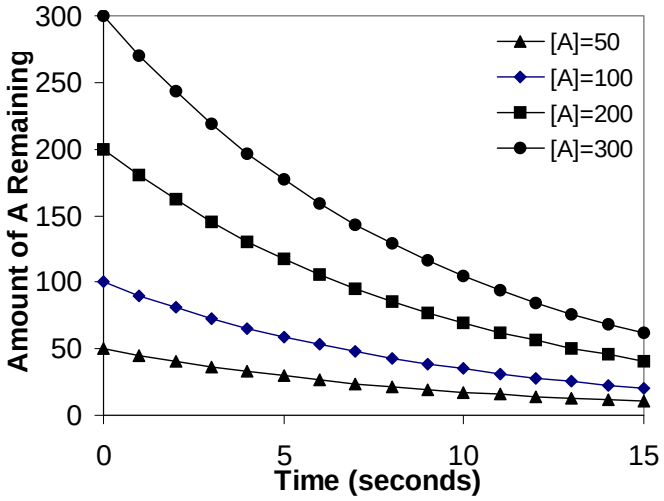
Lecture 4
STEADY STATE KINETICS

The equations of enzyme kinetics are the conceptual tools that allow us to interpret quantitative measures of enzyme activity. The object of this lecture is to thoroughly illustrate the equations we use, the assumptions made and the uses of the equations.

Again, before we begin to analyze enzyme catalyzed reactions it is instructive to review the results and analysis of the kinetics of uncatalyzed chemical reactions. For example, we have already shown that the [P] vs t (or -[A] vs. t) curve for a first order reaction is an exponential decay curve. The time for completion of the exponential decay decreases with increasing concentration of substrate.

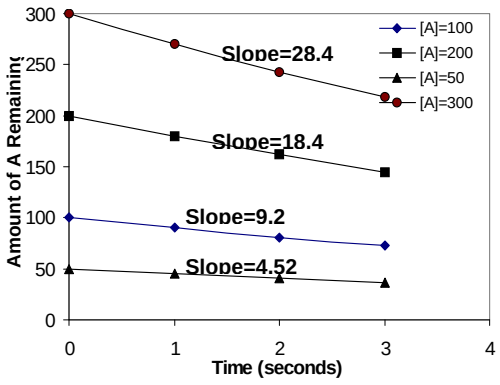
Time	[A]=50	[A]=100	[A]=200	[A]=300
0	50	100	200	300
1	45	90	180	270
2	40.5	81	162	243
3	36.45	72.9	144.8	218.7
4	32.805	65.61	130.32	196.83
5	29.5245	59.049	117.288	177.147
6	26.5721	53.1441	105.559	159.432
7	23.9148	47.8297	95.0033	143.489
8	21.5234	43.0467	85.503	129.14
9	19.371	38.742	76.9527	116.226
10	17.4339	34.8678	69.2574	104.604
11	15.6905	31.3811	62.3317	94.1432
12	14.1215	28.243	56.0985	84.7289
13	12.7093	25.4187	50.4886	76.256
14	11.4384	22.8768	45.4398	68.6304
15	10.2946	20.5891	40.8958	61.7673

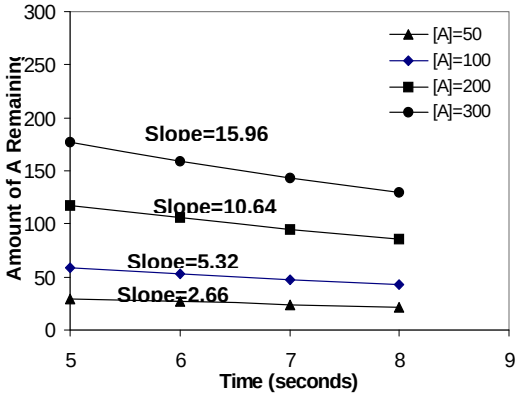


The velocity of the reaction

$$v = -\frac{d[A]}{dt}$$

(i.e., the change in A vs t) can (must) be measured from the slope of the exponential curve at early times in the reaction. It must be done at the *instantaneous initial velocity* because the concentration of A is always changing.



