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

**Goals:**

1. Measure parameter (KM) related to substrate binding.
2. Measure parameter (kCAT) related to catalytic efficiency.
3. Predict reaction velocity given KM and kCAT and [S].

**Simple Enzyme Kinetic Scheme.**

* *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 and provides information on the mechanism.
* The (ES) complex is also called the "Michaelis complex".**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:** How do we actually measure rates?



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



Assume that the rate = kCAT[ES]

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

low [S]:

high [S]:

ii) How does the rate depend on the total amount of enzyme, [ETOT]?

**4. Analytical Derivation of Rate Law - 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]

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

We want to come up with an equation the gives us the initial velocity as a function of [S], and depends on KM and VMAX. We can then use our data to determine KM and VMAX.

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: | 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.

**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.*



**When [S]=KM the enzyme is ½ saturated with substrate: v = ½ VMax**

**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].*





**Steady-State Assumption:**



**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.*



**When [S]=KM the enzyme is ½ saturated with substrate & v = ½ VMax**

|  |  |  |
| --- | --- | --- |
| **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 |

**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].*

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

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

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

**Step B:** Analyze data

**I:** [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

**II**: [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



|  |  |  |
| --- | --- | --- |
|  | **VMAX** | **KM** |
| **A** | **1** | **10** |
| **B** | **1** | **20** |
| **C** | **10** | **20** |

**Example:** Double reciprocal plots were obtained for an enzyme reacting with three different substrates. The VMAX and KM  values for these substrates are given in the table on the right. Which line on the double reciprocal plot corresponds to each substrate**?**

**Why determine KM and kCAT?**



**A. Mechanistic Information -** about particular enzyme-substrate pairs.

1. The active site region of Trypsin is shown below.

i) Sketch the kCAT as a function of pH.

ii) Sketch the KM as a function of pH.



2. The two substrates shown on the right were presented to trypsin. Their structures and measured KM and kCAT values are given.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Substrate** | **KM** | **kCAT** |
| **A** |  | **10 μM** | **1000 s-1** |
| **B** |  | **100 μM** | **1000 s-1** |

i) Explain the differences in KM of A versus B.

ii) Why are the kCAT  values the same?



**B. Predictive Information:** Given KM, ETOT, kCAT, and [S] you can predict the initial velocity:



**Example:** An enzyme has a kcat of 105/sec and a KM of 0.1 μM towards a certain substrate. One (1) nmole of enzyme is mixed with 0.3 μM substrate in a volume of one ml, what is the initial rate of the reaction (dP/dt)?

