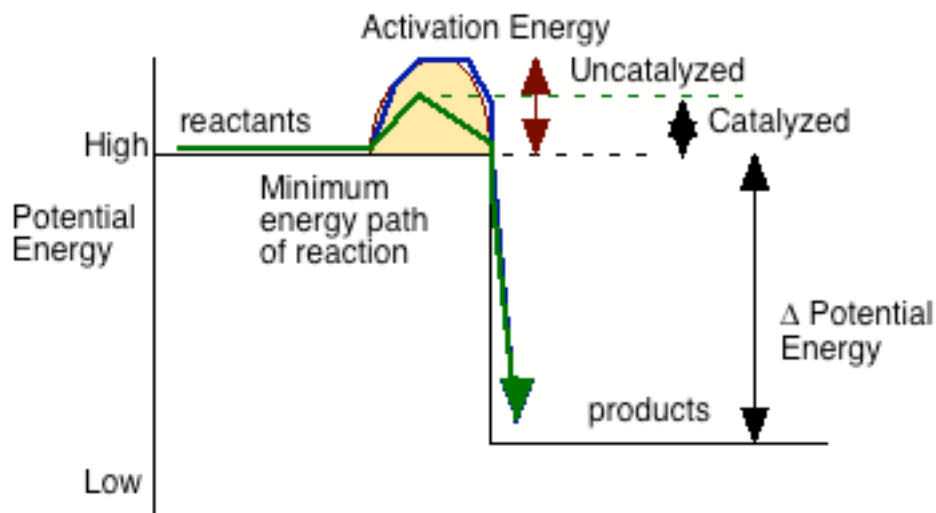


# ENZYME FUNCTION\*

**Reading:** read Campbell pp. 96-100

**Overview:** In the last section, we discussed the nature of chemical reactions. We overviewed the concept of forward and reverse reactions, reaction rates, equilibrium and non-equilibrium conditions. We learned about the concept of Gibbs free energy and learned how to use it to predict whether a particular reaction was feasible under a given set of conditions. We saw that feasibility had everything to do with the displacement from equilibrium. Finally, we looked at the topic of reaction kinetics -- the factors that govern the rate, path, and mechanisms of a reaction. This is where we pick things up today. We want to see how catalysts affect kinetics and how they work. Then, more specifically we will look at the action of protein catalysts -- enzymes and we will see how their exact structure and concentration affects the rate of a particular reaction in an organism.

**Catalysts:** Catalysts participate in reactions but are not consumed. They increase reaction rates by lowering activation energy,  $E_A$  (see last notes). We can visualize this on the same energy hill diagram that we saw in the last set of notes:



How does this work? The key is that the reactants must be adsorbed briefly onto the surface or a crack in the catalyst. So, catalysis is a surface-dependent phenomenon. The adsorption or binding between reactants and catalyst does not take place in a haphazard manner. Instead, what makes the surface of a catalyst effective is that it binds the reactants in the correct steric (position) relationships to allow the reaction to occur.

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Let's delve into this a bit more. The binding of the reactants to the catalyst is typically via some sort of polar or covalent interaction -- or, sometimes it is the result of a hydrophobic interaction. For the moment, let's assume the reactant(s) bind initially via polar interaction (the most common case). The binding often creates a stress in the bond(s) where the reaction is to take place. You can think of the reactant molecule as being distorted somewhat by the catalyst. Notice that this is the equivalent of what happens when we add lots of activation energy -- recall from the last notes that the increased energy caused, randomly, much more stress and distortion in the bonds of the reactants. If there is more than one reactant, the other thing the catalyst does is to ensure that the reactants are very close to each other and in the correct position for the specific reaction that is being catalyzed. Recall that we previously stated that in uncatalyzed reactions, adding more activation energy increased the frequency of sterically correct collisions between the reactants (if there are more than one). Notice what the catalyst has done -- **at a much lower system energy**, it has **stressed the bonds in the reactants and brought them together in the correct configuration to encourage a specific reaction** -- simply by only allowing them to bind in a certain configuration. Notice that this has happened with a much lower requirement of activation energy. Notice also that **the reaction was very specific** -- there was an **exact mechanism leading to an exact product**. To illustrate how specific catalyzed reactions usually are -- recall that in many reactions involving organic molecules, it may be possible to produce two optical isomers of what is otherwise the same compound. In uncatalyzed reactions, both will be produced. However, catalysts generally only produce one.

