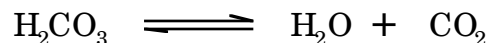


## **Introduction to Enzymes**

Open a can of soda. Breathe out. In both cases, a reaction occurs: the conversion of carbonic acid to carbon dioxide and water.



In the case of the soda, the reaction occurs spontaneously and relatively slowly (which is fortunate, because it allows the soda to remain carbonated for quite some time after being opened). On the other hand, if animals had to wait for the reaction to occur, they would have problems associated with build-up of waste carbon dioxide. To avoid this problem, animals have an enzyme, **carbonic anhydrase**, which accelerates the reaction. (As with most enzymes, carbonic anhydrase catalyzes the reaction in both the forward and reverse directions, and in locations other than the lungs, frequently catalyzes the formation of carbonic acid.)

A single molecule of carbonic anhydrase can catalyze the formation of about  $10^6$  molecules of  $\text{CO}_2$  per second. This represents a  $\sim 10^7$ -fold increase in rate compared to the uncatalyzed reaction.

## **Historical Aspects**

In the 1880s, Louis Pasteur argued that biological reactions (such as the fermentation of grapes) required living organisms. Eduard Buchner disproved this hypothesis in 1896, when he showed that cell extracts could catalyze the reactions of fermentation. (Buchner won the 1907 Chemistry Nobel Prize).

Enzymes were shown to be **proteins** when James Sumner crystallized the enzyme urease in 1926. More recently, Thomas Czeck and others have shown that some **RNA** molecules exhibit enzymatic activity.

## **General Properties of enzymes**

### **Enzymes are catalysts**

An enzyme enhances the rate of reaction without being consumed in the reaction. The catalytic process requires the enzyme to have a three-dimensional structure; unfolded enzymes do not catalyze reactions.

Enzymes have **active sites**. The active site is the specific part of the enzyme molecule where the reaction occurs. The active site is usually comprised of a relatively small number of residues within the overall enzyme structure.

### **Enzymes do not require harsh conditions to perform elaborate chemistry**

Enzymes can catalyze reactions under relatively mild conditions. In humans, these conditions are generally  $37^\circ\text{C}$  at one atmosphere pressure, with a pH near neutral. This is in marked contrast to organic chemistry reactions, which frequently require fairly extreme conditions.

### **(Most) enzymes are proteins**

Although RNA can have catalytic activity, proteins have much more diverse chemistry, because proteins are comprised of 20 amino acids instead of only four nucleotides. In addition, the amino acids have several functional groups that are not present in nucleotides. The chemistry of the amino acid side-chains is critically important when considering enzymatic reactions.

### **Enzymes may have coenzymes or cofactors**

Some enzymes consist solely of a polypeptide chain. However, amino acid side-chains are not always the best choice for some types of chemistry; enzymes may therefore bind other molecules in order to alter the chemistry of the active site. These other molecules are called “**coenzymes**” or “**cofactors**” (the terms are often used essentially interchangeably).<sup>1</sup>

Metal ions are frequently used as cofactors. For example, carbonic anhydrase uses zinc ions as cofactors, and a number of enzymes exhibit altered activity depending on the presence or absence of metal ions (especially calcium, but also magnesium, zinc, and other ions).

Some enzymes require reversibly bound organic compounds for activity. Some enzymes have very tightly bound or irreversibly bound compounds, in which the compound remains associated with the enzyme as long as the enzyme remains in its native conformation). A tightly bound compound that always remains associated with an enzyme and is required for activity is often referred to as a **prosthetic group**. Prosthetic groups may be covalently or non-covalently associated with the enzyme.

