(with embedded Q&A)

Lec. 8. Biol C2005/F2401 2010 L. Chasin September 30, 2010 Last updated: Thursday, September 30, 2010 12:35 AM

What is the function of these enzymes in the cell? The enzymes are **central** to the whole problem of building two new E. coli cells from one old one. Remember the flow of glucose carbon atoms into the 50 different small molecules, which go on to form the 5000 different macromolecules? Each arrow in those biosynthetic pathways represents an enzyme, a particular protein with a particular amino acid sequence, with a particular 3-D structure, which can bind and catalyze the chemical transformation of particular substrates into particular products (and having particular Km's and t.o.n.'s). (Draw arrows and letters emanating from glucose).

(Enzyme inhibition)

Let's continue the discussion of enzymes by considering the **modulation** of enzyme activity, through 3 types of inhibition of an enzyme's catalytic action.

The 3 types are inhibitors I want to discuss are :

- competitive (e.g., illustrating the action of drugs)
- non-competitive (e.g., poisons)
- allosteric (e.g., characteristic of natural feedback regulators)

(competitive inhibition)

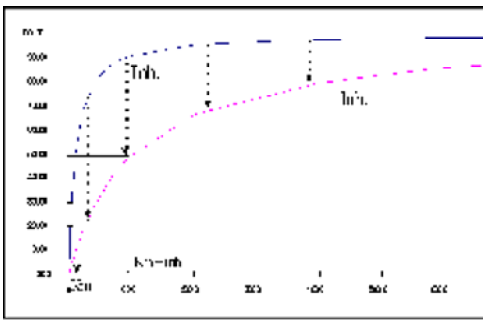
A competitive inhibitor is a molecule that competes with the natural substrate for the substrate binding site of the enzyme. {Q&A} In the presence of a **COMPETITIVE INHIBITOR:**

At low [S], you may get good effective inhibition as long as the substrate concentration is low,

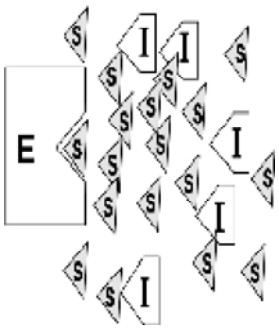
but at high [S], the comp. inhibitor (I) will be swamped out. {Q&A}

Let's look at this situation on our Vo vs. S plot.

At a given concentration of I, adding more and more S will eventually bring you back to Vmax, as the inhibitor (I) is swamped out.



Competitive inhibition



The **APPARENT Km** in the presence of the inhibitor, will be increased, reflecting the difficulty the substrate is having in getting bound to the enzyme in the face of all this competition from the inhibitor. {Q&A}

It follows that since the competitive inhibitor must bind to the same site as the substrate it should have a structure VERY SIMILAR TO THE SUBSTRATE. See [Purves 6.21]. {Q&A}

Why is competitive inhibition important? Because since we know the structure of the substrate, we, with the help of organic chemists, can design and synthesize candidate organic molecules as specific enzyme inhibitors, which can be useful in medicine.

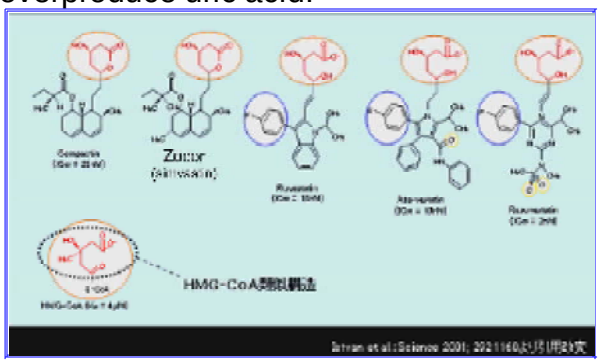
For example, this morning, as every morning, I took 100 mg of the drug allopurinol, which has the 2-ring structure as seen on the handout.

I took it because my uric acid (see the uric acid structure in the handout) levels are too high. ..... Uric acid is not very soluble, so if the concentration in the blood is too high, it can precipitate and settle, collecting in capillaries of your feet, and you have gout, a painful condition. Or it can precipitate in the urine as it is formed (thus the name), leading to kidney stones.

Uric acid is produced by the action of the enzyme xanthine oxidase, that catalyzes the reaction :

hypoxanthine (from nucleic acids we eat) -------> uric acid (which is excreted) (see handout)

For some unknown reason, this enzyme becomes overactive in some people, so that they overproduce uric acid.



The allopurinol can bind to the xanthine oxidase, but it cannot be oxidized (Note differences in the 5-membered ring and where the OH's are introduced). The binding of allopurinol prevents the binding of the true substrate.

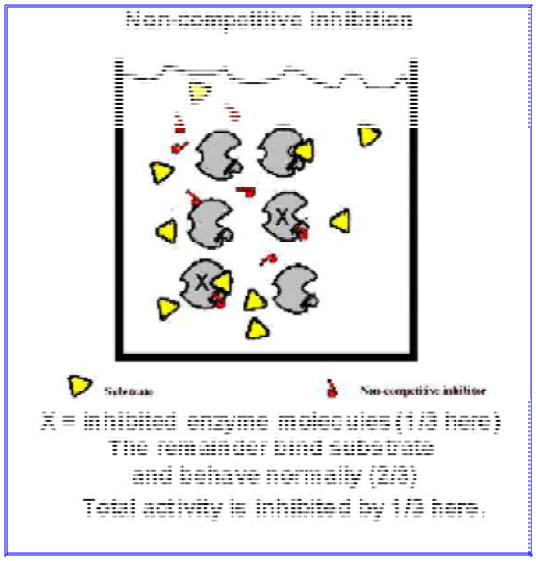
Zocor (simvastin) is another popular competitive inhibitor drug. The enzyme it inhibits an early enzyme in the pathway of cholesterol biosynthesis. It resembles HMG-CoA, the substrate of this enzyme, and can lower blood cholesterol in persons making to much of this lipid. Many of the anti-HIV drugs [e.g., AZT, ddC] are competitive inhibitors of the enzymes that polymerize the viral nucleic acid. So competitive

inhibitors are important for pharmaceutical applications. And they are important to the biochemist, as she or he can use various analogs of the true substrate to probe the nature of the active site, to determine just what groups on the substrate are being recognized in the active site.