Let's add some additional details and then summarize. After a reactant binds loosely to the catalyst (for example, by hydrogen bonds or via a hydrophobic interaction, keep in mind the fact that sometimes the reactant will briefly become covalently bonded to the catalyst. This is quite common in enzymes. This covalent binding is generally part of the reaction mechanism. A reactant becomes covalently bonded, its characteristics change because of this bonding, and then it reacts with the other reactant. In the process, the covalent bond between the initial reactant and the catalyst break. Once the reaction is complete, the products are released. Notice that the reactants have been converted to products at a much lower energy level than would be possible than without the catalyst. Also, note that the catalyst has not been used up (unlike the reactants). It is there and now can be used again. Notice on the macro level that a thermodynamically feasible reaction is proceeding under physical conditions (most likely low temperature or pressure) where it normally would not. In other words, the catalyst has altered the reaction kinetics by lowering  $E_A$ . Again, the reaction is occurring at energy levels that are too low to support a noticeable reaction in the absence of the catalyst. Finally, note that the reaction rate will be proportional to the amount of reactant present, the "distance" from equilibrium, and most importantly, the amount of catalytic binding sites.

**Questions** (some review, some to try to figure out):

Do catalysts change the equilibrium point?

Do catalysts alter the size of the change in the Gibbs Free Energy of a reaction and therefore its thermodynamic feasibility?

What does adding more catalyst do to the rate of a reaction? If more catalyst is added, does that further lower the  $E_A$  or does  $E_A$  have more to do with the structures of the catalyst and reactants?

How can a catalyst participate directly in a reaction, even to the point of in some cases covalently binding to the reactants, and yet not be consumed by the reaction?

In catalyzed reactions, the reaction rate is sometimes proportional to the displacement of the reaction from equilibrium -- sometimes not. See if you can explain this. As part of your answer -- does displacement from equilibrium strictly predict the rate of an uncatalyzed reaction? This topic will be discussed in more detail later in these notes.

**Enzymes -- Protein Catalysts:** As we learned earlier in the section on proteins, they can come in a bewildering variety of shapes. We have seen that their higher order structures are influenced by interactions between their amino acids with each other and with the broader physical and chemical environment.

When a polypeptide gains tertiary structure, it is not uncommon for it to have **clefts** or openings leading to cavities of various shapes. In principle, any shape might be possible. **Beyond shape**, the amino acids that line the inside of any such cavities can have many different characteristics. Some parts of the cavity wall could be polar, others non-polar. There could be regions where the amino acid side groups are relatively reactive with certain other compounds, other regions where they are inert. The term for this cavity and its opening is the **ACTIVE SITE**.

Except for the fact that active sites are catalytic, they have many properties in common with "**binding**" or "**recognition**" sites on other proteins such as receptors. In all cases, "recognition" by the protein is based on complementary shapes and charge arrangement between the ligand<sup>1</sup> and the binding (or active) site.

**Step 1. Binding:** The first step is for the enzyme and substrate to bind. Initially, this binding is weak and is accomplished by **complementary shapes of enzyme and substrate**, **hydrogen bonding** in most cases, and in some cases by **regions of hydrophobic interaction**. Due to the weakness of these interactions, the **enzyme-substrate complex** is relatively easily broken up -- it is easily reversible. We can view the binding step as:

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<sup>1</sup> Ligand: a chemical that binds to the protein, whether it is termed a substrate or neurotransmitter, hormone or whatever.



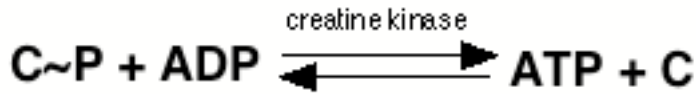
Let's explore this in a bit more detail. Binding occurs as a result of collisions between substrate and active site. Not all collisions will result in an enzyme-substrate complex. That is because the substrate(s) may not hit the active site in the correct (or nearly correct) configuration. Moreover, there will be many cases when some factor will have caused the shape of the active site to be altered in a way that it is not a particular good fit for the substrate (in fact, initially that is always the case). In addition, once the E-S complex has been formed, there is no guarantee that it will persist long enough for a reaction to occur. If there is not a particularly good shape fit between enzyme and substrate (for example, say some environmental factor such as a change in pH has altered the tertiary structure of the enzyme somewhat), the substrate could easily be knocked back out again. The factors that affect whether or not the E-S complex will form and persist long enough for a reaction to occur make up a property of the enzyme called its **AFFINITY**; we will discuss this in more detail later.

One other important thing happens during binding of substrate and enzyme. The shape of both the enzyme and the now bound substrate molecules change somewhat. This is very important. First, it is exactly what we would expect about a tertiary structure such as an enzyme. These are huge molecules where much of the shape is owed to relatively weak interactions. The addition and binding of (an)other molecule(s) to the enzyme (substrate(s)) will have to affect the overall shape of the enzyme. Likewise, a shape change in the large enzyme molecule will surely affect the shape of the small regions of substrate bound within the active site. The usual result of this initial shape change is to increase the strength of binding between enzyme and substrate align various side groups within the active site with the substrate in a way that will promote the reaction about to be catalyzed, and realign the substrate molecules with respect to each other. Moreover, the bending of the substrates tends to stress certain bonds and make them more reactive.

We call this sort of an adaptive shape change in a protein (which can be caused by lots of things binding to a protein besides a substrate) an **ALLOSTERIC CHANGE**. The overall picture we have just described could be called an **INDUCED FIT**. Initially E and S do not bind well, but with interaction, an allosteric change results in better binding and the potential for rapid catalysis.

