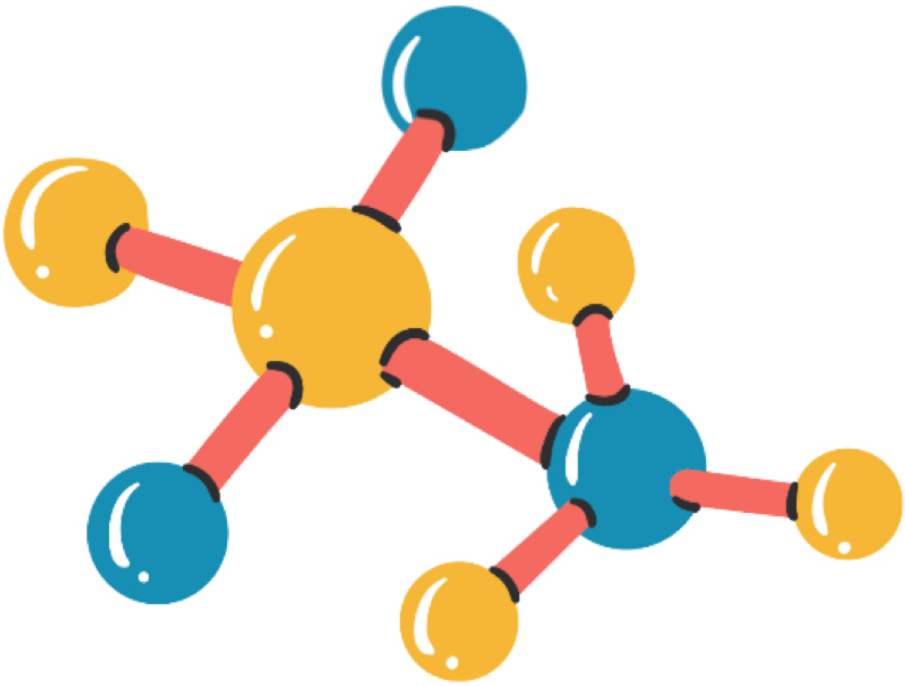
Biochemistry



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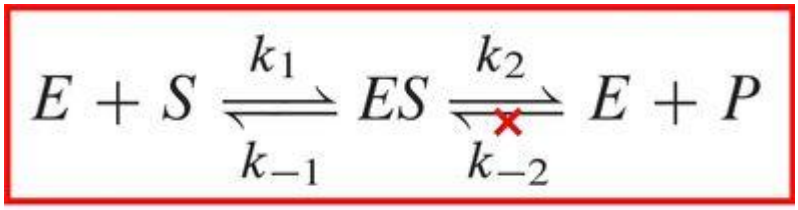
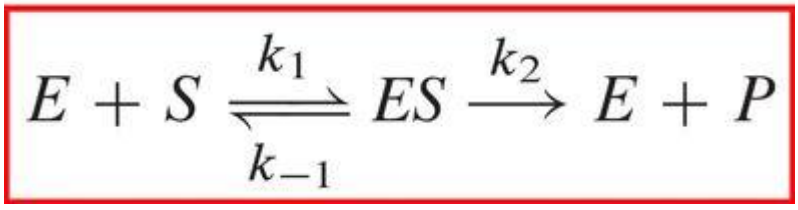
Make sure that you understand these points from previous lecture:

1. Scientists made 2 assumptions to form an equation (Michalis-Menten equation):

A. Enzyme-substrates complex (ES) will turn into enzyme + products without having reversible reaction (products and enzyme won't form ES again)





B. ES is in a steady state (rate of formation = rate of degradation), so ES concentration does NOT change

2. Km (Michaelis constant) equals ES dissociation rate constants over ES association rate constants (in above figure (**$k_{-1} + k_2)/k_1$**). It indicates the affinity of an enzyme (Km is inversely proportional with affinity). However, it is NOT an exact measure. (The exact one is called dissociation constant (**K_d**), it equals **k_{-1}/k_1**)

Michalis-Menten equation

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

V_0: The initial reaction rate.

V_{max}: Maximum reaction rate, i.e. reaction rate when the enzyme is saturated with the substrate.

K_m: Michaelis constant, which is only determined by the nature of the enzyme and has no connection with the concentration of the enzyme. It could be used to identify different enzymes.

$[S]$: Substrate concentration.

This equation is a quantitative description of the relationship between the rate of an enzyme catalyzed reaction (V_0) and substrate concentration [S]

Let's apply this equation practically in different situations:

From previous lecture, the doctor said "At the beginning of the reaction, it will adopt a first order reaction"

1. When substrate concentration is very low, we can neglect the [S] in the denominator because it is relatively small to Km, so the equation will be **V0=Vmax.[S]/Km**. As Vmax and Km are constants, this will form linear equation with a slope equals Vmax/Km and passes through origin point (0,0)

REMEMBER: the general form of linear equation **y=Ax +B**, while **A** represents the slope and **B** represents the interception with Y axis (it equals zero in Michalis-Menten equation).