(non-competitive inhibition)

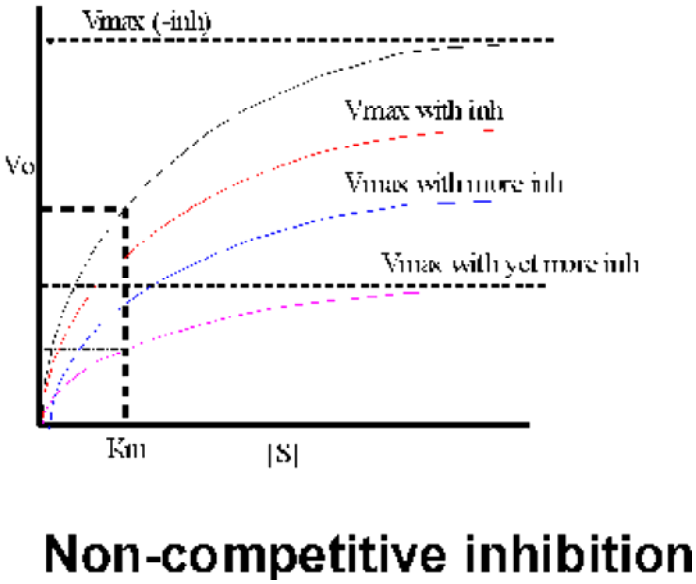
Non-competitive inhibitor, NCI:

But a chemical compound need not always look like the substrate in order to act as an inhibitor. A non-competitive inhibitor can binds to a site other than the substrate binding site and inhibit activity. These inhibitors bind at or near the active site, although NOT in the substrate binding site. These inhibitors usually interfere with the catalytic reaction itself (the formation of a transition state complex on the surface), so the substrate still binds to the enzyme but then the enzyme can't do anything with it. Take mercury (Hg), for example; unlike allopurinol, it is a poison rather than a therapeutic agent. Hg++ ions can bind to free sulfhydryl groups, which in proteins means the side chain of cysteine. Sulfhydryls often take part in the catalytic process, but if Hg is bound the sulfhydryl may not be able to do its job. In this case, adding an excess of substrate will NOT reverse the action of the inhibitor. The Vmax of the reaction will be affected by the non-competitive inhibitor, but not the Km. {Q&A}



How will a non-competitive inhibitor affect the measurement of enzyme kinetics?

Graphic we can see in the graph that at a given concent of inhibitor we get a lower Vmax, and the higher the concentration of the non-competitive inhibitor, the lower is the achievable Vmax.



Non-competitive inhibition

How can we envision this?

Think of a beaker of enzyme. If we add an excess of non-competitive inhibitor, we cannot measure any activity, so we cannot investigate any effect. So let's add a sub-saturating amount of non-competitive inhibitor. At any given moment, a constant proportion of the enzyme molecules will be taken out of action because they are binding the non-competitive inhibitor . Let's say 2/3 are inactive at any moment (but in a dynamic equilibrium):

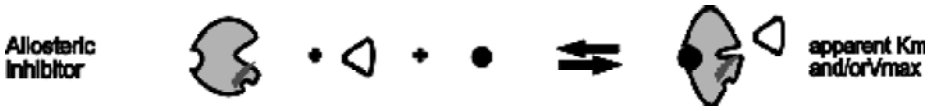
The remaining 1/3 are OK and act perfectly normally with respect to their dependence on S. It is they that contribute to the V being measured on the graph.

So the Km measurement is not affected; this uninhibited fraction of the enzyme population gets to 1/2 of the new (lower) Vmax that can be attained at the usual S concentration. But the Vmax they attain will be only 1/3 of that reached in the absence of the non-competitive inhibitor. So here the apparent Vmax is lowered and the Km is unaffected, just the opposite of what we saw with a competitive inhibitor.

To test your understanding of competitive vs. non-competitive inhibitors, try problem 3-1 part B, and problem 3-13.

(allosteric inhibition)

The third type of inhibitor is called an **ALLOSTERIC INHIBITOR**. This is the most important natural enzyme inhibition. Living cells use enzyme inhibitors to regulate the activity of many enzymes. This kind of inhibition is similar to non-competitive inhibitor, in that the inhibitor binds to a site different from the substrate binding site. In this case it binds to its very own special site, a site that has evolved for this purpose, remote from the substrate binding site. The inhibitor acts by deforming the enzyme so that it either can no longer bind its substrate or cannot catalyze its reaction. {Q&A} Enzymes that are regulated by allosteric inhibition are usually hetero-multimers composed of regulatory subunits and catalytic subunits. The allosteric inhibitor binds to the regulatory subunits in a reversible manner changing their conformation. {Q&A} The catalytic site on another subunit is turned on or off depending on the conformation of the regulatory subunits. The quantitative treatment of this inhibition is beyond our scope here. But see Purves6ed 6.23 for a picture.



SO: Allosteric inhibitors (black ball in the diagram) can affect the apparent Km, Vmax, or both.

{Q&A} {Q&A}

How does the cell use this allosteric inhibition? {Q&A}

(Feedback inhibition)

The most obvious roles for allosteric inhibiition in the E. coli cell are for those enzymes that catalyze the reactions of a biosynthetic pathway.

E. coli growing on glucose as the only source of carbon must synthesize all of its small molecules by a series of chemical reactions that emanate from glucose. In general, glucose is first broken down from its 6-carbon state to smaller molecules of 3, 2 or 1 carbons, and then these smaller molecules are used to build all the necessary monomers and co-factors in the cell.

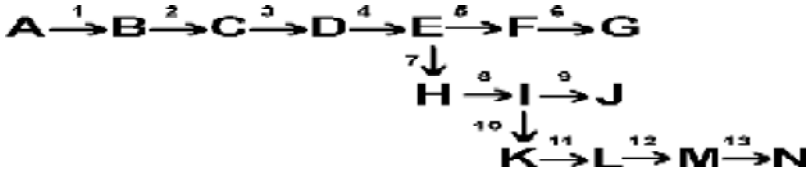
All the monomers, the 20 amino acids, the prosthetic groups like heme, virtually every molecule in the cell has a pathway that can be traced back to glucose. These pathways do not simply run at equal and constant rates, but rather are tightly controlled and coordinated. Much of this coordination is brought about by the cell's judicious use of allosteric inhibition. The very molecules that are being synthesized (the end-products of the pathway) are often used as the mediators of this inhibition; they are the allosteric inhibitors, as we will see in a moment.

