

## Topic 7: METABOLISM: THERMODYNAMICS, CHEMICAL EQUILIBRIA, ENERGY COUPLING and CATALYSIS (lectures 9-10)

### OBJECTIVES:

1. Understand the concepts of kinetic vs. potential energy.
2. Understand the concepts of free energy and entropy; use these concepts and thermodynamic principles to show whether a particular reaction is going to be spontaneous or not.
3. Be able to define equilibrium constant and how this relates to degree of spontaneity of a given reaction.
4. Understand the process by which an endergonic reaction is coupled to a highly exergonic reaction and the role of ATP in biological systems.
5. Understand the principle of mass action.
6. Draw a free energy diagram to explain the concept of activation energy ( $E_a$ ) and then show the impact of enzymatic catalysis on  $E_a$ .
7. Understand the concepts of enzyme velocity, maximal velocity ( $V_{max}$ ) and "affinity" as well as the factors (substrate concentration, pH, temperature etc.) which impact the rate of enzyme catalyzed reactions.

**Energy-** physico-chemical term for the capacity to do work (work = moving a force over a distance); units are in calorie or more commonly in Joule. (note: force = mass x acceleration). There are two forms of energy:

- (1) **kinetic-** energy that is actively engaged in doing work
- (2) **potential-** energy that is not actively engaged in doing work but has the potential to do so.

**Energy transformation-** the process by which energy is converted from one form to another

- chemical energy into mechanical energy as would take place during muscle contraction
- chemical energy into covalent bonds as would take place during the biosynthesis of macromolecules

**Bioenergetics-** the study of energy conversion in biological systems

**Metabolism-** the sum total of all the chemical reactions taking place in an organism; consists of a network of chemical reactions often called pathways. Two general types of pathways:

- (1) **catabolic-** breakdown complex molecules into simpler molecules
- (2) **anabolic** - form complex molecules from simpler molecules; biosynthesis requires energy input

**Thermodynamics-** the study of energy transformations as applied to all physico-chemical systems including biological.

Consider the following model chemical reaction:



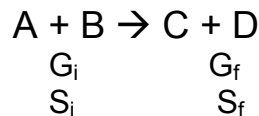
we ask the simple question, what determines whether this reaction takes place spontaneously or not? The principles of thermodynamics help us to understand this question. First of all we need to define yet another term-

**free energy**- as applied to molecular reactions, it is the energy available to do work; often denoted by the symbol G (for Gibb's free energy)

**first law of thermodynamics**- energy transformations do not create nor destroy energy but simply result in the interconversion from one form to the other

**second law of thermodynamics**- all energy transformations result in an increase in disorder; **entropy** is a term which is a measure of the extent of disorder in a system. Thus, the second law can be restated by saying that all energy transformations result in an increase in entropy in the system.

Now let's apply the above two laws to defining whether a reaction is spontaneous or not-



where  $G_i$  = free energy at initial state;  $G_f$  = free energy at final state and  $\Delta G = G_f - G_i$

and  $S_i$  = entropy at initial state;  $S_f$  = entropy at final state and  $\Delta S = S_f - S_i$ . Thus, when

$\Delta G$  = negative value, reaction is spontaneous; it is said to be **exergonic**; spontaneous reactions lead to a decrease in free energy.

$\Delta S$  = positive value, reaction is spontaneous; spontaneous reactions lead to an increase in entropy.

**Exergonic reactions lead to a decrease in free energy and an increase in entropy.**

**Endergonic** reactions- are not spontaneous; movement in this direction would lead to an increase in free energy and a decrease in entropy. A good example is biosynthesis of large molecules. We'll see in a few minutes how it is possible to drive endergonic reactions by coupling them with exergonic reactions.

Fig. 6.5- relationship of free energy to stability, work capacity and spontaneous change

Fig. 6.6 – exergonic vs. endergonic reactions

Chemical equilibria.