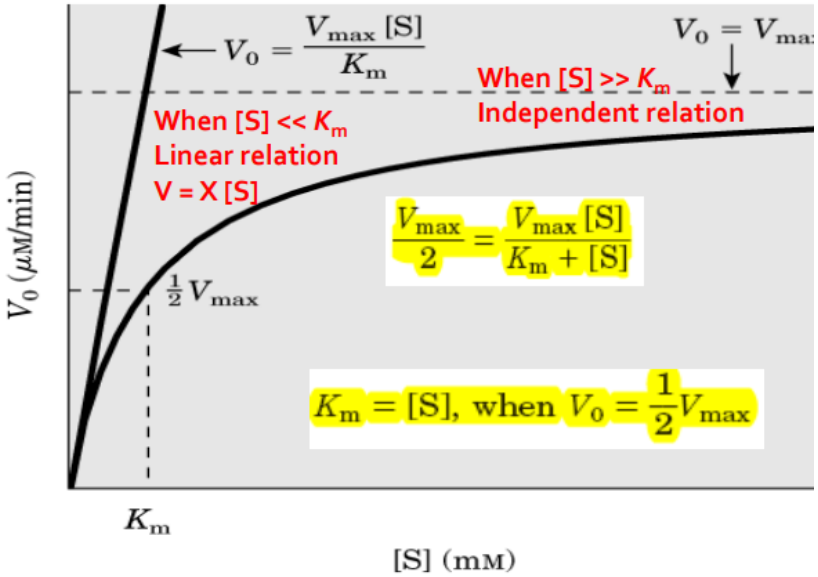
REMEMBER: we can neglect small values **unless** they are multiplied or divided by another value. That is why we don't neglect [S] in numerator.

And the linear equation proves what doctor said above in the last lecture.

From previous lecture, the doctor said, "when we have very high substrate concentration, the reaction will occur at maximal rate". Let's prove that:

2. When substrate concentration is very high, we can neglect the Km in the denominator because it is relatively small to [S], so the [S] in numerator and denominator will cancel each other and the equation will be **V0 = Vmax**.

3. When substrate concentration equals Km, the equation will be **V0=Vmax/2.** From this case, we can define Km as the concentration of substrate value needed to make the reaction rate equals half Vmax



When we have an enzyme catalyzed reaction with more than one substrate, each one has a different structure. Accordingly, each one will have different affinity and K_m.

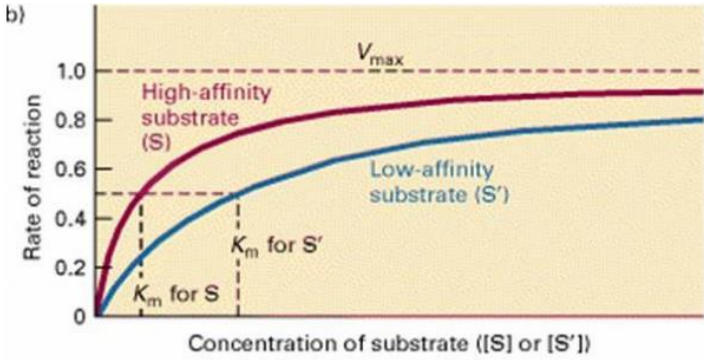
For example, **hexokinase** enzyme which transfers a phosphate group from ATP (usually) to a hexose like glucose. The active site must bind **ATP** and **glucose** together in order to produce **glucose-6-phosphate**. Each substrate has different affinity and K_m, so there will be two curves (one for each substrate). However, they are in the same reaction which has only one V_{max} which means in the end the two lines will merge.

The curve that represents the high-affinity substrate will be shifted to the left. However, the curve that represents the low-affinity substrate will be shifted to the right.

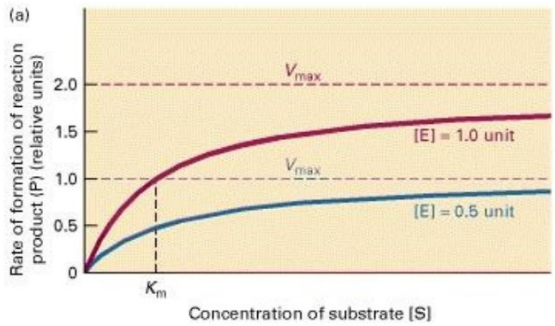
When we have an enzyme that can work on multiple substrates such as hexokinase which can work either on glucose to form glucose-6-phosphate or fructose to form fructose-6-phosphate. Each reaction has different V_{max}.