(A pathway traced back from an end-product to glucose can usually be divided into two parts, the

breakdown part, called **catabolism**, and then the build-up part, called **anabolism**.

Or catabolic pathways vs. anabolic pathways (the anabolic pathways are more often called biosynthetic pathway or biosynthesis).

So: Glucose ---->



Each arrow in the diagram represents a specific chemical transformation, such as the reduction of fumarate by succinic dehydrogenase, or the oxidation of hypoxanthine by xanthine oxidase.

To coordinate all this activity, E. coli has evolved a sophisticated and sensible way of controlling the flow of glucose carbons through its biosynthetic pathways. This is called end-product inhibition, and usually works by having the end-product of a biosynthetic pathway act as an allosteric inhibitor of the first step in the pathway that is **committed** to the biosynthesis of that molecule (e.g., rxn. 5 for G, rxn 10 for N, rxn. 9 for J in the diagram above). That is, the first enzyme in a pathway has evolved to have, *built into its structure*, a site for the ultimate end-product of the pathway it is starting. {Q&A} Since the end-product is usually many steps down the road of chemical transformations, it does not bear much resemblance to the substrate or the product of the reaction it is inhibiting. See [Purves6ed 6.24].

For example, the pathway to the amino acid isoleucine can be considered to start as a branch after the synthesis of the aa threonine:

Thr deaminase
glucose ..... --> --> threonine ----------> alpha-ketobutyric acid --> A --> B --> C --> isoleucine (and no other aa)

Isoleucine turns out to be an allosteric inhibitor of the enzyme threonine deaminase

In this way the pathway to isoleucine is shut off when enough isoleucine is present - the cells has feedback information about the amount of end-product it is synthesizing. This mechanism of feedback inhibition or end-product inhibition is used extensively in E. coli to avoid the waste of running a pathway when it is not needed.

Moreover, this end-product inhibition works whether the end-product (e.g., isoleucine) was home-grown (the product of E. coli's biosynthetic pathway) or if was simply provided in the the medium or in E. coli' s natural environment. Does this mean that E. coli could divide a bit faster if we gave it isoleucine in addition to glucose in minimal medium? Could it shave its doubling time from 60 minutes down to say 55 minutes by shutting off that path? While we're at it, why not add all 20 amino acids, and a few vitamins, some fatty acids, some DNA and RNA precursors. Now would E. coli grow any faster? Indeed they do, in such a rich medium they will double every 20 minutes, instead of once an hour. A whole new E. coli cell in 20 minutes. Now feedback inhibition is not the only reason for this increased efficiency, but it is playing a large part. And you can imagine that this ability to shut-off off and turn on biosynthetic pathways fits the life style of E. coli as it sits in your intestines waiting for the next Big Mac. The more efficient doublers will soon take over the population, so there probably has been great selective pressure for the evolution of control

mechanisms that respond to the environment in this way.

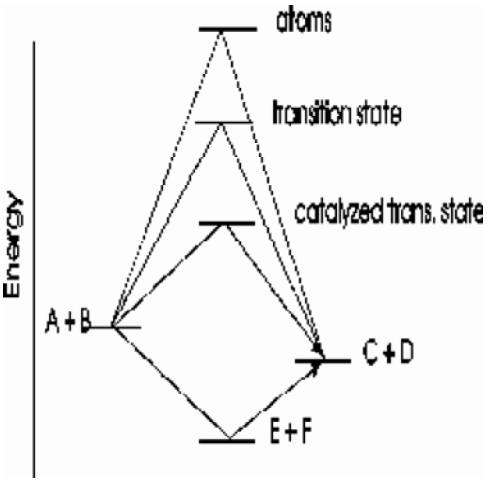
One can see why you need the allo-steric ("different in space") inhibition mentioned above for the feedback regulation of this biosynthetic pathway. Note that the inhibitor, isoleucine, bears little resemblance to the substrate (threonine) for the reaction it inhibits. Isoleucine binds not to the catalytic site (active site) but to an allosteric site, a special regulatory site that is remote from the catalytic site. Indeed, in the regulatory site is located on a separate polypeptide in threonine deaminase, a protein with quaternary structure.

For problems involving allosteric inhibition, and regulation of pathways, see problems 3-8 & 3-10.

(Free energy, Delta G and Delta G^{o})

We now have some idea of how the **rate** of chemical reactions in the cell are speeded up so that they can occur in time frames consistent with cell growth. But what about this problem of **DIRECTION**, a problem the catalysts cannot address. Are all the chemical transformations in these biosynthetic paths, for example, exergonic, so that the direction of carbon flow from glucose is a spontaneous reaction? The answer is no, quite the opposite. Most of the chemical transformations needed in a biosynthetic pathway are by themselves **ender**gonic, and will not go from left to right unless something is done about it. Thus the building of a new E. coli cell must deal with this energy problem, and much of the cell machinery has indeed been devoted to the solution. To understand the problem, we must discuss it some more quantitative detail. and for this purpose, the use of the concept of FREE ENERGY CHANGE associated with a chemical reaction is very useful.

We can define a unit of energy such that it is tied to the directionality of a chemical reaction as follows:



{Q&A}

For the model reaction A + B <--> C + D, written in the left-to-right direction indicated:

IF Delta G IS <0 THEN A AND B WILL TEND TO PRODUCE C AND D.

IF Delta G IS >0 THEN C AND D WILL TEND TO PRODUCE A AND B.

IF Delta G IS 0 THEN THE REACTION WILL BE AT EQUILIBRIUM: NOT TENDING TO GO IN EITHER DIRECTION IN A NET WAY. {Q&A}

In an energy diagram:

Note that the Delta G is independent of the route between the starting reactants and the final products (say, 3 kcal/mole, for all 4 routes shown here).

Free energy is that part of the energy change associated with a chemical reaction that can be harnessed to perform work.

We can measure Delta G according to the following equation [must take this on faith here]:

Delta G = Delta G^{o} + RTln([C][D]/[A][B])

where A, B, C and D are the concentrations of the reactants and the products AT THE ***MOMENT*** BEING CONSIDERED.