The organic or organometallic coenzymes are frequently vitamin derivatives. Some examples of vitamin-derived coenzymes are **nicotinamide adenine dinucleotide** (NAD), which is derived from niacin (vitamin B<sub>3</sub>), **flavin adenine dinucleotide** (FAD), which is derived from riboflavin (vitamin B<sub>2</sub>), **coenzyme A**, which is derived from pantothenic acid (vitamin B<sub>5</sub>), **pyridoxal phosphate**, which is derived from pyridoxal (vitamin B<sub>6</sub>), and **tetrahydrofolate**, which is derived from folic acid. On the other hand, not all coenzymes are derived from vitamins; **heme**, the prosthetic group of cytochrome P450 enzymes, peroxidases, and hemoglobin is synthesized from amino acids and iron ions.

An enzyme lacking its cofactor, coenzyme, or prosthetic group is called an “**apoenzyme**”; the enzyme with its associated compound is called a “**holoenzyme**”.

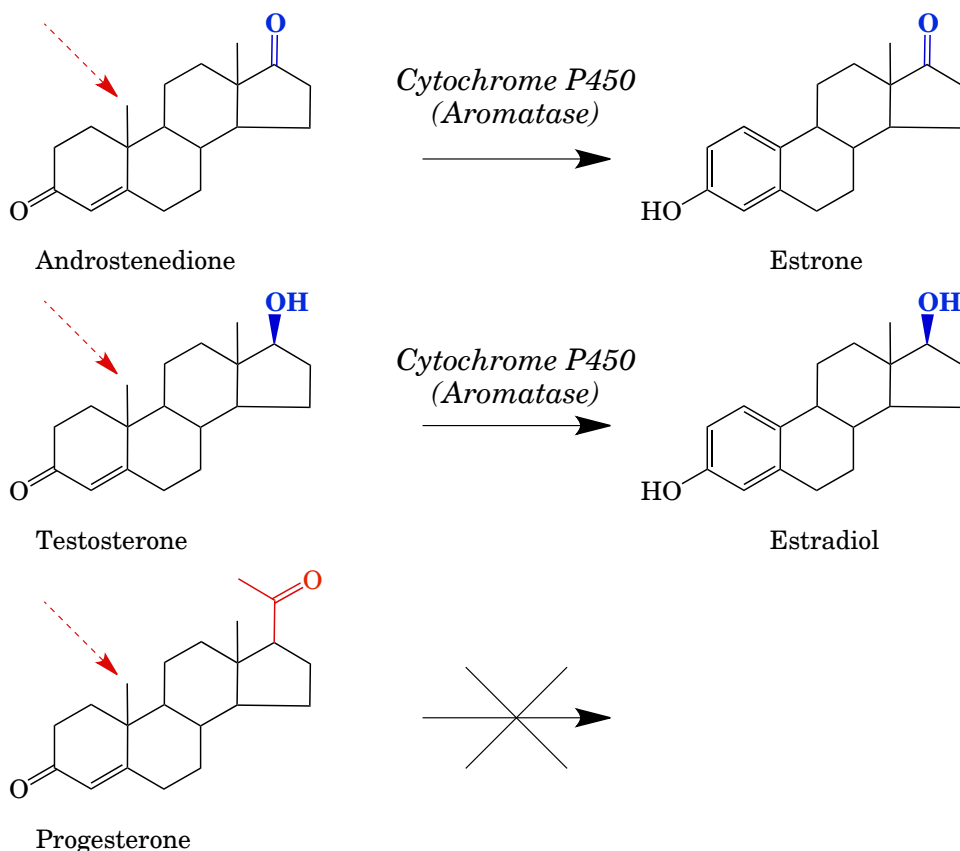
### **Enzymes are specific**

An enzyme will only catalyze one reaction, or at most, a very small number of closely related reactions. In addition, most enzymes will discriminate between potential substrates. As an example, consider the reaction below: the cytochrome P450 enzyme **aromatase** catalyzes the conversion of androstenedione to estrone, and of testosterone to estradiol. It will not, however, catalyze a reaction using