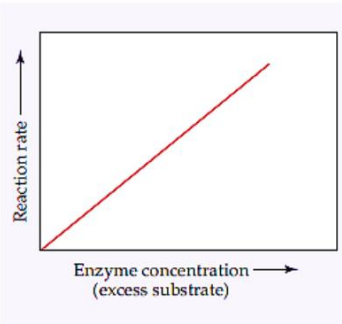
To conclude, the maximum velocity of an enzyme is an inherent property of an enzyme with respect towards its substrate (differs from substrate to another)

K_m values have a wide range. Mostly between (10^{-1} & 10^{-7} M) in physiological enzymes.



Substrate concentration has no relation with V_{max}, K_m. However, enzyme concentration will affect the V_{max} because if we increase enzyme concentration, there will be more active sites and the ability of binding more molecules at the same time will increase and more reactions will occur per time unit, so the rate of reaction will increase.





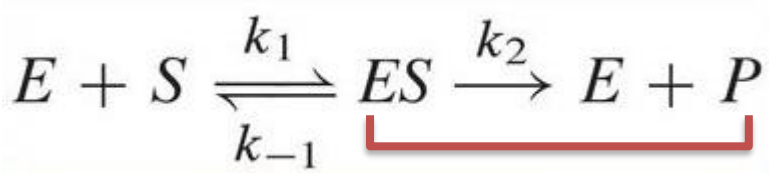
The relation between enzyme concentration and the rate of reaction is linear proportional. If we double enzyme concentration, the rate will be doubled.

K_m doesn't change by changing enzyme concentration.

Q. If we did 2 experiments for two different enzymes to catalyze the same reaction with excessive substrates. The first one made the reaction runs by rate=20 units, the second one made the reaction runs by rate=5 units, which enzyme in more efficient?

A. we can't know without determine the enzyme concentration. Maybe the higher V_{max} in the first enzyme is due to higher enzyme concentration, so we must find out a new concept which determines the efficiency of enzymes which is **K_{cat}**

From previous lecture, we know that we can calculate the rate of reaction by multiplying the substrate concentration by rate constant.



In the above reaction, the making products reaction is the right part whose rate equals to **K_2.[ES]**. In saturation situation, there will be no free enzymes and all of them will be ES. Also, the rate of reaction will be at its maximal point, so we can write the equation like this: **$V_{max} = K_2$ [E]T** and accordingly **$K_2 = V_{max}$/[E]T,** so **K_2** is a constant which considers maximum velocity and total enzyme concentration and that is what we need to describe enzyme efficiency.

K_2 is called here **K_{cat}** or **Turnover number.**

The higher **K_{cat}** is, the more is the efficiency of enzyme.

K_{cat} & K_m values vary a lot between enzymes, so we need a constant which combines these two constants together which is **specificity constant** which describes:

1. specificity (the higher the specificity constant is, the higher is the specificity)
2. enzyme catalytic efficiency (the higher the specificity constant is, the higher is the efficiency)

specificity constant=K_{cat}/K_m

The range of specificity constant is only 4, while the range of K_{cat} & K_m is very large

$$V = \frac{V_{max}[S]}{K_M + [S]} = \frac{k_{cat}[E_T][S]}{K_M + [S]} \qquad V = (k_{cat}/K_M)[E][S]$$

Enzyme concentration

- a 10^{-6} M solution of carbonic anhydrase catalyzes the formation of 0.6 M H_2CO_3 per second when it is fully saturated with substrate

V_{max}

Sol. $K_{cat} = V_{max}/[E]_T$

- ✓ Hence, k_{cat} is 6×10^5 s^{-1}
- ✓ 3.6×10^7 min^{-1}

- Each catalyzed reaction takes place in a time equal to $1/k_2$, which is 1.7 μs for carbonic anhydrase
- The turnover numbers of most enzymes with their physiological substrates fall in the range from 1 to 10^4 per second

Reaction rate: measures the concentration of substrate consumed or product produced per unit time (mol/{L.s} or M/s)

Enzyme activity: measures the number of moles of substrate consumed (or product produced) per unit time (mol/s)

Enzyme activity = rate of reaction × reaction volume

Specific activity: measures moles of substrate converted per unit time per unit mass of enzyme (mol/{s.g})

Specific activity = enzyme activity / actual mass of enzyme

This is useful in determining enzyme purity after purification

Turnover number: measures moles of substrate converted per unit time per moles of enzyme (min-1 or s-1)