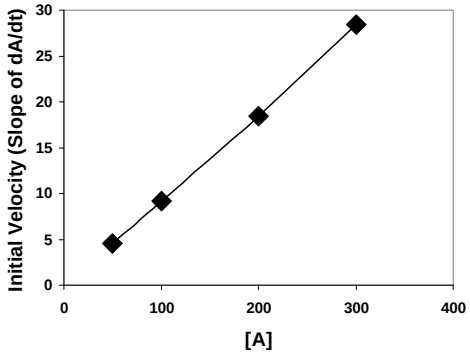


The velocity (formation of product/unit time) of a first order reaction, is also, as we have already shown can be derived from the equation:

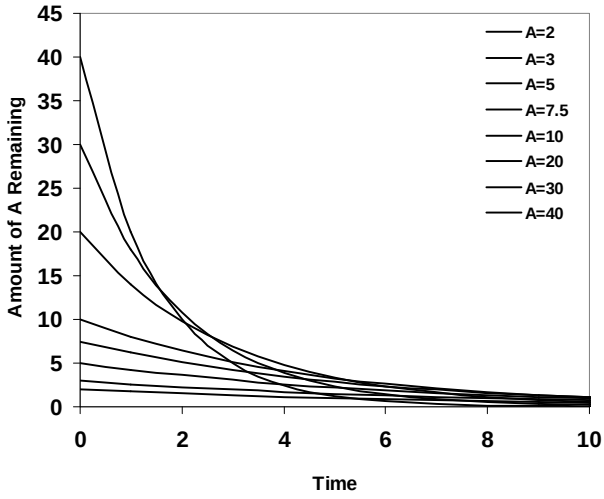
$$v = k [A]$$

Thus a plot of v *vs.* [A] can be obtained by plotting the *instantaneous initial* velocity of the reaction, as determined by the slope of the tangent to the curve at the initial time against the [A]. This plot is a straight line whose slope is equal to k, the rate constant for the reaction. This reaction continues *ad infinitum*.

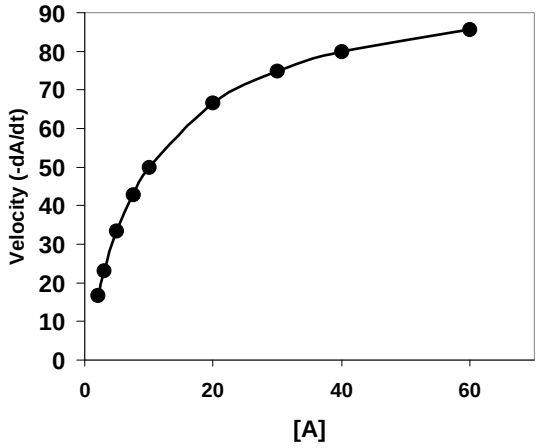
[A]	V initial
50	4.52
100	9.2
200	18.4
300	28.4



Now let us consider the plot of P vs t (or -A vs t) for an enzyme catalyzed reaction under the conditions where [E]<<<[S]. At relatively low [S], the [A] vs t curve looks very much like an exponential decay curve, i.e. a first order reaction. At very high concentrations, however, this curve looks distinctly different; there is a long linear portion where the slope of the [A] vs t curve is not changing. Moreover, at still higher [S] concentrations, there is no further change in the [A] vs. t plot.



Well, now what does this all mean? To analyze this further, lets plot the initial velocity of the reaction as determined from the slope of the tangential line (i.e. instantaneous initial velocity) or the slope of the linear portion of the curve.



The curve is a hyperbolic one. At low [S], v is nearly linearly dependent on the [S], i.e., the rate of product formed is constant per unit increase in [S]. This is reminiscent of the situation we see for a first order reaction. At increasingly higher [S], the rate of increase in v/unit increase in [S] decreases. The velocity in not linearly dependent on the concentration of [S]. At very high [S], the velocity is independent of [S]. The velocity of the reaction reaches *saturation.* The reaction is said to be zero order, independent of the concentration of any reactant in the mass action expression. The rate saturation phenomena is characteristic of catalyzed reactions, and for us in particular, enzyme catalyzed reaction.

In 1907, Michaelis and Menton used this observation to postulate that enzymes catalyze reactions via a complex of the enzyme with the substrate. That is, the enzyme binds the substrate and the limiting step is an intramolecular catalysis step. This rate saturation observation is one of the best evidences that enzymes bind substrates. Subsequently, x-ray crystal structures and active site labeling experiments have shown that enzymes do indeed bind the enzyme-the enzyme-substrate complex has been visualized.

The Michaelis-Menton postulate suggested the enzyme catalyzed reactions can be described by the following scheme:

$$E + S \underset{k_2}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{k_3} E + P$$

binding catalysis

Thus, the enzyme reaction can be separated into two discreet parts, the binding of the enzyme and substrate and the catalytic step in the ES complex to form product.

Michaelis and Menton postulated that the ES complex is in equilibrium with E + S. That is, the rate of formation of the ES complex, k_1 is much greater than the rate of the catalytic step. In fact this is a very specialized case of enzyme catalyzed reactions, so their treatment was modified for the more generalized case of steady-state.

Definition of Steady-State (Briggs-Haldane Approach)

The formation of the ES complex is characterized by the rate constant k_1. This rate constant is a second order constant describing the collision of E & S. Once formed the ES complex can be broken down by either of two pathway, dissociation, governed by the rate constant k_2, or catalytic turnover k_3. These two rate constants describe first order processes.

