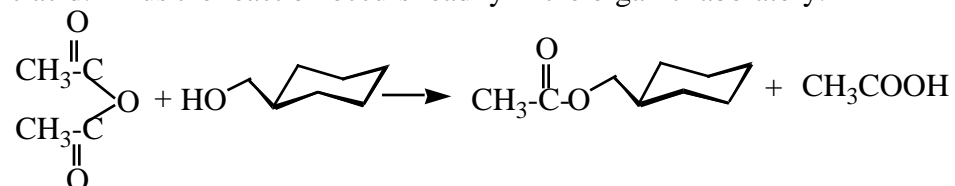


CHAPTER 16 BIOLOGICAL REAGENTS

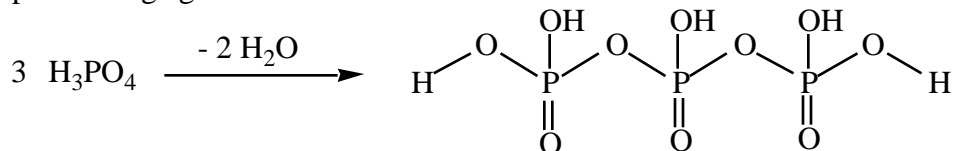
Reactions that occur in living systems require a very defined set of reaction conditions (aqueous, dilute, pH 7) and reagents. The reagents, also called cofactors or coenzymes, combine with the enzyme and substrate (and sometimes metal ions) to form an enzyme substrate complex that converts the substrate to products. Both the enzyme and the cofactor can be reused for further reactions, perhaps after some modification. Some biological reactions synthesize new larger molecules in a process called anabolism. Metabolism is the breakdown of large molecules and the process is called catabolism.

16.1 Anhydride Reactions

A common reaction found in organic systems and discussed the chapter on carboxylic acids is the reaction of an alcohol with an anhydride to form an ester. Acetic anhydride, which is a dehydrated form of acetic acid, is considered a high energy molecule because it is ready to react with hydroxyl functions and return to acetic acid. Thus the reaction occurs readily in the organic laboratory.

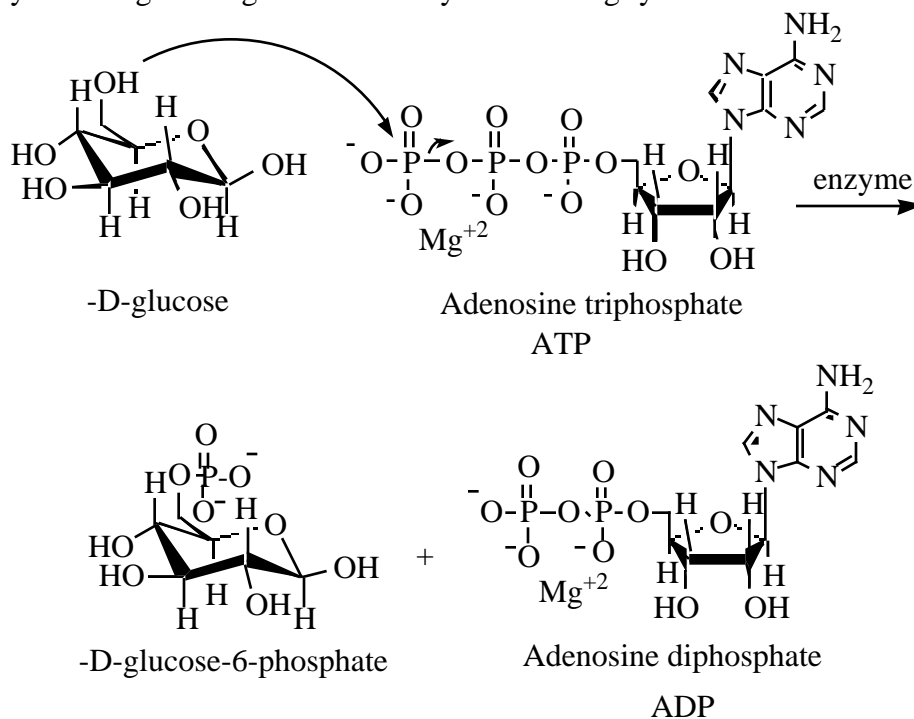


Living systems perform a similar task but usually instead of using anhydrides of carboxylic acid, anhydrides of phosphoric acid are used. Phosphoric acid may be dehydrated to polyphosphoric acids, of which triphosphoric acid is shown below. These are used in organic chemistry as dehydrating and phosphorylating agents.