Suppose you mix A and B together; they will react to form C and D which will accumulate. C + D will begin to react to form A + B. Eventually,

$A + B \rightarrow C + D$  reaction rate =  $C + D \rightarrow A + B$  reaction rate; at this point we can say that the reaction has reached **chemical equilibrium**. Each kind of reaction has its own unique chemical equilibria which can be defined by the equilibrium constant (Keq)-

$$\text{Keq} = \frac{\text{(product of concentrations of products at equilibrium)}}{\text{(product of concentrations of reactants at equilibrium)}}$$

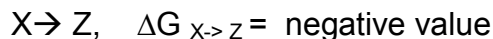
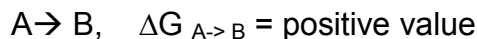
in our example above  $\text{Keq} = \frac{[C] \times [D]}{[A] \times [B]}$

### Equilibria and spontaneity.

- (1) reactions which have  $\text{Keq} \gg \gg \gg 1$  are highly exergonic
- (2) reactions which have  $\text{Keq} \ll \ll \ll 1$  are highly endergonic

However you can make an endergonic reaction go in a non-spontaneous direction by coupling it with an exergonic reaction.

Energy coupling- the use of an exergonic process to drive an endergonic process  
suppose



$\Delta G_{\text{net}} = \Delta G_{A \rightarrow B} + \Delta G_{X \rightarrow Z}$ ; **if  $\Delta G_{\text{net}}$  is negative, then the  $A \rightarrow B$  reaction will proceed.**

Energy coupling is extremely common in biological systems. By far, the most common coupling reaction is the reaction which involves the hydrolysis of a compound known as ATP, adenosine triphosphate:



fig. 6.8- ATP is very unstable and is spontaneously hydrolyzed by water; this reaction, however, can be coupled to another reaction as shown in fig. 6.9 ( glutamine formation).

ATP is often referred to as the energy currency of cells; it is constantly being utilized to drive endergonic processes. In addition, ATP is unstable. If you were to add ATP to a beaker of water, it would spontaneously hydrolyze so that at chemical equilibrium, 99.99% of the ATP would have been hydrolyzed to ADP and Pi. In cells, the concentration of ATP is 100 times greater than ADP. **Thus, cells keep the ATP hydrolysis reaction far displaced from chemical equilibrium.** This is accomplished

by a process known as **cellular energy metabolism** (energy metabolism = catabolism of organic molecules yielding ATP [and other useful forms of chemical energy]).

Fig. 6.10- the ATP hydrolysis/regeneration cycle in cells

### Rates of reactions.

For a chemical reaction like  $A \rightarrow B$  the rate of the reaction is a function of the concentrations of reactants and products. Thus, the **principle of chemical mass action** tells us that we can increase the rate of  $A \rightarrow B$  by increasing [A], decreasing [B] or both. However, biological systems have evolved enzymes which function as catalysts to speed up chemical reactions (**enzyme** = catalytic protein; **catalyst** = an agent which accelerates a chemical reaction without being consumed).

Energy barriers for a reaction to proceed- fig. 6.12; reactants must absorb energy to reach a critical state at which the reaction will proceed. This amount of energy is known as the activation energy ( $E_a$ ) and is unique for each chemical reaction. What an enzyme does is that it lowers the  $E_a$  by bringing the reactants very close together as well as by using the special chemistry of its amino acids to create a new chemical "pathway" for the reaction to take place (see fig. 6.13). The net effect is to speed up the reaction tremendously.

Enzyme terminology;

- (1) substrate = reactant
- (2) enzyme velocity = the rate of the enzyme catalyzed reaction
- (3) substrate specificity = the degree of selectivity for a substrate molecule; enzymes are typically very specific. This is due to the unique structure of the **active site** (**active site** = region of protein where catalysis takes place)