From the equation

$$E + S \underset{k_2}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{k_3} E + P$$

the overall velocity of the reaction is given by the rate law:

$$v = k_3 \bullet [ES].$$

The only problem with this rate law is that we cannot directly measure the concentration the ES complex, only the appearance of product or disappearance of substrate. Nevertheless, we can analyze an enzyme-catalyzed reaction by making some surmises.

Starting with the relationship, $v=k_3[ES]$, in order to mathematically analyze an enzyme reaction, we must express [ES] in terms of known quantities.

From our above discussion, the velocity of ES formation is:

$$k_1 \bullet [E]_{free} \bullet [S],$$

while the rate of breakdown of ES is given by:

$$(k_2+k_3) \bullet [ES].$$

During the period of the reaction where we measured our initial velocities on the [A] vs t curve to derive our v vs [S] plot, we observed that there was a period on the A vs t curve where it was straight, i.e., the velocity of the reaction-amount of product vs. time was not changing:

$$d(d[A])/dt = 0.$$

Since we said that the velocity of the reaction is $k_3[ES]$, this means that during the period of the initial velocity measurement, the concentration of the ES complex is constant. THe concentration of the ES complex is a said to be in **steady-state**- the rate of formation of the ES complex is equal to its rate of breakdown. During steady-state, the amount of A and S are changing, but the ES complex is constant. Thus at steady-state

$$d[ES]/dt$$

meaining that the velocity of ES formation, $k_1 \bullet [E]_{free} \bullet [S]$, is the same as the velocity for its breakdown $(k_2+k_3) \bullet [ES]$

Thus: $k_1 \bullet [E]_{free} \bullet [S] = (k_2 + k_3) \bullet [ES]$

Rearranging this equation we get:

$$[ES] = \frac{k_1 \bullet [E]_{free} \bullet [S]}{k_2 + k_3}$$

Dividing through by k_1 gives

$$[ES] = \frac{[E]_{free} \bullet [S]}{(k_2 + k_3)/k_1}$$

where $\frac{k_2 + k_3}{} = K_m$, (Michaelis constant)

This new constant, K_m, has the form of a dissociation constant, its units are molar concentration (M). Although it is not really an equilibrium constant, the Km can be used as an *apparent* equilibrium constant, one which measures the affinity of the enzyme for substrates. Since the Km is a dissociation constant, the lower the Km, the higher the affinity of the enzyme for the substrate. (WILL BE REINFORCED LATER).

Now we still do not have the velocity equation in terms of things we can measure, for that we must calculate where all the enzyme is during steady-state, for it is not all free and it is not all bound by S

$$[E]_{free} = [E]_{total} - [ES]$$

Substituting this equivalence into the above equation and solving for ES again gives:

$$[ES] = \frac{([E]_{total} - [ES]) \bullet [S]}{K_m}$$

multiplying through gives

$$[ES] = \frac{[E]_{total} \bullet [S]}{K_m} - \frac{[ES] \bullet [S]}{K_m}$$

Making the steady-state assumption:

$$[ES] = \frac{[E]_{total} \bullet [S]}{K_m} = \frac{[ES] \bullet [S]}{K_m}$$

and factoring

$$[ES] = \frac{[E]_{total} \bullet [S]}{K_m} = [ES] \bullet \left(1 + \frac{[S]}{K_m}\right)$$

Dividing through the left side of the equation by the right side gives:

$$[ES] = \frac{[E]_{total} \bullet [S]/K_m}{1 + [S]/K_m}$$

Multiplying top and bottom by K_m gives:

$$[ES] = \frac{[E]_{total} \bullet [S]}{K_m + [S]}$$

We began with $v = k_3 \bullet [ES]$, so substituting the above into this equation gives

$$v = \frac{k_3 \bullet [E]_t \bullet [S]}{[S] + Km}$$

Since the maximal velocity of the enzyme reaction is obtained when $[ES]=[E]_t$, then $Vm = k_3 \bullet [E]_t$ Thus,

$$v = \frac{Vmax \bullet [S]}{[S] + Km}$$

Note this equation accounts for the data given by a v vs [S] plot for an enzyme catalyzed reaction. At very low [S], ([S]<<<<Km), the [S] in the denominator is negligible and the value of the denominator is constant. Thus the velocity is directly proportional to [S]. At very high [S] ([S]>>>Km), the quantity [S]/[S]+Km --> 1, and thus, v=Vmax, and v is independent of the substrate concentration.

The value and meaning of Km is also apparent from inspection of the Michaelis-Menton equation. Under the condition where [S]=Km, the quantity, [S]/[S]+Km =1/2. Thus, the Km is the [S] where the velocity of the reaction is equal to 0.5•Vmax.