This CD/AB ratio is sometimes called Q for Quotient (so Delta G = Delta G^{o} + RTlnQ).. Q is **NOT** the equilibrium constant, though it *looks* like it...:

Here are the terms:

R = UNIVERSAL GAS CONSTANT = 1.98 CAL / DEG K MOLE (or ~2)
T = ABSOLUTE TEMP. (0^{o} C = 273^{o} K; Room temp = 25^{o} C = 298^{o} K) (or ~ 300)
ln = NATURAL LOG
Delta G^{o} = A constant, a QUANTITY RELATED TO THE INTRINSIC PROPERTIES OF A, B, C, AND D. {Q&A}

The first term, Delta G^{o}, relates to the QUALITY of the reactants and products, and the second term, RTlnQ, relates to the QUANTITY of the reactants and products, how much of each is present.

What is this Delta G^{o}? It is called the: ***STANDARD*** **FREE ENERGY CHANGE** of a reaction.

One useful way to define it is to consider the special case when all the reactants and all the products are present at 1 unit concentration. The change in free energy of this reaction is:

Delta G = Delta G^{o} + RTln(1), or Delta G = Delta G^{o} +RT x 0,

or Delta G = Delta G^{o} (a special case when all participants are at a concentration of 1)

So Delta G^{o} is the free energy change that occurs when all participants in the chemical reaction are at unit concentration. What is unit concentration? 1 M, for most reactants and products (water and hydrogen ions are treated differently, as we'll see.)

One way to think of this Delta G^{o} is to picture 1000 moles of A, B, C and D in 1000 liters of solution. So all components are at 1M. One mole of A + B is now converted to C +D. The energy absorbed is the standard free energy change. Note that the concentration of reactants has not appreciably changed in this hypothetical condition.

Why make such a fanciful and arbitrary situation? By defining the conditions for a STANDARD free energy change, all reactions can be compared to one another, regardless of the particular conditions; it puts them all on an equal footing. The Delta G^{o} reflects the **nature** of the reactants and products without regard to their concentration. Note that the actual free energy change of a reaction **does** depend on these concentrations, and the SECOND TERM of the equation for Delta G takes these into account. {Q&A}

Please note from the above explanation that Delta G and Delta G^{o} are not the same thing. That

distinction is important.

(Equilibrium)
Delta G^o can be calculated, although not accurately, from tables of free energies of formation from simple atoms or molecules, comparing these values for the reactants and the products. But it is most easily and accurately determined experimentally, by measuring the concentration of the reactants and products at equilibrium. For at equilibrium,

Delta G = 0
and
Q = K_{eq}, (a special case). So: Delta G^o = -RTlnK_{eq}.

If we measure A, B, C and D at equilibrium, let's say we get: $[C]_{eq}[D]_{eq}$ /$[A]_{eq}[B]_{eq}$ = 2.5 x 10^{-3} . Note very little C and D are formed from A and B in this case.

Then Delta G^o = -2 x 300 x ln (0.0025) = -600 x -6 = 3600

So the calculated Delta G^o is +3600 cal/mole, or +3.6 kcal/mole. So 3.6 kcal will be absorbed when 1 mole of A + B goes to C + D. Energy is absorbed rather than released, confirming the lack of a tendency of C and D to be formed from A and B.

That is, since the standard free energy change is **positive**, this reaction does not tend to go to the right, but rather to the left. That is, if we start with 1M A, B, C and D, then C + D --> A + B. So A and B will build up at the expense of C + D. {Q&A}

Note: If we write A + B --> C + D, Delta G^o = +3.6 kcal/mole, then we can also write:
C + D --> A + B , Delta G^o = -3.6 kcal/mole (the equilibrium constant for this C+D --> A+B reaction is just 1/2.5 x 10^{-3}, or 400)

Note also, that if the reactants combine in ratios other than 1:1, we can write:
aA + bB <--> cC + dD, Delta G = Delta G^o + RT ln $[C]^c[D]^d / [A]^a[B]^b$

(Summary of free energy changes)
And to reiterate: the free energy change of an overall reaction is independent of the route taken by the reactants, it could be direct or indirect [see energy diagram with routes that go up or down to intermediates].

The reaction will go to the right if Delta G is <0 and to the left if Delta G is >0. At Delta G = 0 the reaction is at equilibrium; under this special condition (Delta G=0) Q is equal to the equilibrium constant K_{eq}.

We stated that Delta G^o is called the STANDARD free energy change and represents the free energy change when these reactants and products are observed to react with each being at a concentration of 1M, which is the standard condition, by definition. However, biochemists make 2 exceptions to the definition of these STANDARD CONDITIONS in the cases of water and hydrogen ion concentration. Since these two components in a reaction are usually constant in a biological reaction, they are **defined** to be equal to 1 for the purpose of Delta G^o calculation when $[H_2O]$=55M

(pure water) and [H+] = 10^{-7} M (i.e., pH7, or neutrality). Strictly speaking, one should acknowledge this use of these 2 exceptions by designating the free energy change as Delta $G^{o'}$ rather than Delta G^{o}, but we will not bother to do that here.

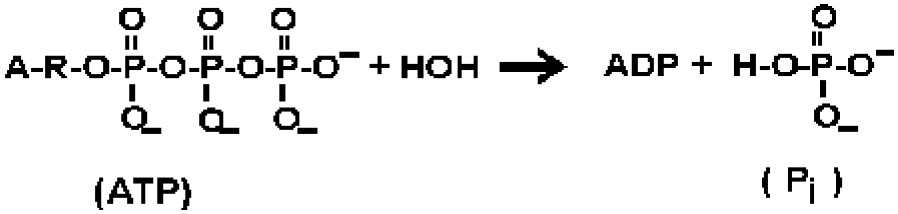
All chemical reactions have a Delta G^{o} associated with them**,** a value that can be written down in a book. Thus any two reactions can be compared under the standard condition of unit concentration.

The equilibrium constant of a reaction can be easily measured by measuring the concentration of all reactants and products after the reaction has reached equilibrium. From the equilibrium constant, one can calculate the Delta G^{o} of the reaction, since at equilibrium, Delta G = 0, and so **Delta G^{o} = -RTlnK_{eq}.** {Q&A}

Whereas the first term in the equation for Delta G takes into account the nature of the reactants, the second term deals with the amounts of the reactants and products in the situation under consideration. Thus if you know what you have and how much of each, you can predict the direction in which the net reaction will go.