Fig. 6.15 -catalytic cycle

Fig. 6.14- concept of induced fit; substrate causes 3-D change in enzyme

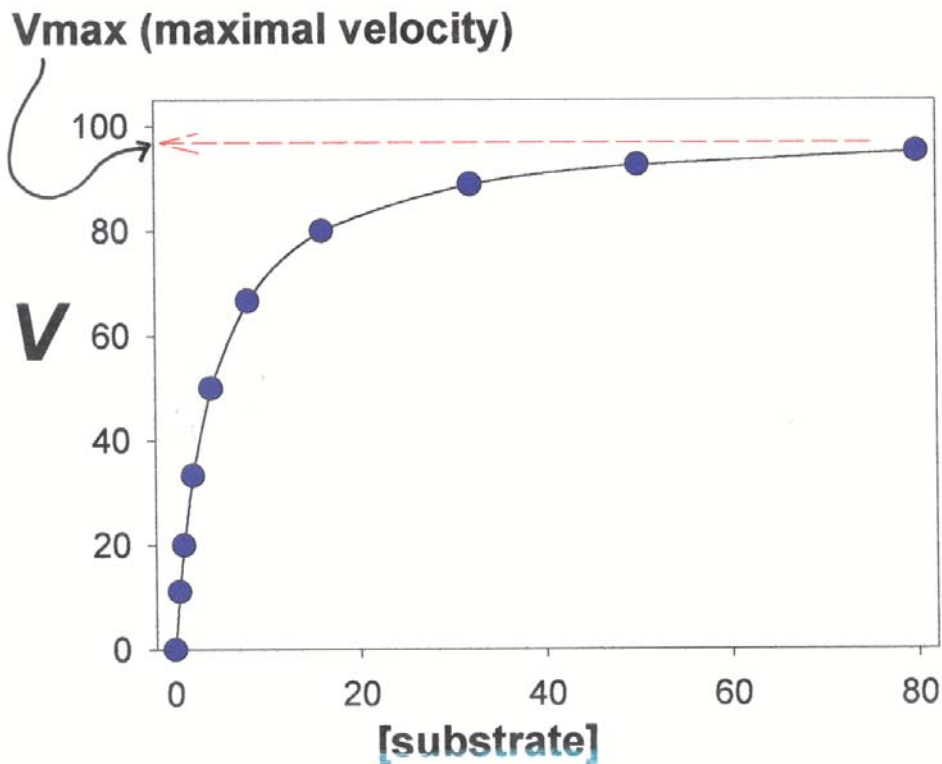
- (4) substrate affinity = a measure of the how readily an enzyme binds a substrate molecule for catalysis

Factors influencing enzyme velocity

- (1) **Substrate concentration ([S])**- as [S] increases, enzyme velocity (V) increases; however, the relationship (below) is a rectangular hyperbola.

Thus, velocity increases rapidly at low [S]'s but the rate of increase decreases at higher [S]'s and reaches a maximum and longer increases even if [S] is increased. Under

these conditions, the enzyme is said to be **“saturated”**; at any given point in time all enzyme molecules are in the act of catalysis.



(2) **“affinity” of enzyme for substrate** (substrate affinity = *a measure of the how readily an enzyme binds a substrate molecule for catalysis or alternatively, the strength of binding of S to the enzyme*)- enzymes differ in terms of how readily they bind substrate molecules. This in turn influences the impact of [S] on enzyme velocity. Let’s consider two enzymes that differ in terms of their affinity for substrate. This would be reflected in terms of the shape of their V vs. [S] curves. The low affinity enzyme would have a curve shifted to the right which means that at lower [S]’s, this enzyme would have a lower V than the V for the corresponding high affinity enzyme.