Turnover number = specific activity × molecular weight of enzyme

A solution contains initially **25X10- 4 mol L -1** (initial substrate concentration) of peptide substrate and **1.5 µg** chymotrypsin (enzyme actual mass) in **2.5 ml** (volume). After **10 minutes** (time), **18.6X10- 4 mol L -1** (final substrate concentration) of peptide substrate remain. Molar mass of chymotrypsin is **25,000 g mol-1** (molar mass)

1. How much is the rate of the reaction? ▪ (conc./time)
   sol: (initial – final) substrate concentration/time
   answer: 6.4 X 10- 5 M/minutes

2. How much is the enzyme activity? (mol./time)
   Sol: rate X volume
   Answer: 1.6 X 10- 7 mol/min

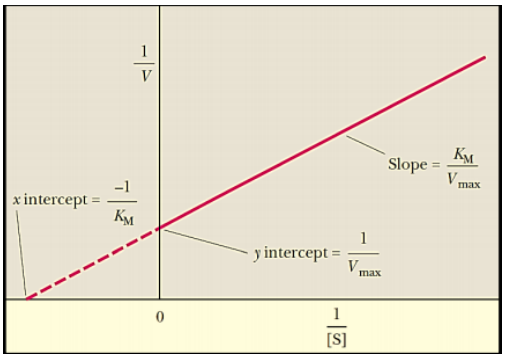
3. How much is the specific activity?
   Sol: enzyme Activity/enzyme Mass
   Answer: 0.10667 mol/(min.g)

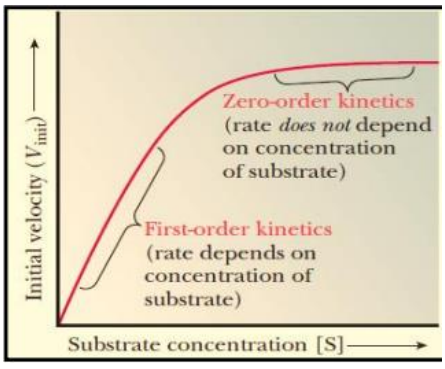
4. How much is the turnover number?
   Sol: specific Activity X enzyme molar mass
   Answer: 2666.67 (1/min)

In the past, it was difficult to deal with **Michalis-Menten equation** because: (disadvantages)

1. it is not linear equation
2. we need large amount of substrate to reach Vmax (money and time consuming)
3. not accurate values

Lineweaver-Burk or double-reciprocal plot: A plot of $1/V_0$ versus 1/[S] which means (1/[S] represents X, $1/V_0$ represents Y), yields a straight line with an y-intercept of 1/Vmax and a slope of K_M/V_{max} and the intercept on the x-axis is $-1/K_M$





Lineweaver-Burk or double-reciprocal plot made it easier to find out V_{max} & K_M by a smaller number of experiments and with higher accuracy.

In the 1990s, using of Lineweaver-Burk equation was declining, and scientists returned to Michalis-Menten equation because of development of computer software that can deal with it.

- **A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetics. Approximately, V_{max} of this enzyme is … & K_m is …?**
  - A. 5000 & 699
  - B. 699 & 5000
  - C. 621 & 50
  - D. 94 & 1
  - E. 700 & 8

Substrate Concentration (μM)	Initial velocity (μmol/min)
1	49
2	96
8	349
50	621
100	676
1000	698
5000	699

- **You are working on the enzyme "Medicine" which has a molecular weight of 50,000 g/mol. You have used 10 μg of the enzyme in an experiment and the results show that the enzyme converts 9.6 μmol per min at 25°C. the turn-over number (k_{cat}) for the enzyme is:**
  - A. 9.6 s^{-1}
  - B. 48 s^{-1}
  - C. 800 s^{-1}
  - D. 960 s^{-1}
  - E. 1920 s^{-1}

The first question, we can take any two points from the table and put it in Michalis-Menten equation or Lineweaver-Burk equation solve the question mathematically, but the doctor said we shouldn't do that because the question says that the numbers are approximated and mathematics will give us exact numbers. While we were increasing the substrate concentration, the velocity increased at the beginning by same ratio (first order reaction). Then the velocity started to increase by less proportion relative to substrate concentration. Then, we the velocity reached 670, the increasing of velocity had become almost neglegible, so V_{max} is around 670

Second part of the same question is K_m, its defined as the concentration of substrate value needed to make the reaction rate equals half V_{max}. V_{max} is around 700, so K_m the substrate concentration which makes the velocity around 350, so K_m=8 from the table

The right answer is E

The second question, enzyme activity = 9.6 μmol per min = 0.16 μmol per second
actual mass of the enzyme = 10 μg, the molar mass = 50000 g/mol

Turnover number = enzyme activity X molar mass/ actual mass of the enzyme

Be careful in using units **The right answer is C**