change (See handouts)

- Large amount of substrate is required to reach max. rate.

- At high substrate conc. the effect of the inhibitor will be reduced

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- Non-competitive Inhibition
  - Non-competitive inhibitors interact with enzymes in many different ways
  - They can bind reversibly or irreversibly to active sites or other regions
  - Non-competitive inhibitors combine with either the free enzyme or the enzyme-substrate complex to produce a dead-end complex

$$E + S \underset{k2}{\overset{k1}{\rightleftarrows}} ES$$

$$E + I \underset{k4}{\overset{k3}{\rightleftarrows}} EI$$

$$EI + S \underset{k6}{\overset{k5}{\rightleftarrows}} EIS$$

$$ES + I \underset{k8}{\overset{k7}{\rightleftarrows}} EIS$$

$$ES \xrightarrow{k9} E + P$$

$$r_p = \frac{r_{I,\max} C_S}{C_S + K_S}; \quad r_{I,\max} = \frac{r_{\max}}{1 + \frac{C_I}{K_I}}$$

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-Non-competitive inhibitors decrease the maximum reaction rate

- The Michaelis constant remains the same

- See handouts

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- **Evaluation of Monod Kinetic Parameters**
  - Use a Chemostat:

$$\frac{1}{\mu} = \frac{1}{D} = \frac{K_S}{\mu_{\max}}\left(\frac{1}{C_S}\right) + \frac{1}{\mu_{\max}}$$

  - Plot $1/\mu$ vs $1/C_s$ to obtain K_s and μ_{max}
  - The following equations can also be used:

$$\frac{C_S}{\mu} = \frac{K_S}{\mu_{\max}} + \frac{C_S}{\mu_{\max}}; \quad \mu = \mu_{\max} - K_S \frac{\mu}{C_S}$$

  - Several chemostat runs to estimate the parameters

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- Problem with the chemostat approach:
  - Length of runs (days or weeks to get steady-state values)
  - Risk of contamination

Batch runs can also be used

Project

Bring questions related to the project!