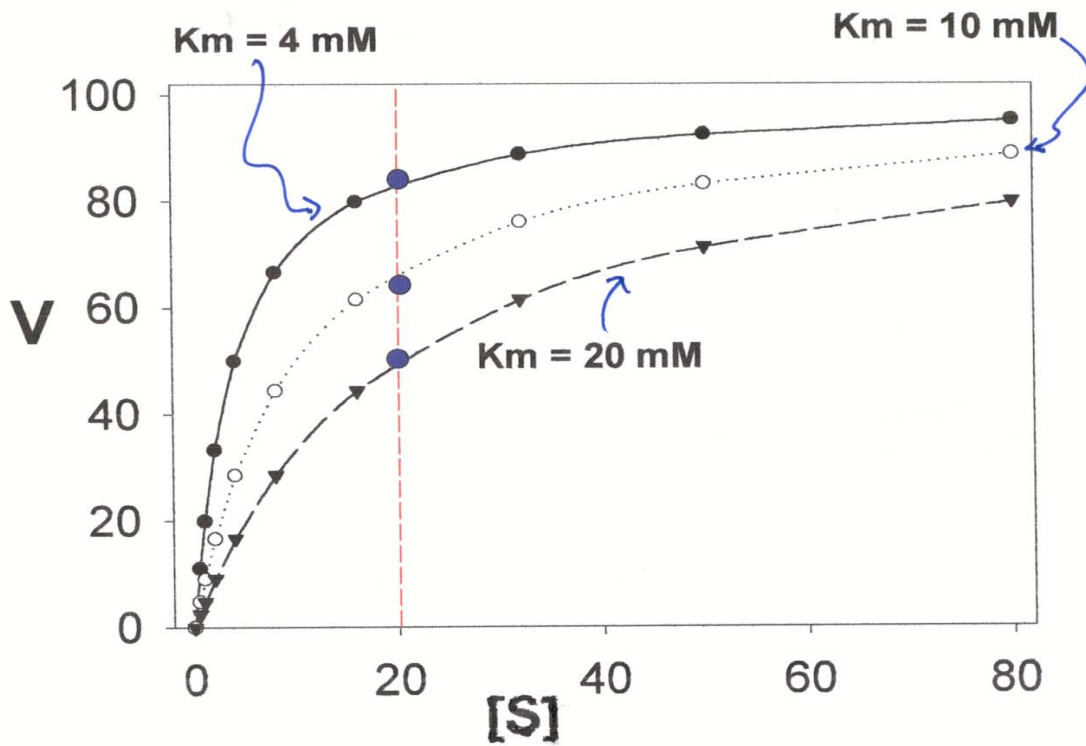
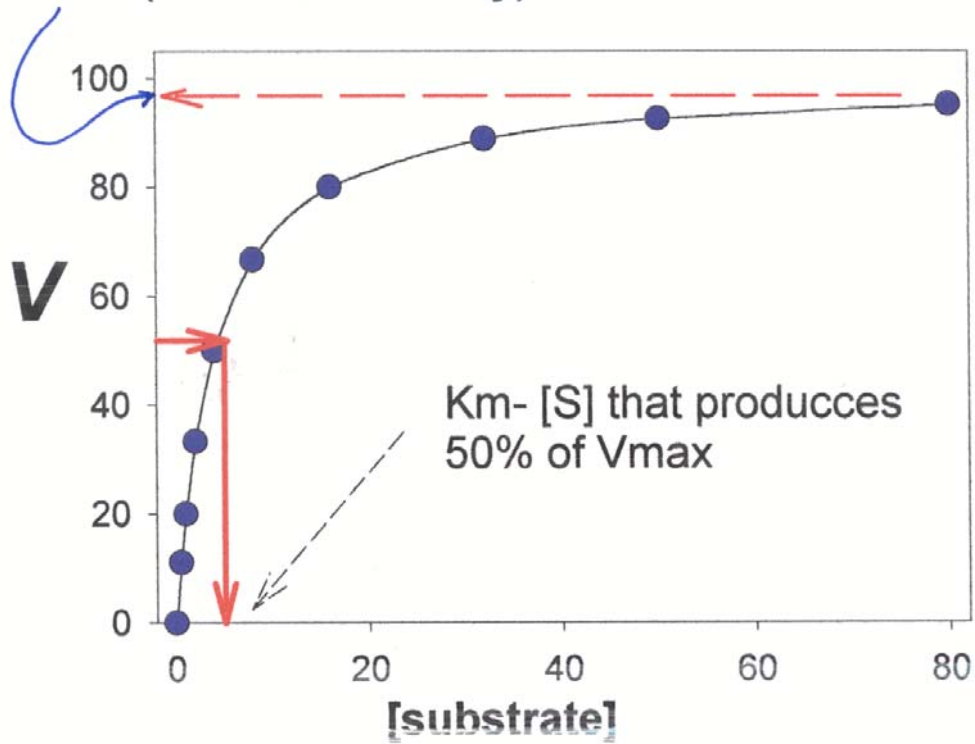
a quantitative index for affinity- “high” and “low” affinity are imprecise, qualitative terms. Thus, it is useful to use a more quantitative index. The useful index is the  $K_m$  (Michaelis constant; named after a 19<sup>th</sup> century physical chemist)

$K_m$  = [S] that produces 50% of maximal velocity ( $V_{max}$ ,  $V_{max}$  = maximal velocity of the enzyme catalyzed reaction; enzyme is “saturated”).