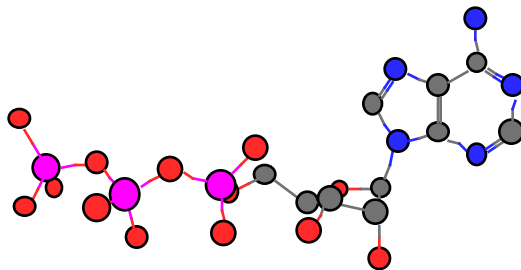


Most living systems cannot use such a highly reactive substance as triphosphoric acid, but living systems use a reagent called adenosine triphosphate (ATP). ATP is used to make phosphates of molecules in the body so that the

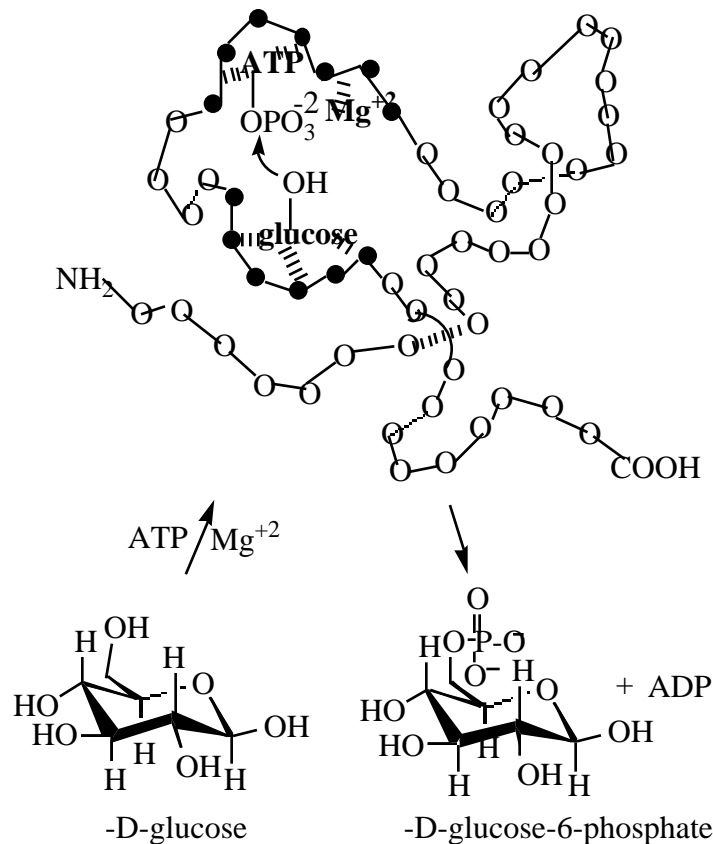
phosphate can be used as a substrate for an enzyme reaction. Below is a sketch of the first reaction in the metabolism of glucose, which ultimately yields carbon dioxide and water. Glucose is not a substrate for the metabolism and it must be converted to the higher energy phosphate in order for enzymes to use it. Thus glucose-6-phosphate is generated and used further in the metabolism scheme (not shown here). The biological reagent ATP functions as an anhydride of phosphoric acid and delivers a phosphate to the glucose and gives up the energy in the process. The ATP is converted into ADP. ADP can phosphorylate other molecules and produces adenosine monophosphate (AMP), or it can be reconverted to ATP. Nearly all biological reagents can be recycled in living systems.



A model of ATP without hydrogen atoms is shown below.



A sketch of the reaction proceeding through an enzyme-substrate complex is shown below.