[To review Delta G, Delta G^{o}, and K_{eq}, do problems 4-1 and 4-2]

(ATP hydrolysis)
To start our consideration of the free energy changes that are associated with biochemical reactions, let's consider one of the most important and fundamental energy-related biochemical reaction, the hydrolysis of adenosine triphosphate, or ATP. Hydrolysis means the breakage of bonds by the addition of water. Here:



where A = adenine (a somewhat basic 2-ring structure, called a "base" here); R = ribose, a 5C sugar; joined to 3 phosphate groups, which are themselves joined together. See [Purves picture]. The bond between the phosphates is called and acid anhydride bond, formed by the extraction of molecule of water between 2 acids.
The Delta G^{o} of this reaction is about -7 kcal/mole. {Q&A}

("High energy" bonds ("~"))
Most HYDROLYSIS reactions in the cell do release free energy, but usually less than 7 kcal/mole. The few, like ATP hydrolysis, that release at least 7 kcal/mole, are important, because this amount of energy can be harnessed for useful work, as we'll see. The bonds whose HYDROLYSIS RELEASES 7 KCAL/MOLE OR MORE ARE CALLED "HIGH ENERGY BONDS" for this reason. These bonds are not stronger than other bonds, so this is somewhat of a misnomer, but it turns out to be a convenient term. It is denoted by a SQUIGGLE when we are talking about it: AR-P-P~P. Looking at the structure of ATP we can rationalize the high energy release by seeing that the addition of water relieves the electrical repulsion between the negatively charged (acidic) phosphate groups. By such reasoning we would predict that the bond between the first and second phosphates should also be a high energy bond, and indeed it is, so:

AR-P~P~P. That is, hydrolyzing between P atoms 1 and 2 also releases about 7 kcal/mole (i.e.,

Delta G^o = -7 kcal/mole)

On the other hand the bond between the ribose and the first phosphate is not a high energy bond.

Given the Delta G^o, one can calculate the equilibrium constant, which turns out to be about 10^{+5}.

(-7 = -RT x 2.3 log K = -0.6 x 2.3 log K; K = $10^{(-7/-1.4)}$ = 10^5).

You should practice these types of calculations by doing the problems in Problem Set 4.

(In doing these problems, note that the universal gas constant is usually given as 1.98 cal/deg-mole, whereas Delta G^o and Delta G are usually expressed in kilocalories, so you usually have to divide the RT term by 1000 to get an answer in kcal.)

I will just note again that the concentration of WATER is not taken into account, as it is present at 55 M in aqueous solution and does not CHANGE in aqueous reactions, and by convention is defined as 1. (We can make such arbitrary definitions because we are only considering **changes** here [concentration changes, free energy changes], not absolute values.) {Q&A}

So if we start with 1M ATP we will end up with only about 10^{-5} M ATP remaining at equilibrium.

Yet a 1 ml. solution of ATP will last for days on this bench top. We still must add a catalyst to get this reaction to go in a reasonable time.

So we add an enzyme, say a pure preparation of **ATP-ase** (note nomenclature).

Now all the ATP is hydrolyzed in a few minutes. The reaction has been allowed to reach its equilibrium and has gone far to the right, because of the very favorable (large and negative) Delta G.

And 7 kcal/mole of energy, free energy, is released. Free energy, so it could be used for useful work, but what work did it do here? Nothing? Energy had to go somewhere. It is released as HEAT. The test tube solution warmed by about 7^{o}C, by my calculation

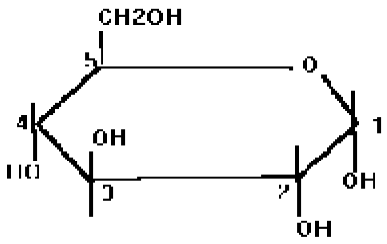
[since 1 ml of solution here, at 1 M, so 0.001 moles or 1 millimole; 7 kcal/ mole = 7 cal/ mmole; 1 cal will raise the temp. of 1 gram (ml.) of water 1 degree C or K].

This hydrolysis of ATP is in fact the most common reaction the cell uses to produce usable energy. The trick is to harness this energy chemically, to put it to useful chemical work. Keep in mind the overall problem, to make a new E. coli cell that requires many endergonic transformations.

See problem 4-1 for more on the hydrolysis of ATP.

(Coupled reactions)

So let's take an example of one of these endergonic energy-requiring reactions: One such reaction is the very first transformation that a glucose molecule undergoes upon entering an E. coli cell:



+ P_i --> glucose-6-P + H_2O; Delta G^o = +3.6 kcal/mole.
(Note that Pi is used as an abbreviation for PO_4^{---})

So the Delta G^o is unfavorable in the very first reaction in the utilization of glucose in the cell.

How bad is the situation?

G + Pi --> G-6-P, Keq = [G6P]/[G][Pi]

If [G] and [Pi] are ~ 0.01M (typical) and K_{eq} (from Delta G^o of +3.6) = 2.5 X 10^{-3} then [G6P] at equilibrium would be 2.5 x 10^{-7}, which is even below the Km's of most enzymes (for example, for the next reaction taking G6P further). Now let's consider putting the two reactions we've talked about together:

ATP + H_2O --> ADP + P_i Delta G^o = -7 kcal/mole
Glucose + P_i --> G6P + H_2O Delta G^o = +3.6 kcal/mole

Glucose + ATP --> G6P + ADP Delta G^o = -3.4 kcal/mole overall = net sum of the two considered reactions

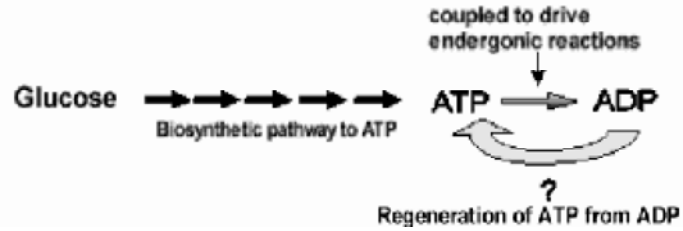
So let's mix the reactants together and hope for the best.... I guess we should add a couple of enzymes to catalyze these 2 reactions: say: "glucose phosphorylase" and "phosphatase".... But under these conditions the 7 kcal/mole produced by the hydrolysis of ATP is lost mainly to heating the surroundings (H_2O). And besides, in mammals, where E. coli lives, the temperature is kept constant, so we cannot influence reactions by heat. The problem is solved by an enzyme, **hexokinase**. This enzyme binds both ATP **AND** glucose. The **very** phosphate from the ATP is transferred to the glucose molecule. So the overall reaction written here on the bottom line is not merely the net sum of the two reactions written above it, rather, it **IS** the reaction catalyzed by hexokinase. The phosphorylation of glucose has been **COUPLED** to the hydrolysis of ATP. {Q&A} {Q&A}

For more about these reactions see problems 4-4 and 4-5. (Delta G^o values are slightly different there, as they came from a different source.)