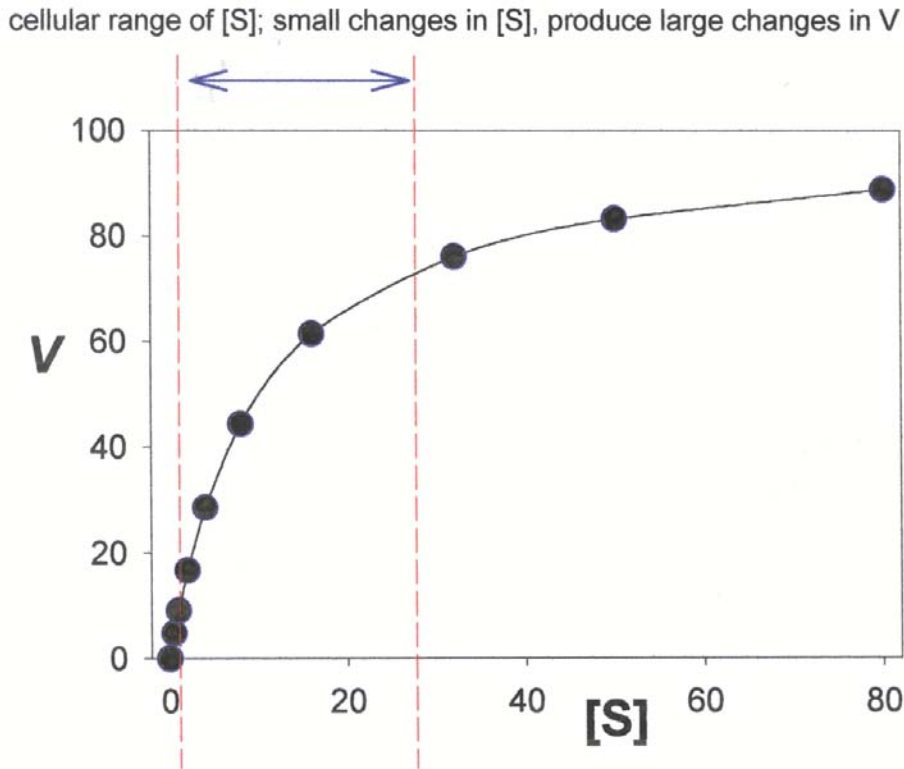
The higher the  $K_m$ , the lower the affinity ( and vice versa). Affinity is important when viewed in the context that the enzyme is functioning, namely inside of cells.

$K_m$  is an index of affinity

**$V_{max}$  (maximal velocity)**



One typically finds that the  $K_m$  for a typical enzyme falls in the range of concentrations of the substrate in the cell. This means that small changes in the physiological  $[S]$ 's produce large changes in the velocity of the enzyme catalyzed reaction.



- (3) **temperature**- temperature increases cause an increase in the rates of enzyme catalyzed reactions (fig. 6.16); however, at critical temperatures weak bonds start to break and activity will fall and reach zero when the protein is denatured.
- (4) **pH**- hydrogen ions may be participants (literally substrates) in enzyme catalyzed reactions; further, changes in pH may alter the ionization states of amino acid residues. Thus, it is not surprising that pH influences enzyme activity. Typically, enzymes show pH optima (fig. 6.16).
- (5) **low molecular weight activators and inhibitors**- often the activity of enzymes is regulated by low molecular weight organic (and sometimes inorganic molecules). These serve to change activity by altering  $V_{max}$  and/or  $K_m$ .
  - a. activators- bind to some binding site other than the active site; typically increase affinity (observed decrease in  $K_m$ ).
  - b. inhibitors- reduce enzyme velocity - Fig. 6.17

**competitive inhibitors**- mimic the structure of the substrate; compete with the substrate for binding at the active site. In effect, the inhibitor raises the  $K_m$  thereby reducing its apparent affinity for substrate

**non-competitive inhibitors**- bind to some site other than the active site