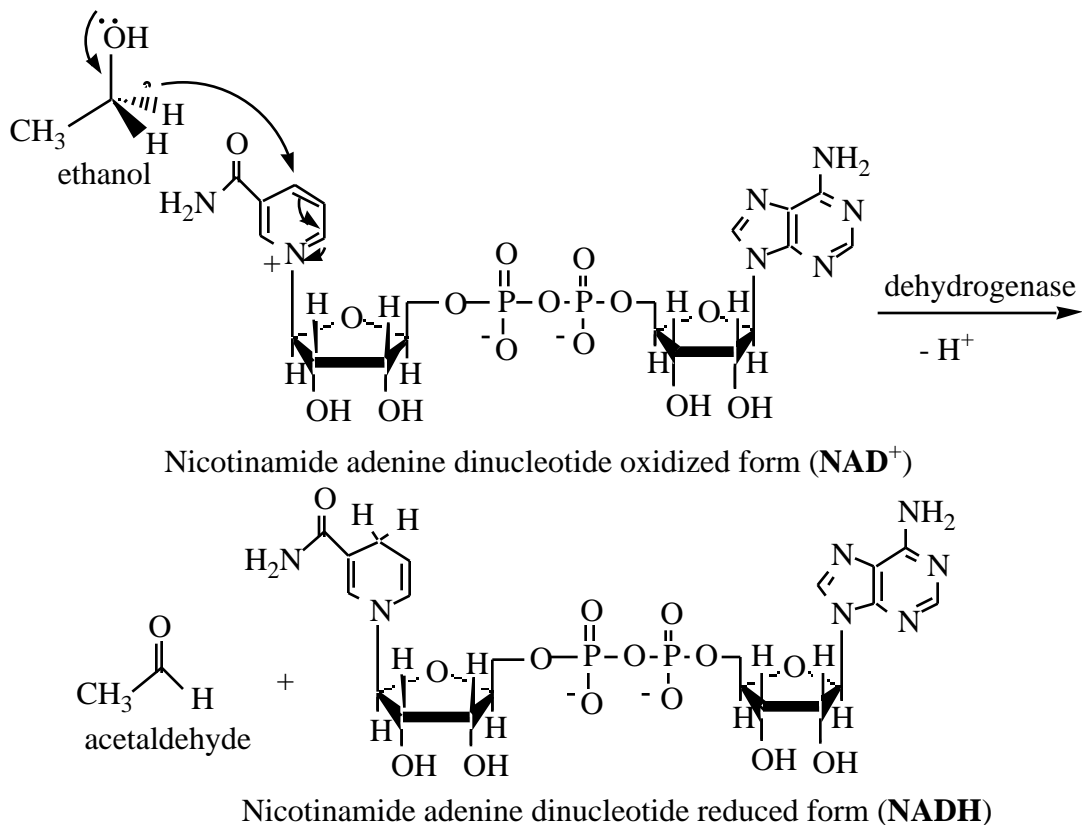


16.2 Oxidation-Reduction

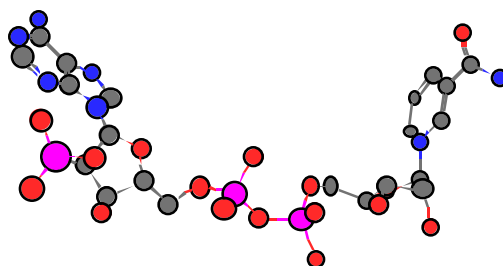
The reductions of the carbonyl group to an alcohol or the oxidation of an alcohol to a carbonyl group represent very important transformations in organic chemistry. The reagents used for these transformations have been discussed in previous chapters. Common reducing agents include $NaBH_4$, $LiAlH_4$ and H_2 with a catalyst. Common oxidizing agents include compounds of chromium and manganese. Although these procedures work well in preparative organic chemistry, none of them would work in a biological setting because of their toxicity and lack of specificity.