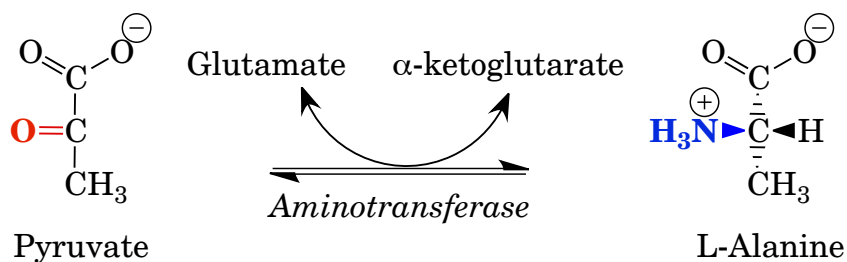
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<sup>1</sup>Some biochemists define “cofactors” as “metal ions”, and “coenzymes” as “organic” and “organometallic” compounds; others use the terms interchangeably.

progesterone. Note that androstenedione, testosterone, and progesterone all have the same structure at the “left” side of the molecule (as shown here using the conventional representation of steroids). The methyl group removed during the reaction (indicated by the arrow) is present in all three of these compounds; the only difference between progesterone and androstenedione is the type of group present at the other end of the molecule. The enzyme aromatase can recognize this difference, and will not use progesterone as a substrate. (Note: the aromatase reaction below is quite complex, and involves other substrates and other products that are not shown.)



As another example, consider the conversion of pyruvate to alanine:



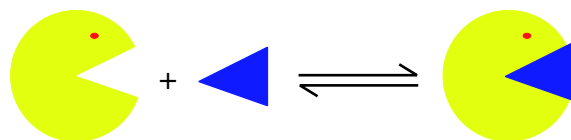
Physiological amino acids have L-stereochemistry because enzymes can take a non-chiral compound such as pyruvate, and **stereospecifically** form a product (in this

case, L-alanine, and not its D-stereoisomer). The reaction shown uses a common representation, in which the molecules of interest (in this case, pyruvate and L-alanine) have their structures shown, while the less directly relevant molecules (the co-substrate glutamate and co-product  $\alpha$ -ketoglutarate) are shown as participating the reaction with only their names shown. This depiction is especially common in drawings of multi-reaction pathways, because it focuses on the molecules with primary roles as intermediates in the pathway.

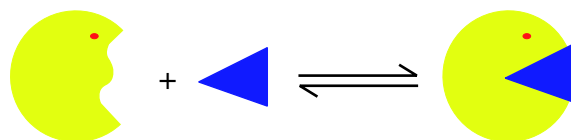
Life depends on specificity. The specificity for particular substrates, and the ability to always produce one stereoisomeric product are crucial for living organisms. These specificities are required to allow cells to control the outcome of the *many* reactions going on simultaneously within the same cell.

### Substrate recognition

Emil Fischer proposed the original “**lock-and-key**” hypothesis for substrate recognition when he suggested that each enzyme contains a pre-formed substrate-binding site. In the Fischer model, the substrate fits this site in the same way that a key fits into a lock. The enzyme can then catalyze the reaction using the bound substrate.



Additional experiments led Daniel Koshland to propose a modified hypothesis in 1953, in which the binding of substrate to the enzyme caused a conformational change by the enzyme that resulted in the formation of the active site.



The potential problem with the Koshland **induced fit model** is that, in the absence of a pre-formed binding site, the substrate will not actually bind. The current consensus is that, for some enzymes, the lock-and-key model is fairly close to reality; for other enzymes, the binding of substrate to a pre-formed pocket results in the somewhat altered conformation that is appropriate for catalysis.

### How do enzymes alter the rate of a reaction?

The factors listed below are, in very general terms, the factors used by different enzymes to enhance reaction rates. Most enzymes use at least one of these mechanisms.

