Enzyme kinetics

BIOS 10016

24 June 2025

Objectives

- Describe the principles behind enzyme kinetics.
- Describe the criteria and assumptions required to analyze a system using Michaelis-Menten kinetics, and the parameters that are yielded.
- Prepare Michaelis-Menten and Lineweaver-Burk plots from raw data.
- Calculate elements of the Michaelis-Menten equation when provided sufficient information.
- Calculate the turnover number and specificity constant.
- Extract kinetic data from the Lineweaver-Burk plot.
- Describe the limits at which the turnover number and the specificity constant drive the reaction.

Enzymes

General properties of enzymes

- Have high reaction rates (10^6 to 10^{12} times faster than uncatalyzed reactions)
- Mild reaction conditions

$$2N_{2(g)} + 6\,H_2(g) \rightarrow 2\,NH_3(g)$$

- Regulated activity
- Reaction specificity
  - Absolute specificity: only one substrate (urease → urea)
  - Relative specificity: few related substrates (e.g. hexokinase → glucose and fructose)
  - Stereospecific specificity: only one isomer (D or L form)

Active sites

Substrates bind using noncovalent interactions to **active sites**. Active sites are a unique microenvironment that binds the substrate and catalyzes the reaction.

Induced fit = changing the shape of the enzyme to fit the substrate.

Initial binding of the substrate to the active site depends on IMFs.

Structural complementarity

Geometric: substrate and enzyme structures match structurally

Electronic: substrate and enzyme structures match electronically (opposite charges)

Stereospecificity: chiralities match

Binding energy and transition states

or ΔG_B

The energy associated with substrate binding to the active site that pays for the entropy associated with binding.

Energy comes from:

1. Enthalpy from IMFs
2. Entropy of H_2O
3. Entropy of the substrate

Transition states may block spontaneous processes. The transition state is short-lived, high-energy, and determines reaction rate.

To speed up: lower activation energy or increase ΔG of S.

Transition state analogs are excellent enzyme inhibitors. The molecules mimic a transition state and bind to the active site, preventing substrate binding.

Dissociation constant - how tightly a substrate binds to a protein or enzyme. A smaller K_D means stronger binding. (K_i is K_D for an inhibitor.)

Arrhenius equation

$$k = Ae^{-\frac{E_a}{RT}} \tag{1}$$

where k is the rate constant, A is the pre-exponential factor, E_a is the activation energy, R is the gas constant, and T is the temperature in Kelvin.

Kinetics

Enzyme kinetics

Consider a first-order reaction: an enzyme E converts one substrate S into one product P.

$$S \rightleftharpoons P$$

$$\text{rate} = \frac{d[P]}{dt} = \frac{-d[S]}{dt}$$

An enzyme is like a factory assembly line - rate of product formation depends on the time it takes to make a product (V_{max}, k_{cat}) and the time it takes for materials to arrive

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightarrow E + P$$

$E + S \rightarrow ES = k_1$
$ES \rightarrow E + S = k\_1$
$ES \rightarrow EP = k_2$
$EP \rightarrow ES = k\_2$

Dependence of reaction rate on [S]

The more substrate, the greater the chance for collisions

rate = velocity = $V \propto [S]$

To study enzyme kinetics, study it far away from equilibrium

v_0 = initial rate of reaction (initial velocities)

Reaction order

The number of molecules that react in the rate determining step (slowest step)

first-order reaction rate = k[S], rate $\propto$ [S]

zero-order reaction reaction rate is not proportional to [S], observed when [S] >>> [E]
on the flat line

rate = k_{cat}

second-order reaction reaction rate $\propto [S]_1$ and $[S]_2$

$$\text{pyruvate} + HCO_3{}^- \rightarrow \text{oxaloacetate}$$

rate = $k[S]_1[S]_2$

pseudo first-order reaction reaction rate $\propto [S]_1$ only (the independent substrate is present in excess amounts)

$$\text{pyruvate} + HCO_3{}^- \text{ (excess)} \rightarrow \text{oxaloacetate}$$

rate = $k[S]_1$

Michaelis-Menten kinetics

- Simplest, but very useful model
- Requires collection of initial velocity data
- Can be applied to study enzyme kinetics providing..
  - 1st or pseudo 1st order
  - hyperbolic
- Based upon the reaction E + S $\rightleftharpoons$ ES $\rightarrow$ E + P or binding $\rightleftharpoons$ processing
- **Michaelis-Menten complex = $\mathbf{K}_m$ = enzyme-substrate complex** (ES)
- Looking only at initial velocities (no $k_{-}2$)
- Assumptions:
  - Minimal amount of enzyme [E] <<< [S]
  - No product (<10%) and the forward reaction predominates, V = V_0
  - $k_{-1} >> k_2$ (ES $\rightarrow$ E + S is much faster than ES $\rightarrow$ E + P)
  - Steady state condition ([ES] will not change)

The Michaelis-Menten equation

$$v_0 = \frac{V_{max}[S]}{[S] + K_m} \tag{2}$$

where:

- v_0 = initial velocity
- V_{max} = maximum velocity, line approaches this value asymptotically
- [S] = substrate concentration
- K_m = Michaelis constant (the substrate concentration at which the reaction rate is half of V_{max})

Extracting K_m and V_{max} values

Linear plots:

- **The Lineweaver-Burke Double Reciprocal Plot**
- Woolf-Hofstee Plot
- Hanes-Woolf Plot
- Eadie-Scatchrd Plot

Deriving the Lineweaver-Burk relationship:

$$v_0 = \frac{V_{max}[\mathrm{S}]}{[\mathrm{S}] + K_m}$$

$$\frac{1}{v_0} = \frac{[\mathrm{S}] + K_m}{V_{max}[\mathrm{S}]}$$

$$\frac{1}{v_0} = \frac{K_m}{V_{max}}\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$

Slope: K_m/V_{max}

x-int: $\dfrac{-1}{K_m}$

The Michaelis constant, K_m

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

k_2 is negligible. Cancel and rearrange to get:

$$K_m = \frac{k_{-1}}{k_1} = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]}$$

Units: M

Small value = tighter binding (high affinity)

Large value = weaker binding (low affinity)

The constant value depends on

- enzyme type
- substrate
- reaction conditions (pH, temperature, ionic strength)

K_m is an approximation of substrate affinity, provided $k_2 <<< k_{-1}$.

$\mathbf{V}_{max}$

A value that depends on enzyme properties

Has units: amount/time

Turnover number, $\mathbf{k}_{cat}$, $\mathbf{k}_2$

$$k_{cat} = \frac{V_{max}}{[E]_T}$$

units: s^{-1}

The specificity constant

A useful term to describe enzyme-substrate systems

Quantitative term to assess **catalytic efficiency** (how efficiently an enzyme econverts substrate to product

Specificity constant = $\frac{k_{cat}}{K_m}$ (rate of processing/binding of S)

Units: $M^{-1}s^{-1}$

$$\frac{k_{cat}}{K_m} = \frac{\text{higher rate}}{\text{higher affinity}} = \frac{k_{cat} \text{ is bigger}}{K_m \text{ is smaller}}$$

Dual nature of the Michaelis-Menten equation

Combination of 0-order and 1st-order kinetics

The Michaelis Menten equation describes a hyperbolic dependence on [S]

Low-substrate concentration: $v = \frac{k_{cat}}{K_m}$

High-substrate concentration: $v = k_{cat}$