Living systems have developed several reagents for oxidation and reduction. A commonly used oxidation agent is nicotinamide adenine dinucleotide (NAD^+). The positively charged pyridinium ring attracts a hydride from the α -carbon in alcohols in an enzyme catalyzed reaction that produces the carbonyl compound. A

common reaction in living systems is the conversion of ethanol to acetaldehyde. Acetaldehyde is responsible for the "hangover" and other long term effects in alcohol intoxication.

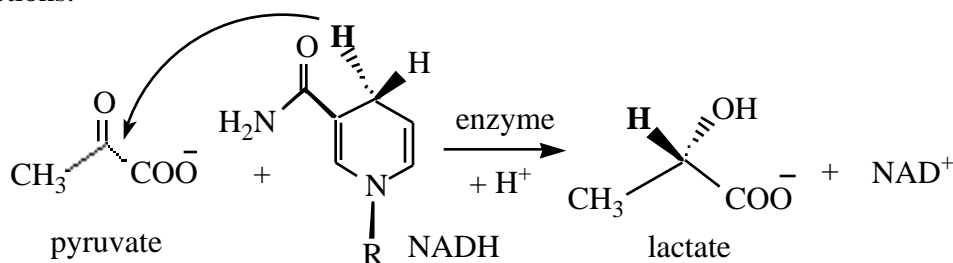


A ball and stick model of NAD⁺ is shown below.



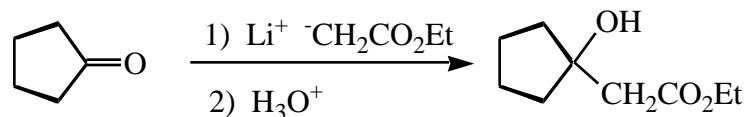
Reduced nicotinamide adenine dinucleotide (NADH) is also a product of the reaction. Living systems use NADH as a reducing agent in the conversion of carbonyl groups to alcohols. NADH is used in an enzyme catalyzed reaction to reduce pyruvate to lactate. NAD⁺ is produced in this reaction and is now available for oxidation reactions. Both the oxidation and reduction reactions are stereospecific

in that only the hydrogen atom indicated by the arrows are transferred in the reactions.

