Lecture 17: Enzyme Kinetics

Assigned reading in Campbell: Chapter 6.6

Key Terms:

- **Steady State Kinetics**
- **Michaelis-Menton Equation: $v_o = V_{max}[S] / (K_M+[S])$**
- **$V_{max} = k_{cat}[E_{total}]$**
- **$K_M = (k_{-1}+k_2) / k_1$**

Enzyme kinetics: the study of the rates of enzyme-catalyzed reactions:
Provides indirect information regarding the specificities and catalytic mechanisms of enzymes.

17.1 **Chemical Kinetics:**

Consider a simple bimolecular reaction: $E + S \rightarrow P$

The rate is proportional to the product of the reactants:
rate $[E]^1[S]^1$ (2^{nd} order because sum of exponents is two)
A 2^{nd} order reaction has the following properties:

- The forward rate constant (k_1) has units of $M^{-1}sec^{-1}$.
- The reverse rate constant (k_{-1}) has units of sec^{-1}.

The rate, or velocity, of any reaction can be determined by measuring the increase in the amount of product formed $\Delta[P]$ during a given amount of time Δt:

$$v = \frac{\Delta[P]}{\Delta t} = \frac{d[P]}{dt}$$

17.2 Steady State Enzyme Kinetics:

For many enzymes, the rate of catalysis, v, varies with substrate concentration [S]. At fixed enzyme concentrations, v is almost linearly proportional to [S]. However, at high [S], v is nearly independent of [S].

A model to account for these observations was proposed by Michaelis & Menton. The critical feature of this model is that ES complex formation is a necessary intermediate in catalysis. This model accounts for the kinetic properties of many enzymes.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

- k_1 is the forward rate constant for substrate binding.
- k_{-1} is the reverse rate constant for substrate binding.
- ES is the enzyme-substrate complex.
- k_{cat} is the catalytic rate constant (containing terms related to stabilization of the transition state).

The goal is to use this model to generate an expression that relates the rate of catalysis to the concentrations of enzyme, substrate, and the individual rate constants.

The starting point is:

$$v = \frac{d[P]}{dt} = k_{cat}[ES]$$

Now we need to express [ES] in terms of readily measurable experimental parameters, such as:

1. The total amount of enzyme E_T = [E] + [ES]
2. The concentration of substrate: [S]
3. The rate of the reaction v = k_{cat} [ES]

[ES] is determined by the rates of its formation and breakdown:

$$\text{Rate of [ES] formation} = k_1[E][S]$$
$$\text{Rate of [ES] breakdown} = k_{-1}[ES] + k_{cat}[ES]$$

To simplify a complicated kinetic equation, we make a key assumption. The **steady state assumption** postulates that there is a period of time (called the steady state) (coincides with the initial velocity period) when [ES] does not change:

d[ES]/dt = 0

Then $k_1[E][S] = k_{-1}[ES] + k_{cat}[ES]$

If we express the rate of product formation as

$$v = k_{cat}[ES]$$

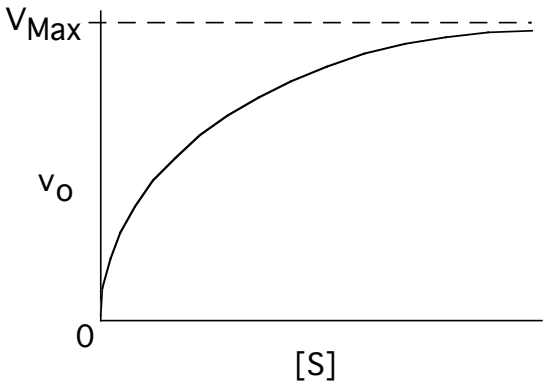
and express [ES] in terms of the kinetic rate constants, [S], and the total amount of enzyme present $[E_T] = [E]+[ES]$,

$$v = k_{cat}[E_T]\frac{[S]}{\frac{k_{-1} + k_{cat}}{k_1} + [S]}$$

Now if we abbreviate $\frac{k_{-1} + k_{cat}}{k_1}$ as K_M

And substitute V_{MAX} for $k_{cat}[E_T]$, we get the following simple equation:

$$v = \frac{V_{MAX}[S]}{K_M + [S]}$$ This is the **Michaelis-Menton** equation.



17.3 Important constants that describe the enzymatic reaction:

1) The K_M or Michaelis constant:

- $$K_M = \frac{k_{-1} + k_{cat}}{k_1}$$

- This is *almost* the same as the K_D ($=k_{-1}/k_1$) dissociation constant, except for the presence of the k_{cat} term. Therefore it is related to the affinity or strength of binding of a substrate to the enzyme.

- K_M is equal to the substrate concentration that gives 1/2 of the maximal velocity, in a similar manner that K_D is given by the [L] that gives Y=0.5.

$$v = \frac{V_{MAX}[S]}{K_M + [S]}$$

2) $V_{max} = k_{cat}[E_T]$:

This is the highest rate of product production possible. It is obtained at high substrate levels ([S]>>K_M).

$$v = \frac{V_{MAX}[S]}{K_M + [S]}$$

Under these conditions all of the enzyme is in the [ES] form (i.e. [ES]=[E_T]). Thus V_{MAX} is a reflection of the ability of an enzyme to perform the catalytic step.

3) Turnover number: $k_{cat} = V_{max} / [E_T]$

This is the number of reactions performed by a single enzyme molecule in a certain period of time (e.g. moles of product formed in a defined period by a mole of enzyme) *when the enzyme is fully saturated with substrate*.

17.4 **Throughput, or efficiency, of enzyme systems**

$$v = \frac{V_{MAX}[S]}{K_M + [S]} \qquad V_{MAX} = k_{cat}[E_T]$$

Low Substrate: At low substrate concentrations ([S]<<K_M) the overall rate of product formation depends on both the total amount of enzyme [E_T] and substrate [S]. The rate constant is comprised of both K_M and k_{cat} (k_{cat}/K_M). Thus under these conditions, the efficiency of an enzyme will depend both on how efficiently it can bind substrate (K_M), as well as how well it can perform the chemical step (k_{cat}). In other words, the intrinsic efficiency of an enzyme at low substrate levels is given by **k_{cat} / K_M**.

$$v = \frac{k_{cat}}{K_M}[E_T][S]$$

High Substrate: At high substrate concentrations ([S]>>Km) the rate becomes independent of [S] since all of the enzyme molecules are saturated with [S]. Therefore the intrinsic efficiency of an enzyme is given simply by **k_{cat}**. The overall rate depends only on k_{cat} and the total amount of enzyme, [E_T]. **$v = k_{cat}[E_T]$ or $v = V_{MAX}$**.