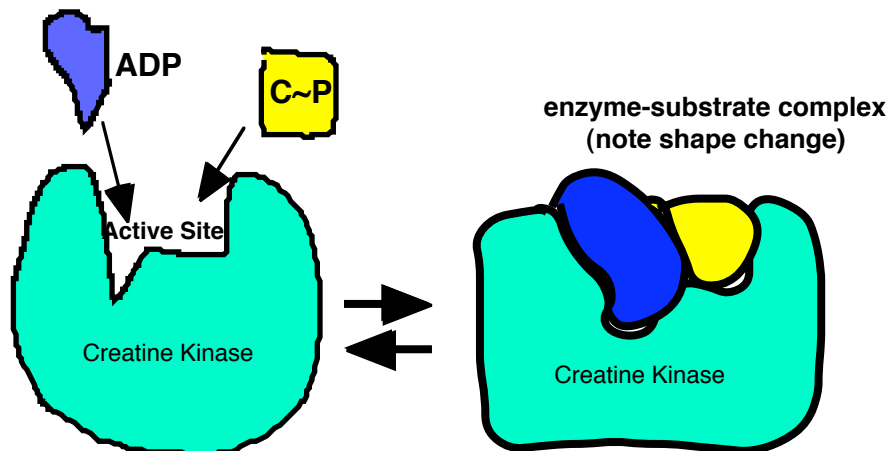
The old model of protein/substrate binding is called "**lock and key**". Although it correctly emphasized the complementary nature of active site and substrate, it also failed to acknowledge the dynamic nature of this interaction. Note the modification is a classic example of the scientific process.

We will use the creatine kinase reaction as an example. Creatine kinase is an enzyme that catalyzes the following reaction:

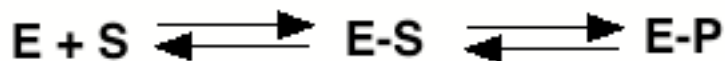


where **C~P** is creatine phosphate (essentially a molecule that stores high-energy phosphates for times of high-energy demand), **C** is creatine, and you already know ADP and ATP. During periods where cells (muscle in this case) demand ATP at a higher rate than it can be produced, ATP decreases and ADP increases. However, ATP is what various contractile proteins in the muscles require. To make up for the shortfall in ATP, cells transfer a high-energy phosphate from C~P to ADP to give ATP. Its kind of like taking money out of your bank account when you spend faster than you earn (where ATP represents a filled wallet and C~P is the banked money). Here is a depiction of the binding step (notice the induced fit):

**Binding of substrates to enzyme**



**Step 2 -- Catalysis:** Due to the allosteric change, the substrate molecule(s) is (are) now in a position to react. In many cases, the actual reaction is a bit complicated -- for instance, one substrate may actually react and produce a covalent bond with one of the side groups of the amino acids lining the active site. Then, part or all of the molecule is again transferred to complete the reaction. However, any covalent bonds between enzyme and substrate(s) are broken by the time the reaction is complete. The enzyme is now, chemically speaking, back to normal. We can talk of there being an enzyme-product (E-P) complex. So, here is the sequence so far:



**Step 3 -- Release of Products:** Now, after the reaction finally happens, the products of the reaction (for example, C and ATP) have slightly different



Which is usually simplified to: 
$$\text{Substrate} \xrightleftharpoons{\text{enzyme}} \text{product}$$

**Questions:**

What features allow enzymes to be catalysts? Why are they so specific in terms of reaction and mechanism?

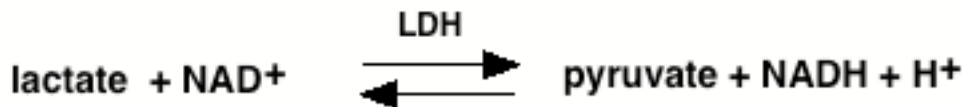
Speculate on the effect of small increases in temperature on the rate of catalysis? Explain. How about large temperature increases?

Compare and contrast the induced fit and lock and key models of enzyme function.

Notice that the enzyme changes shape several times (*i.e.*, its parts move about). Energy is required for motion to proceed orderly (from shape 1 to the E-S shape to the E-P shape back to shape 1). Where did the energy come from?

Should an enzyme catalyze both the forward and reverse reactions? Speculate on whether or not the enzyme will be an equally good catalyst in both directions.