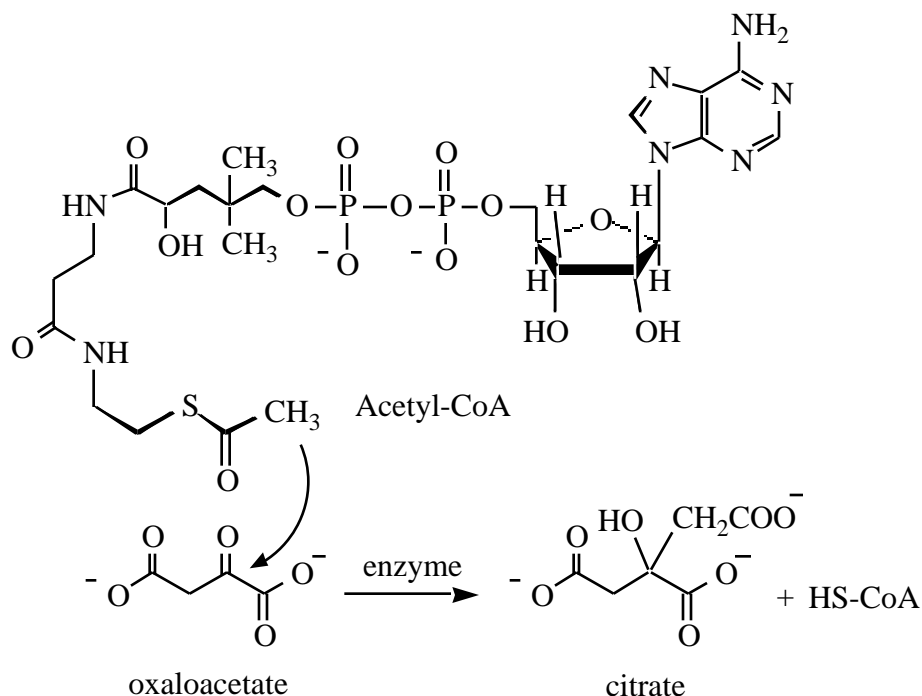


16.3 Claisen Condensations

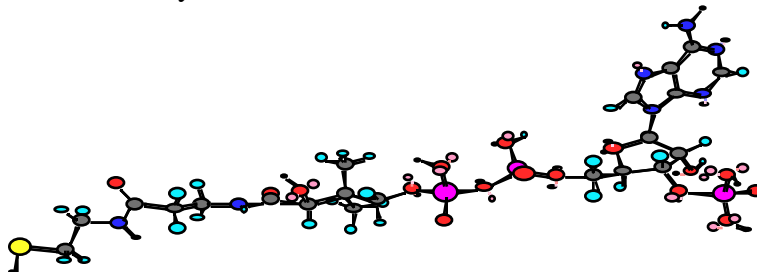
The synthesis of organic compounds in the laboratory requires methods that form new carbon-carbon bonds. Many of these reactions involve the formation of an enolate, a carbanion stabilized by an adjacent carbonyl group, and subsequent addition of the enolate nucleophile to the carbonyl function of an aldehyde or ketone. The Claisen condensation is normally a reaction in which the enolate from an ester reacts with another ester. But a modification of the reaction involves a reaction of an ester enolate with an aldehyde or ketone, and this modification is known as the Claisen reaction. As shown below it is a very useful method for the formation of a new carbon-carbon bond.



Living systems must also synthesize new carbon-carbon bonds for many different anabolic processes. One of these processes is the tricarboxylic acid or Krebs's cycle, shown in the chapter on carboxylic acids. The biological reagent used in this process is known as acetyl coenzyme A (acetyl CoA). Acetyl CoA is a thio ester instead of an oxo ester. The sulfur in acetyl CoA does not efficiently donate electrons to the carbonyl group thus making the acetyl group more reactive than an oxo ester. Thus in the tricarboxylic acid cycle the acetyl group condenses with the carbonyl group of oxaloacetate to produce citrate. Acetyl CoA will also react with alcohols to produce esters.



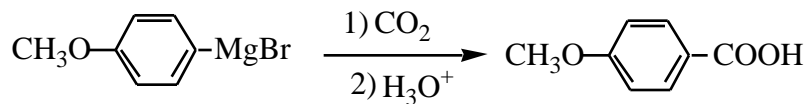
A model of coenzyme A is shown below.



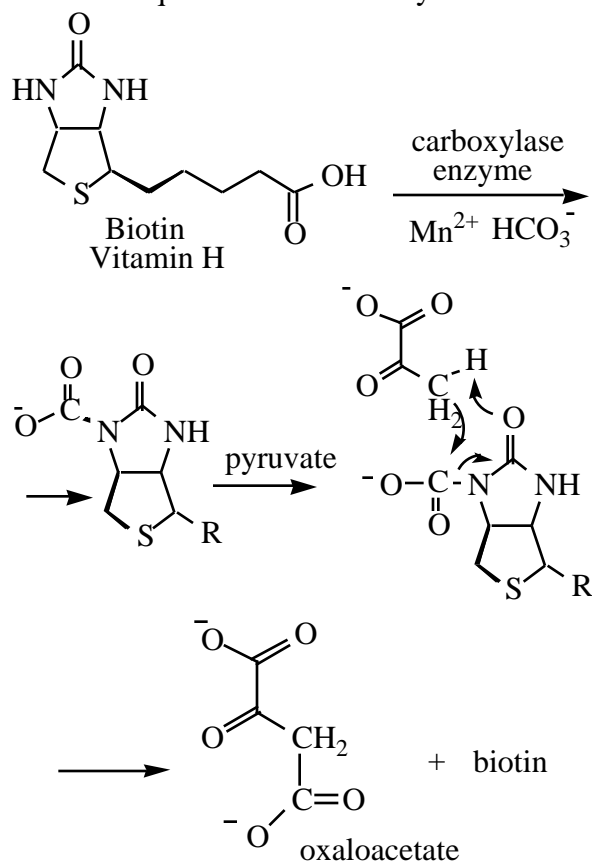
16.4 Carboxylation-Decarboxylation

Carbon dioxide is a useful reagent in organic synthesis and it is easily available from cylinders or dry ice. In living systems carbon dioxide is an essential compound in respiration and in the building (anabolism) or degradation (catabolism) of biological materials.