What about the G6P produced? Does it now contain the high energy phosphate bond? Well, what is the Delta G^o for G6P hydrolysis? Easy: -3.6 kcal/mole, since reverse reaction (second line) is +3.6. But -3.6 falls short of the ~-7 needed to qualify for a high energy bond, so no squiggle here in G6P. Remember, if you write a reaction backwards, you just reverse the sign of the Delta G^o.

This **coupling** to ATP hydrolysis is a very common way the cell uses to drive otherwise endergonic reactions. [Purves picture] Since the ATP is so often used to "pay for" the energy cost of these chemical transformations, ATP is called the **energy "CURRENCY"** of the cell. Coupling to ATP is one of two ways the cell manages to run endergonic reactions; we will discuss the second way a little later.

So is this the solution for E. coli growing on



minimal medium? Far from it; we have just passed the buck. Where's this ATP coming from? Not from the medium, where glucose is the only carbon source. ATP must be synthesized from glucose just like all other small molecules. And that itself takes energy. But, once we have some ATP, most of the molecule can be used over and over again to provide energy for coupled reactions, as long as we can re-phosphorylate ADP back to ATP. The problem has thus shifted to this reaction: ADP + Pi --> ATP (Delta G^o = +7 kcal/mole). If we could find a way to do that, we would have solved our energy problem.

Here is where we have some divergence in the unity of biochemistry: The world is divided mainly into two types of organisms, those who can run this reaction using the energy derived from sunlight, the photosynthetic organisms, like plants; and the rest of us, E. coli, humans, butterflies, who make ATP from ADP by using the energy derived from the breakdown (**catabolism**) of carbon compounds like glucose. Plants are actually also included in this latter category, since when it's dark they derive energy from glucose catabolism, as the rest of us do. We will consider this process of glucose-based energy metabolism in some detail, and not really consider PHOTOSYNTHESIS, for lack of time. Obviously, photosynthesis is the more basic and essential process for the planet, since there would be no glucose if it were not for the plants and their ability to harness solar energy. But photosynthesis is a bit more complex, so it is not a good place to start.

So, ATP by way of glucose: The overall plan is (for E. coli growing in air [dissolved oxygen]): glucose + O_2 --> CO_2 + H_2O, and: ADP + Pi --> ATP

First, an overview: 3 parts: In each of these 3 parts we will be concerned with our goal: to produce ATP's for use in reactions that require energy.

1) GLYCOLYSIS: in which the 6C glucose is broken down to a 3C compound, pyruvic acid [glucose (6C) --> pyruvate (3C)].

2) THE KREBS CYCLE, in which the pyruvate is broken down to CO_2 [pyruvate --> CO_2]

3) The ELECTRON TRANSPORT CHAIN, in which oxygen is taken up and water is produced in a separate series of reactions [utilization of O_2]

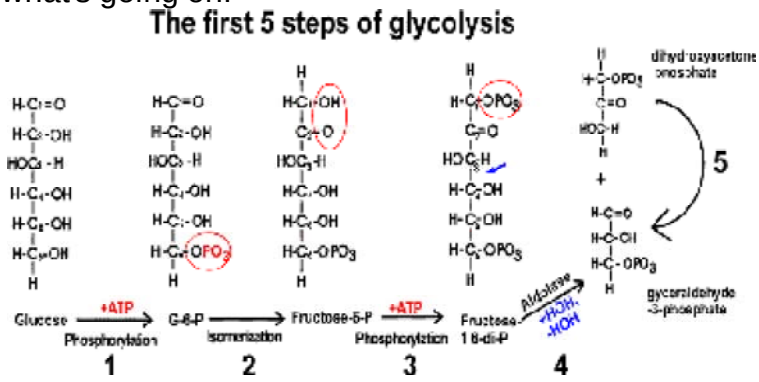
I am now going to discuss **GLYCOLYSIS** in some detail, for two reasons:

1) It will illustrate the problems and the solutions of energy requirements, so one aim is to understand energy metabolism.

2) It is a real life detailed example of a typical metabolic pathway, such as we have been alluding to with all these arrows leading to A's and B's and C's. (The pathway will be characterized by a series of small changes between substrate and product at each step)

(Glycolysis)
GLYCOLYSIS:

I'll show the first few reactions with the sugar in open chain form, because I think it is easier to see what's going on.



1. a phosphorylation (kinase enzyme)

2. an isomerization

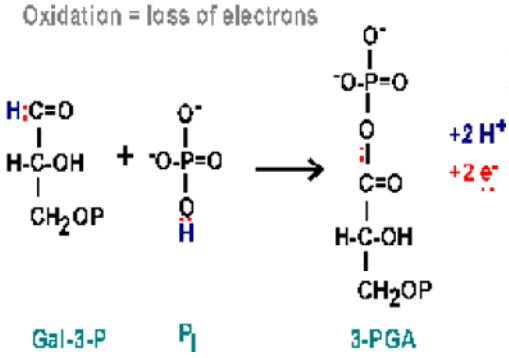
3. phosphorylation again... note that we have now used 2 ATPs: rather than generating energy, we are consuming it, so far.

4. hydrolysis **(For more about this reaction see Problem 4-16)**

5. isomerization of dihydroxyacetone phosphate (as we saw in step 2, but in reverse, transforming the ketone into the aldehyde; and swing it upside down when done: you have another molecule of glyceraldehyde-3-phosphate): note now we have 2 of everything for each glucose molecule that entered the pathway, this will be true from this point on.

So, so far, through rxn 5, we've produced 2 molecules of glyceraldehyde-3-phosphate (GAL-3-P) for each molecule of glucose we used. AND it so far has COST us 2 ATP's This ATP debt is a *loose end* that we will have to deal with sooner or later.....