#### Concentration effects

If a reaction requires more than one substrate, all of the substrate molecules must collide in order for a reaction to occur. In the absence of an enzyme, the rate of

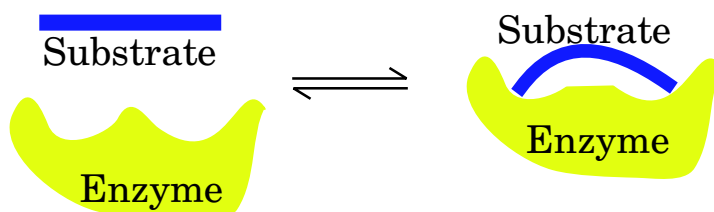
reaction depends entirely on the concentration of the substrates. One role an enzyme can play is in binding the individual substrate molecules. Once all of the substrate molecules are bound to the same enzyme molecule, the reaction can occur very rapidly. The enzyme has two effects: it “collects” the different substrate molecules, and once all are bound, it provides a very small volume (and therefore a high concentration) for the substrates. The result is that, once all of the substrate molecules are bound to the enzyme, the reaction can proceed very rapidly.

### Positioning effects

In most cases, two substrate molecules must collide in a specific orientation in order to allow a reaction to occur. The enzyme can assist this process by binding the substrate molecules and positioning them in the appropriate orientation to allow the reaction to occur.

### Strain induction

In some cases, the binding of substrate to the enzyme results in a change in the structure of the substrate (shown in schematic form below). This attempted alteration of the substrate three-dimensional structure can be important in inducing the desired reaction.



### Acid-base chemistry

Polar amino acid side-chains are in principle capable of donating or accepting protons. Many organic chemical reactions are enhanced by altered pH; although the pH in the overall environment is fixed by other factors, the enzyme can use specific functional groups to alter the effective pH in the active site. In other words, the cell can have an internal pH of 7.4, but due to the presence of a proton donor group in the protein, the effective pH of the active site can be much lower. Alternatively, the local environment within the active site may significantly alter the  $pK_a$  of the groups within the active site, and as a result, alter the likelihood that the group will become protonated or deprotonated. More importantly, by specific placement of proton donor and acceptor groups, an enzyme active site can carry out extremely efficient acid-base catalysis by protonating and deprotonating groups in the substrate in an appropriate sequence to accelerate all phases of the overall reaction.

### Charge stabilization

Many reactions involve a charge separation, either a transient separation during the reaction, or in the final products. By placing side chains of appropriate charge near the active site, the enzyme can stabilize this charge separation, and therefore stabilize transition states and intermediates within the reaction pathway.

## Covalent interactions

In some cases, the enzyme can form a covalent bond to a substrate molecule using either a polypeptide side chain or a prosthetic group. Covalent bond formation may assist in catalyzing the reaction both because it prevents dissociation of the substrate and because it alters the chemistry of the relevant portion of the substrate molecule.

Aminotransferases (such as the enzyme catalyzing the interconversion of pyruvate and alanine shown earlier) are one example of this phenomenon. During the conversion of alanine to pyruvate a prosthetic group in the aminotransferase forms a Schiff base covalent bond to the amino group. The conversion of pyruvate to alanine requires an amino group from a donor molecule to be covalently attached to the aminotransferase.

Note that in the vast majority of cases (including the “covalent interactions” case) the enzyme is regenerated in fully active form at the end of the reaction. The enzyme is not destroyed during the reaction it catalyzes.

## Nomenclature

Enzyme names usually end in “-ase”. We have seen several examples of this: aminotransferase, aromatase, and carbonic anhydrase. In some cases, the enzyme name predated this convention (trypsin, for example, is an enzyme discovered and named such a long time ago that “-ase” was not routinely included as part of the enzyme name).

## Enzyme Commission numbers

A more formal nomenclature can also be used to designate enzymes. The nomenclature rules are somewhat complex; briefly, the enzyme is placed in one of six major classes of enzyme (listed below), and then given further code numbers based on specific criteria. In addition, the enzyme is given a formal name that describes the reaction it catalyzes. For many enzymes, the formal name is rarely used. As an example, the human enzyme **ferredoxin reductase** has a formal name: **NADPH:ferredoxin oxidoreductase**, and is designated **E.C. 1.18.1.2**. Even people doing research on the enzyme only use the formal name and E.C. number when absolutely necessary.