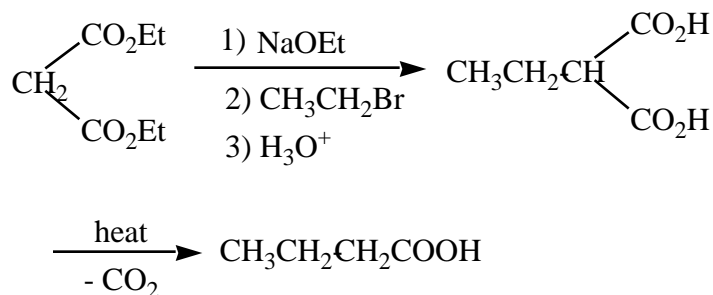
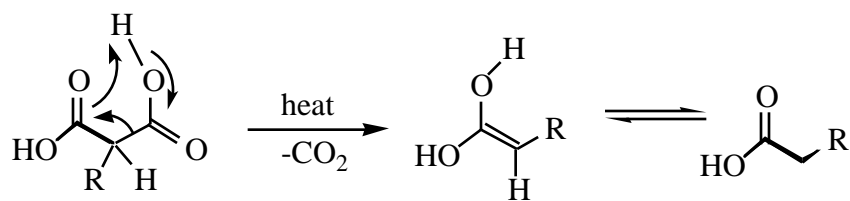
A common organic procedure is the reaction between organometallics (Grignard) with carbon dioxide to produce a carboxylic acid. The procedure is very simple and can be used to make a wide variety of carboxylic acids.



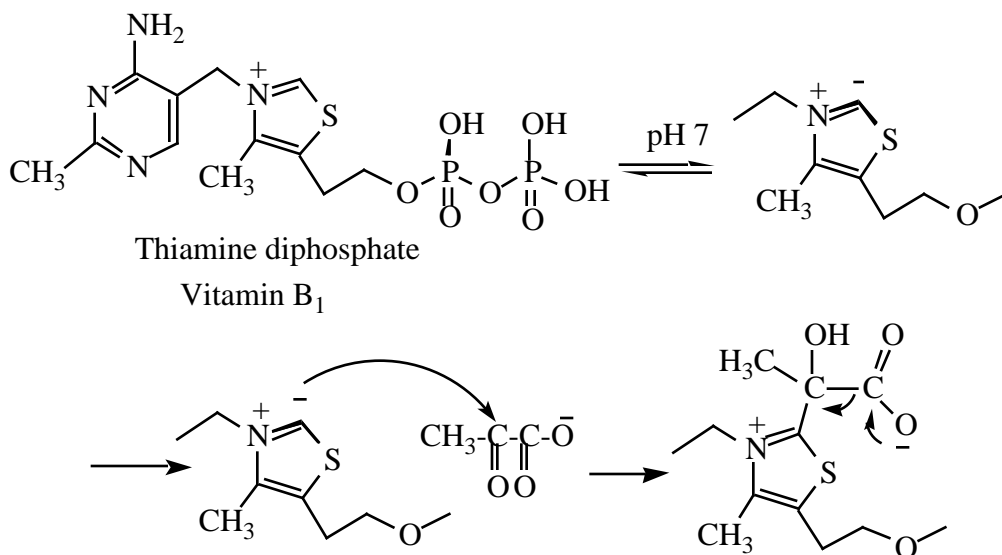
Living systems use enzyme catalyzed reactions of course and mild conditions. The coenzyme used is biotin that reacts with bicarbonate to form a carboxylated biotin that then reacts with pyruvate and transfers the carbon dioxide. The product is oxaloacetate required in the Krebs cycle.