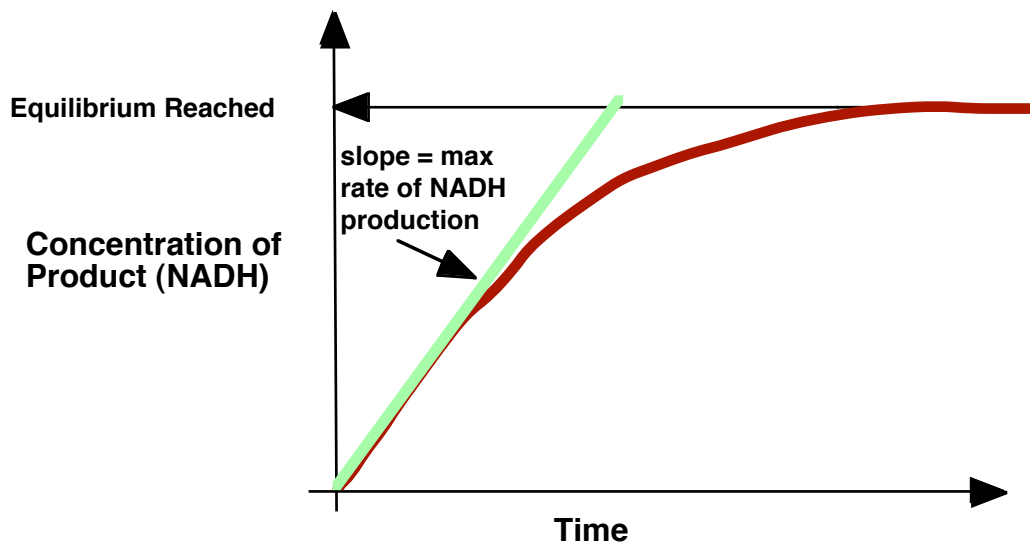
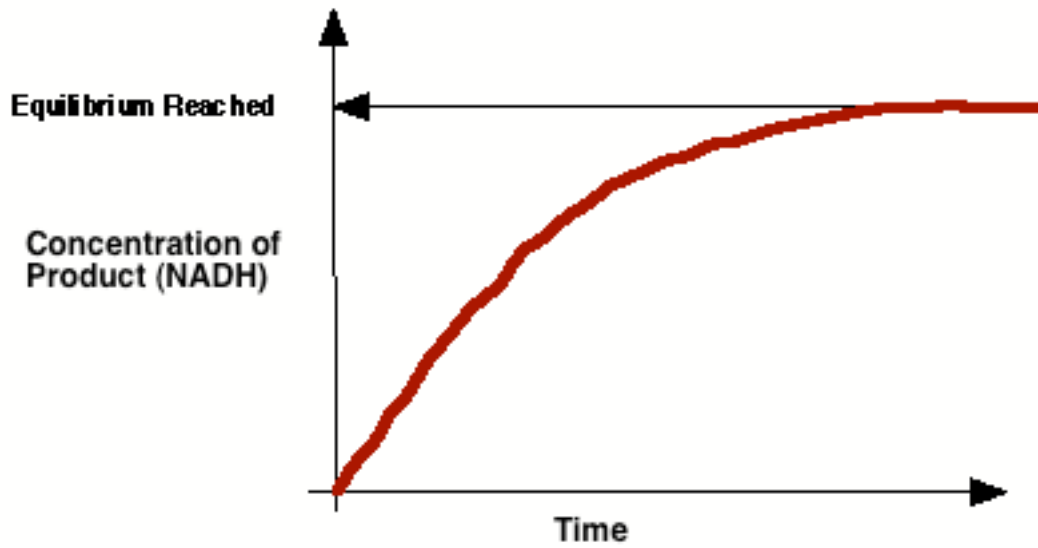
**The Kinetics of Enzymatic Reactions:** Let's suppose that we want to investigate the properties of the enzymes. We pick the specific enzyme lactic dehydrogenase (LDH) as our experimental subject. It catalyzes the following reaction:



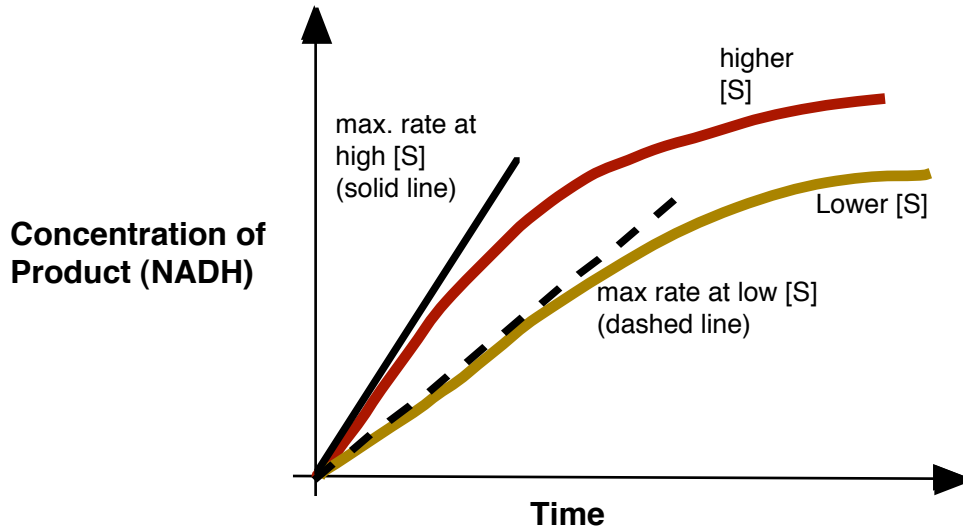
(NAD<sup>+</sup> and NADH are called co-enzymes; their job is accepting high-energy electrons from certain reaction, such as the one above. More about them later.)