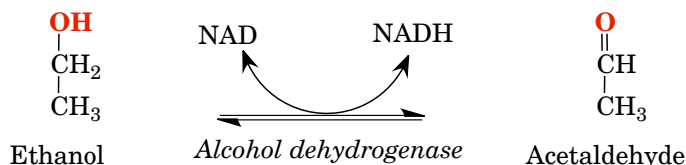
## Classes of enzymatic reactions

### Class 1: Oxidoreductases

Oxidoreductases are enzymes that change the oxidation state of the substrate. Most use NAD or NADP as the electron acceptor, although some use O<sub>2</sub> as the electron acceptor (reducing O<sub>2</sub> to H<sub>2</sub>O in the process).

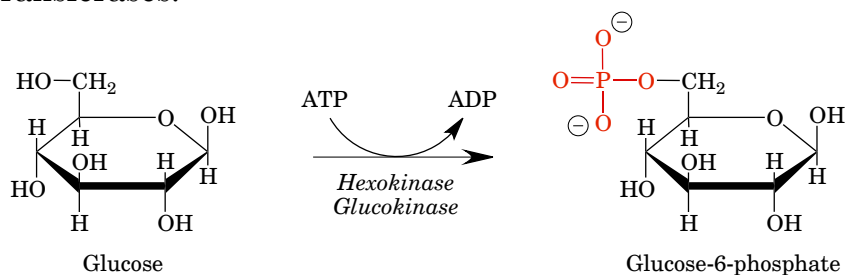
Many of these enzymes are named after their substrate followed by “dehydrogenase”, referring to the fact that the enzyme removes hydrogen atoms from one of the substrates during the reaction. For others the name includes the

term “reductase”. In most cases, reductase enzymes catalyze reactions that are irreversible under physiological conditions, while most dehydrogenases are reversible. The enzyme alcohol dehydrogenase is a good example of this type of enzyme.



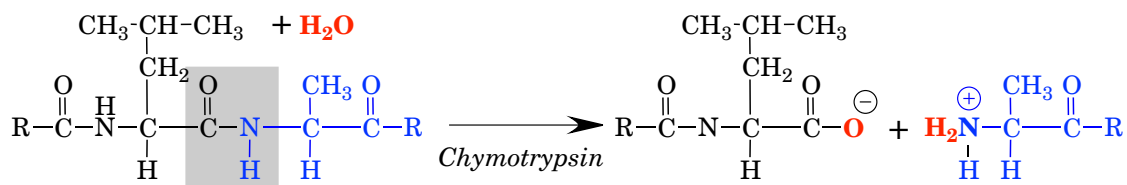
### Class 2: Transferases

Transferases are enzymes that transfer functional groups from one substrate to another. These include enzymes that transfer carbon units, and enzymes that transfer other types of compounds. **Aminotransferases** transfer amino groups (this reaction was shown earlier using the conversion of pyruvate to alanine), and **kinases**, which phosphorylate substrates (such as the isozymes glukokinase and hexokinase, which catalyze the phosphoryl transfer reaction shown below), are examples of transferases.



### Class 3: Hydrolases

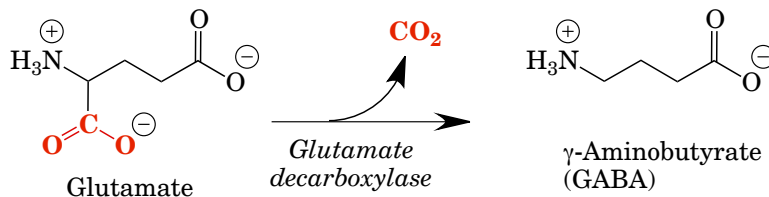
Hydrolases catalyze hydrolysis reactions; they cleave molecules by adding a water molecule across the bond. This class of enzyme includes **phosphatases** (which release inorganic phosphate groups from substrates) and **proteases**, (which hydrolyze peptide bonds).