6. Step 6 is an oxidation, GAL-3P plus another phosphate to form 1,3 diphosphoglyceric acid, (or 1,3-diPGA). {Q&A}



OXIDATION, the loss of ELECTRONS. We saw it before in the formation of the disulfide bond - there the loss of two electrons was in the two hydrogen atoms that were taken away. Here, in reaction 6, we have 2 electrons to be lost from the reactants, GAL-3-P and phosphate:

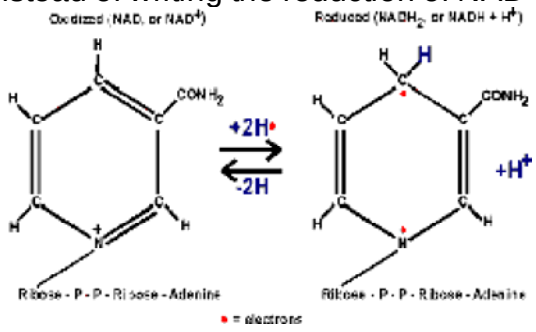
(NAD)

Two electrons have been lost. The protons are not important in oxidation, they are sometimes there with the electrons, and sometimes not. {Q&A} These electrons must go somewhere. They are taken up by the OXIDIZING AGENT which itself will get reduced. As you can see from the NAD handout, this is a compound called NAD, or NICOTINAMIDE ADENINE DINUCLEOTIDE [Purves picture]:

The nicotinamide part is also the vitamin niacin. Niacin sounds less ominous.

In getting reduced, NAD can accept two electrons, but only one proton. The other proton goes into solution as a hydrogen ion. But it is the electrons that are important in oxidations.

Instead of writing the reduction of NAD as NAD+ --> NADH + H+, we will simply write: NAD --> $NADH_2$, referring to the two H's that came from glyceraldehyde-3-phosphate, despite the fact that both protons did not end up on NAD. {Q&A} {Q&A}



This rxn. 6 is rather complicated, involving a phosphorylation as well as an oxidation. The phosphorylation did not require an ATP, but it did require an NAD.... so now have a new loose end analogous to the ATP/ADP situation, we have to worry about restoring NADs from NADH2's as well as ATP's from ADP's.

But at least we have something of value here, a high energy phosphate, as you can see the squiggle at the top of 1,3-di-PGA on your glycolysis handout. That means that enough energy can be released from the hydrolysis of this phosphate even to drive the phosphorylation of ADP in a coupled reaction.

Let's take the money and run... this high energy bond is cashed in the next reaction, rxn 7. Note that the top carbon is now a carboxylic acid in 3-PGA, whereas it had been an aldehyde in GAL-3-P. This change is the result of the oxidation that took place in reaction 6. {Q&A}

So we've now paid off one of the 2 ATPs the debt we ran up at the start .....
Wait a minute, actually, we're all paid up (since we have *two* di-PGA's for every mole of glucose that started down the glycolytic pathway).

Continuing along:

8. Isomerization of 3-PGA to 2-PGA.

9. Dehydration, water removed. The result is an unstable compound, phospho-enol pyruvic acid (PEP), one whose hydrolysis can result in the release of a large packet of free energy.

> [2-PGA, as well as PEP, is at a higher energy level than pyruvate, but the shift in atoms allows cash-in of ATP along the way, since the phosphate bond is a high energy bond in PEP.]

10. Transfer of the phosphate to ADP (X2), resulting in PYRUVIC ACID (pyruvate), which can be considered the end point of glycolysis. [Purves picture], [another Purves picture], [yet another Purves picture]

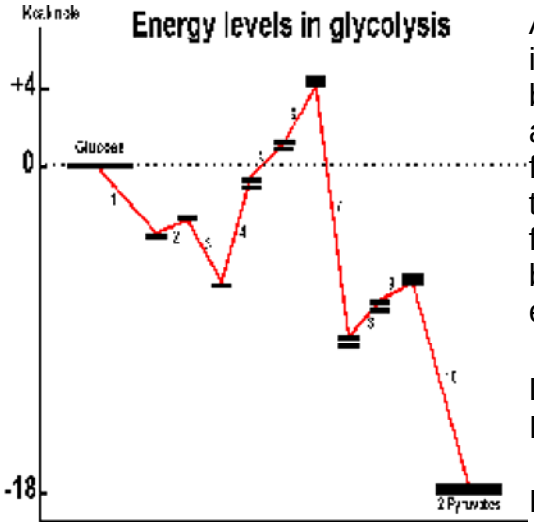
So after the 10 steps of glycolysis we have 4 ATPs produced and 2 invested, for a net gain of 2 ATPs produced from ADP. So glycolysis does produce energy in the form of ATP. And the overall reaction will run far to the right, as the Delta G^o from glucose to 2 pyruvates is -18 kcal/mole (even taking coupled reactions producing the 2 net ATP into account). That is,

1 glucose + 2 ADP + 2 Pi + 2 NAD <--> 2 pyruvate + 2 ATP + 2 $NADH_2$

Delta Go = -18 kcal/mole

The diagram below shows Delta G^{o}'s for the individual steps in glycolysis.

(For a different way of looking at it, see the chart of Delta G's drawn by Purves [Purves picture])



Although the pathway overall is quite favorable, some individual reactions are quite unfavorable, the most extreme being reaction #4. The energy-requiring reactions in this case are being pushed by the build-up of reactants by the more favorable reactions before them, and they are being pulled by the withdrawal of products by the more exergonic reactions further downstream. The actual Delta G's will be influenced by the drain of products, so that the second term in the equation for Delta G is being brought into play here. {Q&A}

Delta G = Delta G^{o} + RTln([products]/[reactants]), where RT~0.6 (in **k**cal/mole)

For instance, if the products are drained such that the ratio of P/R reaches down to 0.00001, this produces ~ 7 kcal/mole of negative Delta G , enough to balance out the unfavorable Delta G_{o} of +6.8 for rxn. 4.

This indirect affect on the Delta G is the second method the cell utilizes to carry out an individual reaction that has an unfavorable Delta G^{o}.

So we have 2 methods:

1.) DIRECT COUPLING of the unfavorable reaction to an energetically favorable one to produce a new coupled reaction with a net negative (favorable) Delta G^{o} (as in the hexokinase reaction #1).