To do our investigation we obtain a sample that contains the enzyme. This would usually be obtained by harvesting cells where the enzyme is found, breaking them apart, and then going through a series of purification steps. We also find the temperature and pH conditions where the enzyme works the best.

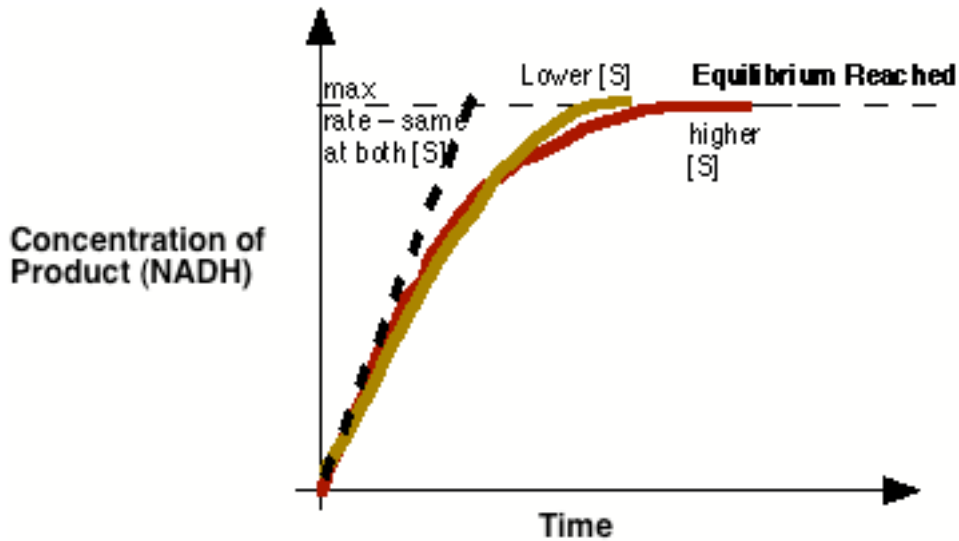
Next, we prepare solutions that all are at the same pH and temperature, that have identical amounts of enzyme, and that have a large excess of NAD<sup>+</sup> (why the large excess?). We put this solution into a cuvette and then into a spectrophotometer. The spectrophotometer will measure the appearance of NADH -- as more accumulates, the solution becomes darker (not to the eye but instead in a region of ultraviolet light). The change in darkness is proportional to the amount of NADH and pyruvate that are produced. Finally, we start the reaction by adding lactic acid. Here is a typical result:



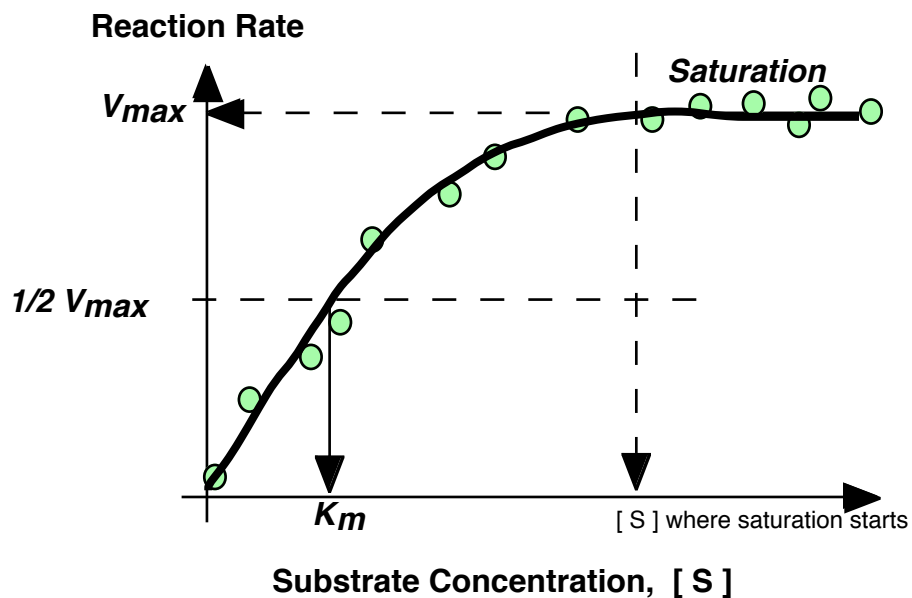
Now, repeat the measurement under identical conditions. Here is an example of the results of the previous measurements combined with another set of measurements at a lower substrate (lactate) concentration. The concentration of enzyme is the same for both:



Notice that the maximum reaction rate (given by the slope of the tangent line for each curve) is less when there is less substrate present. On the other hand, if we continue increasing the amount of lactic acid, we will reach a point where additional lactate no longer causes a greater maximum reaction rate:



Notice that the maximum rate in these cases is much greater than in the previous ones. Now, we take the maximum rate for each [lactate] and make a plot:



- Notice what this graph shows us. For a constant amount of enzyme:  
*Up to a point* the reaction rate increases with an increase in substrate (in this case we only varied one substrate (lactate) but we made sure that there were large amounts of the other (NAD<sup>+</sup>))
- Accordingly, there also exists a [substrate] which when exceeded does not result in any further increase in the reaction rate (for a given amount of enzyme) . At this point we say that the reaction has reached its maximum velocity or  $V_{max}$  .
- Let's explain the general shape of the curve. At very low [S], the reaction rate is low because there is so little substrate. Under these conditions, what substrate there is will have no trouble finding an enzyme molecule. The rate is low simply because there is little substrate present, not because it is hard to find an enzyme molecule. As [S] increases, the reaction rate increases since more substrate molecules per time are finding enzymes that then catalyze the reaction. However, if the [S] is sufficiently high, every enzyme molecule is continuously occupied with substrate catalyzing a reaction. As fast as it completes a reaction and releases products, a new substrate molecule arrives. It is like a production line worker -- it takes a certain amount of time to do the job it is supposed to do. When the conveyor belt moves slowly the worker is not always working and the output of the production line is low. As the belt speeds up, the worker spends more time occupied and the output increases. However, a point is reached where the line goes so fast that the worker cannot work any faster. S/he will let some items pass without working on them. The output of the line will not decrease but nor will it increase. Likewise, when the population of enzyme molecules is fully occupied -- turning over product as rapidly as possible, we say that it is **saturated**.

After reaching saturation, adding more substrate (the equivalent of speeding up the line) will not result in any increase in reaction rate.

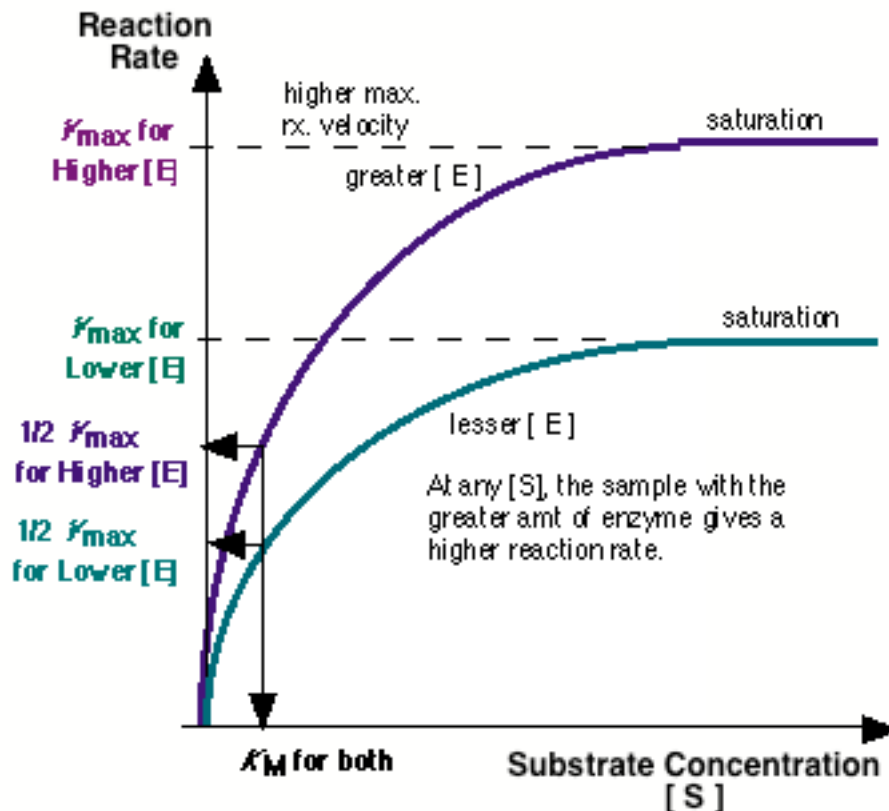
- Working backward from the saturation point we can arbitrarily define another point which will have great usefulness to us. This point, called the **Michelis constant ( $K_M$ )** is the [ **substrate** ] at  $1/2 V_{MAX}$ . We will use the  $K_M$  as a **measure of the affinity of the enzyme for this substrate**. The exact meaning of affinity will be discussed below.

A small note and question -- One can determine the  $K_M$  of each reactant for the forward and reverse reactions that this enzyme catalyzes. Interestingly, they are not all exactly the same even if the stoichiometry of the reaction is say 1:1:1:1 as in our  $A + B \rightleftharpoons C + D$  example. It all has to do with affinity.

