

**PHEN 612**

**SPRING 2008**

**WEEK 11**

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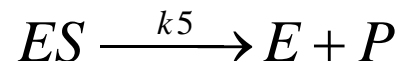
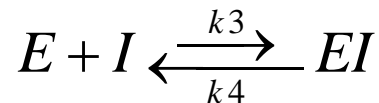
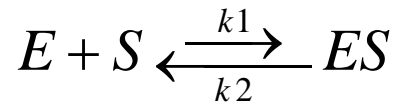
# Collection and Analysis of Rate Data

## How to obtain $K_m$ and $V_{max}$ 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.



## Collection and Analysis of Rate Data

- Slow step is the product formation step:

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

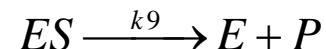
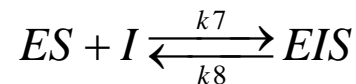
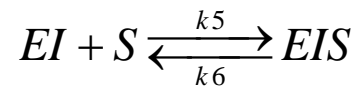
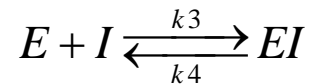
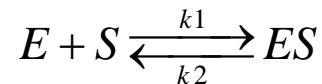
$$V_{\max} = k_5 C_{E0}; \quad K_{MI} = K_S \left( 1 + \frac{C_I}{K_I} \right)$$

- 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

## Collection and Analysis of Rate Data

### - 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



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

## Collection and Analysis of Rate Data

- Non-competitive inhibitors decrease the maximum reaction rate
- The Michaelis constant remains the same
- See handouts

# Collection and Analysis of Rate Data

- **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  $\mu_{\max}$

- 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

## Collection and Analysis of Rate Data

- 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!