2.) INDIRECTLY, via the WITHDRAWAL OF PRODUCTS or buildup of substrates {Q&A}

SO, we have our overall negative Delta G^{o} , and we have generated net ATP, so we should feel pretty good, except for one thing: we have an important loose end to tie up. We borrowed an NAD to oxidize Gal-3-PO_4. That was a key reaction in the path, as oxidations are usually accompanied by large changes in free energy. We were able to get a PO_4 added to our 3-carbon compound, and this phosphate was the one that was used to phosphorylate ADP in the very next step (#7). We used NAD, and it became reduced to NADH2. So now we must consider how we are going to repay that debt. Otherwise the small stores of NAD in the cell would very soon all be converted to $NADH_2$ and glycolysis would quickly grind to a halt.

We need an oxidizing agent to oxidize $NADH_2$. A great one abounds: O_2.

It readily takes up electrons, for instance from Fe++ to make it Fe+++ as steel goes to rust.

(Anaerobiosis)
Does E. coli have access to oxygen? In the lab, yes. We vigorously and constantly shake the E. coli cultures on mechanical shakers to get air dissolved in the culture medium to provide a constant

source of oxygen, called an AEROBIC state. In the gut, sometimes yes, sometimes no (crowded). Do our own cells have access to oxygen? Sure, through the lungs via the blood vessels to all tissues. But when you are running across campus to class so as not to miss the first golden words here, your muscles may need ATP faster than you can deliver oxygen to them for $NADH_2$ oxidation, your muscles will be in an AN-AEROBIC state. And are many organisms that live in naturally anaerobic environments, in mud at the bottom of rivers, e.g. So let's first consider the anaerobic case when no oxygen is available for the oxidation of $NADH_2$. Under these circumstances the cell must make do with what it has available, which is mainly: 2 pyruvates. Fortunately, pyruvate itself is able to act as an oxidizing agent, as seen in rxn #11, where it accepts electrons into its C=O bond, adding two hydrogens as well, to form lactate, or **LACTIC ACID**. It gets the electrons from our $NADH_2$ molecule, which is really NADH and H+, so it is recapturing one proton (an H+ ion) from the aqueous pool. Even the Delta Go is favorable, and we get our NAD regenerated from $NADH_2$. The NAD shuttles back and forth then, getting reduced in rxn 6 and reoxidized in rxn 11 over and over and over again, while glucose runs down to lactate, and ADP's are converted to ATPs to power cell division for E. coli or running up stairs for humans even in the case of insufficient oxygen.

It is thought that it's the build-up of acidity from lactic acid that makes your muscles hurt if they are doing anaerobic glycolysis too long (acidity sensed as pain by neurons in the muscle).

(Fermentation)
So pyruvate is a crossroads: If no oxygen, then if you are E. coli or humans, you carry out a lactic acid fermentation...[Purves picture], [another Purves picture]

If you are yeast, there is a variation on this theme, you break down pyruvate first to acetaldehyde and CO_2, which is not an oxidation, but then use the acetaldehyde as an oxidizing agent for $NADH_2$, with the product here being the 2 carbon alcohol, ethanol (rxns 12 and 13). Beer drinkers appreciate this variation, as lactic acid beer would be pretty bad, and probably not even produce those psycho-pharmacological effects for which ethanol is famous. {Q&A}

Just as in the case of ATP, the NAD - $NADH_2$ case is one of re-generation, not generation. Once a little NAD is made, it can shuttle back and forth millions of times getting alternately reduced and oxidized. [

Let's consider for a change an alternative source of carbon and energy for E. coli, glycerol. Remember glycerol is the tri-alcohol that serves as the backbone for triglycerides (fat) and phospholipids.

See glycolysis handout. From the PowerPoint graphic, it can be seen that glycerol is metabolized as follows:

glycerol + ATP --> glycerol-1-P

glycerol-1-P + NAD --> DHAP (= dihydroxyacetone phosphate) + $NADH_2$

DHAP --> continues in glycolysis (rxn 5). See recitation problems #3, problem 2.

Under aerobic conditions, no problem, the $NADH_2$'s produced will get re-oxidized by oxygen, as we

will see. And so E. coli grow just fine in a glycerol minimal medium using glycerol instead of glucose.

How about under ANAEROBIC conditions?

We used *two* NADs to get down to pyruvate from glycerol, but we get back only *one* in going to lactate. If we try to run the lactate fermentation using glycerol as our only carbon and energy source, we will grind to a halt as all our NAD ends up as $NADH_2$. (You will reach a state where all the NAD is in the form of $NADH_2$, and there is no pyruvate left, just DHAP waiting for an NAD that is not there.) So, although E. coli will grow just fine on glycerol in the presence of air (oxygen), it will NOT, in fact, grow on glycerol in the absence of air (anaerobically). So: these loose-end debts are real, there is no magic, we must play by the rules.

See Problem 4-15 - why was he fired?]

Consider the **efficiency** of glucose fermentation:

For: glucose--> 2 lactates, without considering the couplings for the formation of ATP's (no energy harnessing):
Delta G^o = -45 kcal/mole. {Q&A}.

Out of this comes 2 ATPs, worth 14 kcal/mol. So the efficiency is about 14/45 = 30%, which is not bad, about what a gasoline engine can do.

Where did the other 31 kcal get to? They are released as heat, which is why, after you've run fast to class, in addition to the lactic acid PAIN in your legs, you are also SWEATING.

Now taking the ATPs into account, the overall Delta G^o is about -45 + 14 = -31 kcal/mole, so the lactic acid fermentation runs essentially completely to the right (or clockwise) as written.

(Energy yield)
The efficiency is pretty good, but on the other hand the **YIELD** is poor. What do I mean by YIELD? Well, glucose has a lot more chemical energy in it than we are tapping here. For example, if we BURN glucose (i.e., react it with oxygen) and measure the calories of heat given off, we get no less than 686 kcal/mole. Compare that to the measly 45 kcal/mole we got from converting glucose to two lactates. The lactic acid that we are throwing away at the end could be used for more energy, but in the absence of oxygen there is no way to use it.

With oxygen, it's another story .....and our next chapter.

[To review glycolysis and fermentation, try Problems 4-11 and then 4-13.]

(Krebs Cycle (TCA cycle, citric acid cycle))
So now let us turn to the case when oxygen **is** present. In this case we are headed to the complete oxidation of glucose to 6 molecules of CO_2 and 6 molecules of water:

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ (write high on board and do not erase)

The Delta G^{o} for this reaction is -686 kcal/mole, and we are going to hope to get a lot more ADP --> ATP conversions out of this.

In the presence of oxygen, glycolysis down to pyruvate is the same. But the fate of pyruvate is now different.