### Class 4: Lyases

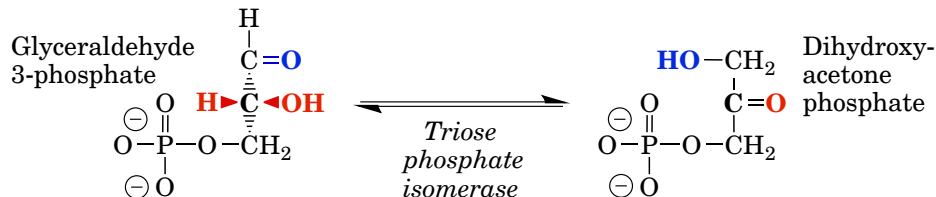
Lyases add molecules to double bonds. An example of this class is carbonic anhydrase (carbonate hydro-lyase) which adds a water molecule across one of the carbon dioxide double bonds to form carbonic acid (and also catalyzes the reverse reaction. Amino acid decarboxylases (such as glutamate decarboxylase, below) are

also lyase enzymes, although their physiological reaction is usually the removal of carbon dioxide rather than its addition.



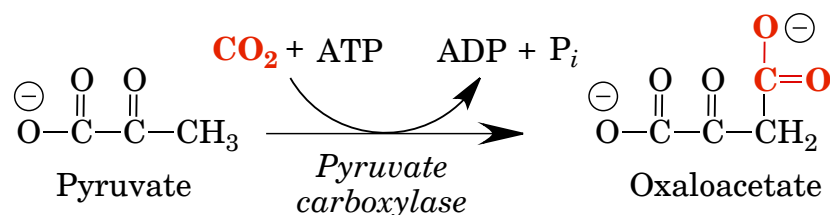
### Class 5: Isomerases

Isomerases interconvert isomers. These enzymes include racemases, which interconvert stereoisomers (for example, enzymes that interconvert D- and L-amino acids), as well as enzymes that interconvert molecules with similar functional groups in different positions, such as triose phosphate isomerase, which rearranges the relative positions of the hydroxyl and carbonyl in its substrates.



### Class 6: Ligases

Ligases are enzymes that connect two molecules together. These include enzymes that splice DNA molecules, and carboxylases, which add carboxylate groups to small molecules. Most, although not all, ligases use ATP to supply the energy required to drive the reaction. An example of a ligase is pyruvate carboxylase, an enzyme that we will see again later in the course.



### Isozymes and isoforms

In many cases, more than one form of an enzyme will catalyze a particular reaction. Different **isoenzymes** or **isozymes** are products of different genes.

Some multimeric proteins can be synthesized from more than one isozyme. The resulting multimers are different **isoforms**.