How would you determine the  $K_M$  for  $NAD^+$  for the LDH reaction? (The LDH reaction is the one considered over the last several pages).

Oh yes, an additional note of no great import -- the graph used above wherein  $K_M$  was determined using a curve is not the way that real biochemists determine  $K_M$ . They convert the data to straight lines (called a Lineweaver-Burke plot) and use some fancy math to find the value. I just mention this for completeness -- I won't be asking you about it!

In the previous experiments we kept the amount of enzyme constant. It would be good to know the relationship between the **amount of enzyme and the reaction rate**. To answer this, we simply repeat the last measurements but this time we do them for a series of different amounts of enzyme. In other words, we put in more or less enzyme than above and then we vary the concentration of lactate and observe the rate. We get a different curve for each [enzyme] – after all it is the important variable in this example. An example of a typical result is given on the next page:



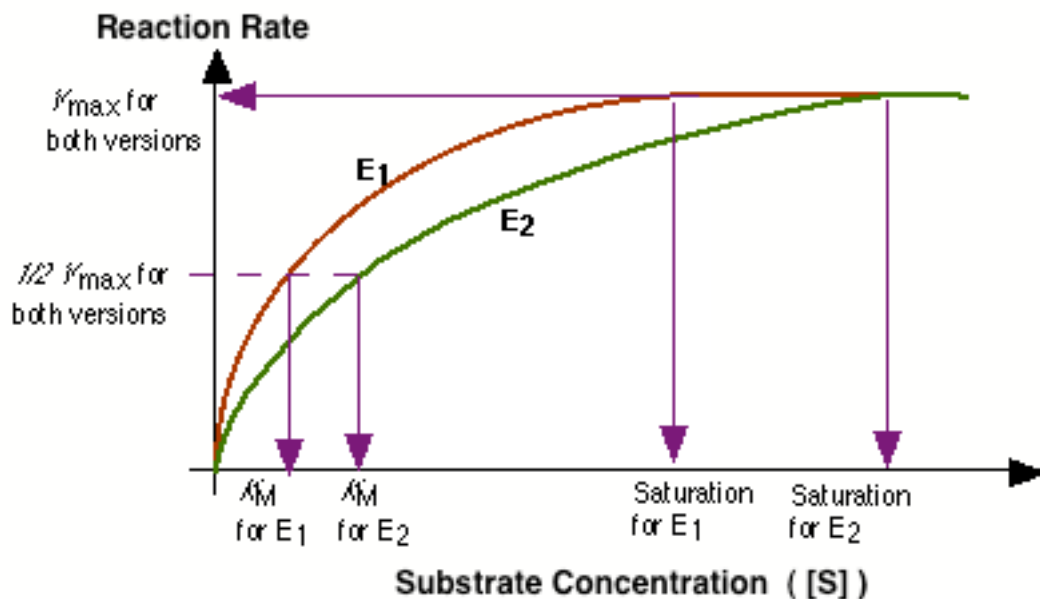
**The more enzyme molecules of a certain type are present, then, for a given concentration of substrate, the faster the reaction will go.** Therefore, a good way to increase reaction rate is to add more enzyme.

The total amount of particular enzyme present in a cell is controlled by three different processes. The first can be called **gene expression**. Various processes that we will soon look at control the rate at which the information in genes is copied into messenger RNA and translated to proteins. The particular genes that help control this rate are called regulatory genes (as compared to the structural genes that contain the information on the primary structure). Second, there is the **rate at which a protein is broken down** by the cell. Finally, cellular processes **can activate or inactivate pre-existing proteins**. In some cases, these are reversible; this is especially common with many types of quaternary structure proteins. In other cases, such as zymogens, the activation is one time affair.

Now, what if we use **different versions of the same enzyme** -- *i.e.*, enzymes that catalyze the same reaction but that have slightly different tertiary structures. Such situations are common. There are **two ways to get differences in enzyme tertiary structure**:

- **Different primary structures** -- these are normally called allozymes or isozymes; they are produced by different alleles for the same gene; *i.e.*, by slightly different versions of the same gene. We have already seen that differences in primary structure, when expressed in the same cellular environment, result in differences in tertiary structure. For the case of lactic dehydrogenase, your body produces several different versions of the enzyme that are based on slight differences in primary structure. You have one kind in your skeletal muscles and another slightly different form in your heart.
- **The same primary structure but different environments** -- we could alter the chemical and/or physical environment in which the enzyme was formed or is being maintained. The result would be differences in tertiary structure.

Let's see what happens if we repeat the first set of experiments again -- we will keep the amount of enzyme exactly the same for each "version" of tertiary structure and as before we will vary the concentration of substrate. Eventually we end up with two summary graphs of the reaction kinetics:



Let's summarize what this graph tells us:

1. First, remember that we have included the same overall amount of enzyme in both cases. Thus, the maximum rate is the same for both versions of the enzyme.
2. However, notice that version#1 (E1) saturates at a lower concentration of substrate than does the other version (E2).
3. Moreover, notice that at any [S] below saturation that E1 gives a higher rx rate than does E2. How does this happen?

