**Lecture 17: Introduction to Steady-State Enzyme Kinetics**

**Key Terms:**

* Steady-State Kinetics
* Vmax = kcat [Etot]
* v=kcat[ES]
* KM = *(kOFF + kCAT)/kON = (k-1 + k2)/k1*
* Michaelis-Menton Equation:

 V=Vmax [S]/(KM + [S])

* Specificity constant: Kcat/KM

**Enzyme Kinetics**

**1. Product Formation:**

The rate, or *velocity*, of the enzyme catalyzed reaction can be determined by measuring the increase in the amount of product formed Δ[P] during a given period of time Δt:



**2. Experimental Measurement of Enzyme Kinetics:**

i) What do we want to find out and why?

ii) How do we actually measure rates?

**3. Empirical Derivation of Rate Law:**

i) How does the rate depend on the total amount of enzyme, [ETOT], and on kcat, when all enzymes have substrate bound (saturated)?

ii) How does the rate depend on the substrate concentration, [S]?

**4. Analytical Derivation of Rate Law - Under Steady-State Conditions**

The goal is to relate the kinetic measurements to *readily measurable* experimental parameters:

i) The total amount of enzyme: ET = [E] + [ES],

ii) the concentration of substrate: [S], and

iii) the measured velocity (v = kCAT [ES]).

The simplest reaction scheme is:

|  |  |
| --- | --- |
| The experimentally obtained velocity of the reaction is: v = d[P]/dt = kCAT[ES]The differential equation that gives the change in [ES] as a function of time is: | * *kON* (= *k1*) is the forward rate constant for substrate binding
* *koff* (=*k -1)* is the reverse rate constant for substrate binding
* kCAT (=*k2*)is the catalytic rate constant (containing terms related to the stabilization of the transition state). It is also called the "**turnover number**", since it is the rate at which one molecule of [ES] converts to product. This will depend on particular substrate-enzyme combinations.
* The (ES) complex is also called the "Michaelis complex".
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If we make the assumption that we are working under steady-state conditions: **d[ES]/dt = 0.**

The last equation is the **Michaelis-Menton** equation, named after the scientists who derived it.

**5. Important Constants that Characterize the Enzymatic reaction:**

**i) The KM or Michaelis constant:** This is *almost* the same as the KD ( = *koff/kon*), the dissociation constant, except for the presence of the *kCAT* term. Therefore it is related to the affinity of a substrate to an enzyme. *It is a constant for any particular enzyme-substrate pair. Substrates with slow off-rates (koff) bind more tightly, and possess a smaller KM.*

**ii) VMAX = kCAT[ET]:** This is the highest rate of product production possible. It is obtained at high substrate levels ([S]>>KM). Under these conditions *all* of the enzyme is in the [ES] form (i.e. [ES]=[ET]). *kCAT*is obtained from VMAX since the total amount of enzyme is known: *kCAT=VMAX/[ET].*

**iii) kCAT/KM** is the rate of the reaction at low substrate and is useful to compare different enzyme/substrate combinations to each other.

$$v=k\_{cat}\left[E\_{T}\right]\frac{\left[S\right]}{K\_{M}+\left[S\right]}\~\frac{k\_{cat}}{K\_{M}}\left[E\_{T}\right][S]$$

**Measuring KM and kCAT** (VMAX):

**Step 1:** Measure the *initial* velocity at different substrate concentrations, keeping the enzyme concentration *constant*.

|  |  |  |
| --- | --- | --- |
| **Exp. #** | **[S] (mM)** | **v (umoles/sec)** |
| 1 | 0.1 | 9.0 |
| 2 | 0.5 | 33.4 |
| 3 | 1.0 | 50.0 |
| 4 | 2.0 | 66.6 |
| 5 | 10.0 | 91.1 |
| 6 | 20.0 | 95.2 |
| 7 | 50.0 | 99.0 |

**Example Data:** The following velocity data was obtained for a number of substrate concentrations ([E]Tot=1 nM).

**Step 2:** Analyze data

**A:** [S] not limiting - Velocity Curve:

i) Plot *v* versus [S].

ii) Obtain VMAX from v at very high [S].

iii) KM is the substrate concentration at gives v=VMAX/2

**B**: [S] *limiting* - Double reciprocal plot

 (Lineweaver-Burk Plot):

Analysis of double-reciprocal plot:

i) y-intercept = 1/VMAX

ii) Slope = KM/VMAX

 KM = slope × VMAX

iii) kCAT = VMAX/ET