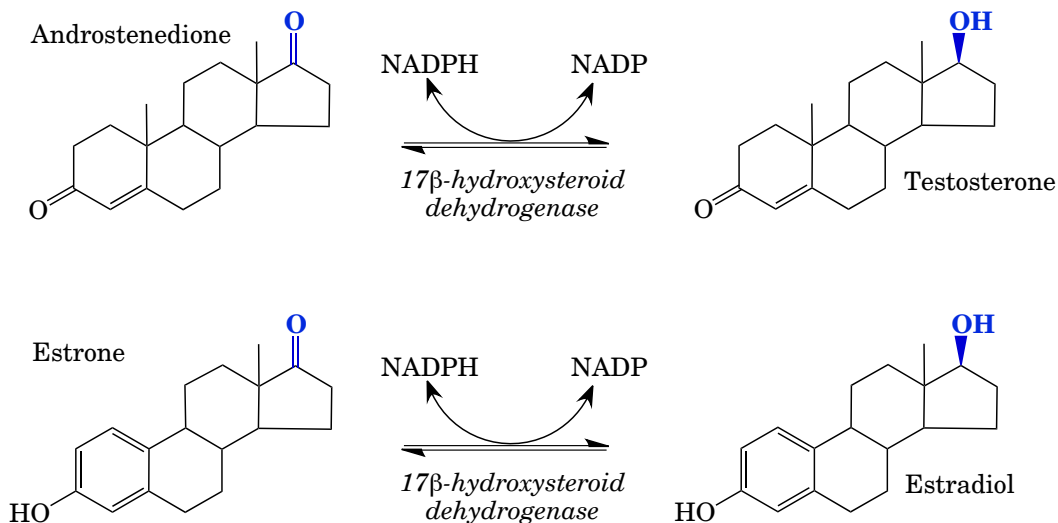
An example of this phenomenon is provided by the enzyme lactate dehydrogenase. Animals have at least two different lactate dehydrogenase genes (the genes are



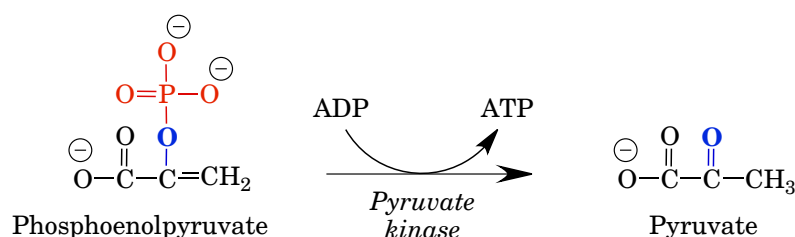
called A and B; the gene products are usually designated M and H for historical reasons). The active form of the lactate dehydrogenase protein is a tetramer, which can be formed in five different ways:  $M_4$ ,  $M_3H$ ,  $M_2H_2$ ,  $MH_3$ , and  $H_4$ . The five different forms of lactate dehydrogenase are **isoforms**, while the M and H polypeptides are **isozymes**, because they are produced from separate genes.

**Why do organisms use more than one enzyme with the same activity?**  
**Different isozymes may be expressed in different tissues.** This is important, because, in most cases, the activity or regulation of different isozymes differs.

**Different isozymes may have slightly different substrate specificities.** As an example, humans have at least five different isozymes of  $17\beta$ -hydroxysteroid dehydrogenase. The major physiological reactions catalyzed by these isozymes are shown below. The different isozymes have varying specificity for the different substrates. Some catalyze the reaction from right to left more effectively than from left to right, and others are specific for estrone and estradiol, and have little activity with androstenedione or testosterone.



**Different isozymes may be regulated differently.** As an example, humans have at least two isozymes of pyruvate kinase. The activity of the isozyme expressed in liver is regulated by several hormones, while the activity of the muscle pyruvate kinase is subject to much less stringent regulation. The reaction catalyzed by the muscle and liver isozymes, however, is identical.



Note: the examples shown above are just that, *examples*. Do not (yet) worry about these specific reactions. You will see some of these reactions again at later times in the course. For now, however, these reactions are merely used for illustrative purposes.

## Summary

Enzymes are biological catalysts, and are capable of dramatic acceleration of the rates of reactions.

Enzymes are specific, in that they catalyze a subset of possible transformations on some substrates, and may have no effect at all on other molecules.

Enzymes catalyze reactions by altering the immediate environment around the substrate(s), and by stabilizing the transition state and, in some cases, reaction intermediates.

Enzymes have names. Most enzymes are named in a fashion that reflects a common substrate or other property, and includes the suffix “-ase”. In addition to the common names used in conversation and in the literature for enzymes, enzymes also have formal systematic names and Enzyme Commission numbers used in databases and to avoid ambiguity.

There are six main types of enzymatic reactions; the specifics of those types of reactions allow for the diverse chemistry required for living organisms.