At any given [S] both E1 and E2 are being hit by the same number of substrate molecules per unit time (remember our earlier discussions of how enzymes work).

The difference is that E1 is:

(a) better able to bind the substrate to itself than is E2 and/or

(b) **E1 more likely to hold onto to a substrate molecule long enough for it to react.**

Thus, we say that E1 has a higher affinity than E2 for its substrate. We measure this difference in affinity by the difference in  $K_M$  -- the lower the  $K_M$ , the higher the affinity. In the example above, the  $K_M$  for enzyme 1 (the substrate concentration at  $1/2 V_{MAX}$  is considerably less than E2.

4. What is the significance to different  $K_M$  values? Since for most reactions in cells the [S] are below saturation, we can see that E1 (with the lower  $K_M$  higher affinity) will typically give a faster reaction rate.

Why aren't all enzymes selected either for very high affinities or for their regulatory genes selected to give high rates of transcription and high concentrations of enzyme? The answer is almost certainly related to conservation of materials and demands shaped by other processes. For instance, it makes no sense to have very large aerobic capacity (ability to use  $O_2$ ) at a cellular level when if it is impossible for the rest of the body to deliver oxygen at a rate sufficient to match this ability. Thus, there would be no selection to increase the frequency of super high affinity versions of an enzyme by causing its possessors to have more children. Likewise, regulatory genes that caused exceptionally large amounts of enzyme to be made would waste amino acids that could be used for other things.

**REGULATION OF ENZYMES.** Many but certainly not all enzymes are regulated. This means that certain chemical signals can alter the shape of their active sites and make them better or poorer enzymes -- by changing the  $K_M$  or changing the ability to act catalytically. Put another way, when an enzyme is regulated it is possible to turn the rate of its reaction up or down.

#### Questions

At typical cellular concentrations of a substrate, what effect would lowering an enzymes  $K_M$  have on the reaction rate?

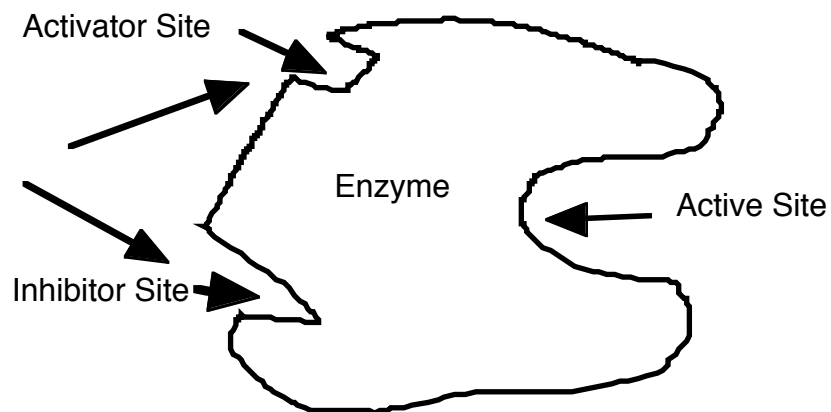
Explain what a lowered  $K_M$  does to the stability and ease with which the E-S complex is formed.

As  $K_M$  is lowered, what happens to the "goodness of fit" between the enzyme and its substrate?

Given your answers to the questions immediately above, why then aren't  $K_M$  values always as low as possible for every enzyme?

Chemical substances that cause the enzymes ability to act as a catalyst to be enhanced are called **ACTIVATORS** while those that diminish its catalytic ability are called **INHIBITORS** (or deactivators).

Activators and inhibitors work by binding to specific sites away from the active site. We call these sites **REGULATORY SITES**. Like the active sites, regulatory sites are specific to certain substances. You should be able to imagine how different substances binding to different specific places on a protein could cause the protein's shape to change so that the active site is better or worse for catalyzing the reaction. Removing an activator or inhibitor from their binding (regulatory site will remove the activation or inhibition). Here's a schematic of an enzyme that whose catalytic ability can be turned both up and down:



Although the protein shown above is tertiary, it is in fact more common for "regulated enzymes" to show 4<sup>o</sup> structure where there are separate subunits for each regulatory site that are different from the polypeptide that contains the active site.

One final word about inhibition. The figure above shows one specialized type of inhibition called **NON-COMPETITIVE INHIBITION**. Another common type, although used less in regulation, is called **COMPETITIVE INHIBITION**. It features an inhibitor that mimics the part of the substrate that would normally enter the active site. It can therefore bind to the active site. However, when it does so, it is not broken down at any appreciable rate and therefore, to the extent that the competitive inhibitor remains bonded to the active site, the enzyme cannot bind its normal substrate and the normal reaction proceeds slowly. By contrast, as is evident in the diagram, non-competitive inhibitors do not have to have a shape that mimics the normal substrate. They bind elsewhere and have their effect allosterically.