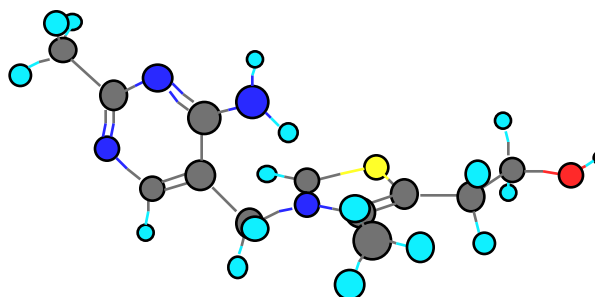
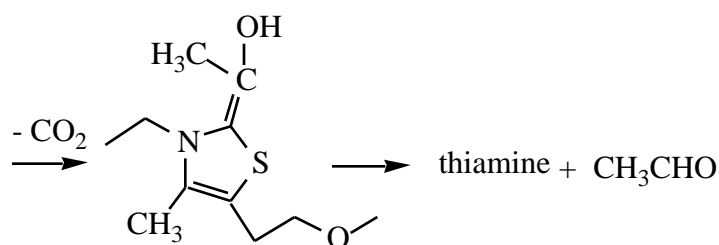


Removal of carbon dioxide from a substance in organic chemistry is often a thermal process. α -Ketoacids decarboxylate readily to yield acetic acid derivatives, a process used in the Malonic ester synthesis of organic compounds. The decarboxylation occurs in a cyclic six-membered ring process.



Thiamin (Vitamin B₁) is the reagent used in living systems to catalyze decarboxylation reaction. Thiamin forms a ylid at biological pH. The ylid adds to the carbonyl group of pyruvate to give an intermediate that resembles a α -ketoacid, a β -iminoacid in this case, that easily decarboxylates. The thiamin intermediate is hydrolyzed to acetaldehyde which eventually becomes acetyl CoA.





Thiamin Diphosphate

Many other biological reagents are used in biological systems, and the reactions are too numerous to elaborate now. All of the vitamins can be considered as a biological reagent as they combine with an enzyme to complete a very specific reaction. The specificity of biological reactions as a result of enzyme catalyzed and enzyme-cofactor catalyzed reactions is one of the most remarkable phenomenon that occurs in nature.

16.5 Summary

Biological systems must conduct reactions under mild conditions. The reactions are similar to those observed in organic chemistry, but *special biological reagents are needed*.

Enzymes recognize phosphate derivatives of many substrates. By analogy with organic chemistry that uses high energy anhydrides to make acetyl derivatives, biological systems use *adenosine triphosphate (ATP)*, a high energy reagent, to make the phosphate derivatives.

Biological oxidations are conducted with the coenzyme *nicotinamide adenine dinucleotide (NAD⁺)* which accepts a hydride to produce *reduced nicotinamide adenine dinucleotide (NADH)*. NADH is used as a hydride reducing agent, delivering its hydride stereospecifically.

Claisen-like addition of an acetic acid unit is accomplished by *acetyl coenzyme A (Acetyl Co-A)*.

Addition of carbon dioxide to organometallic reagents is a very useful organic reaction. Biological systems cannot tolerate an organometallic reagent but instead use *biotin* to activate carbon dioxide for incorporation into biological systems. Decarboxylation occurs readily in α -keto acids (malonic acid) systems in organic reactions. Biological systems form activated carboxylic acids that undergo decarboxylation by using *thiamin*. Biotin and thiamin are both vitamins and coenzymes.

16.6 Problem Set

16.1 The first step in the metabolism of glucose (glycolysis) involves the formation of the glucose 6-phosphate shown in this chapter. The next step is the conversion of glucose-6-phosphate by an enzyme and ATP to fructose 1,6-diphosphate. Show the structure of fructose 1,6-diphosphate.

16.2 The oxidation of ethanol by NAD^+ is stereospecific. Only the pro-R hydrogen atom is removed. Which hydrogen atom is the pro-R hydrogen?

16.3 Show a crossed Claisen reaction between benzaldehyde and acetyl-CoA. Do you expect this to be a significant biological reaction?

16.4 The decarboxylation of acetoacetic acid produces acetone and carbon dioxide is lost. The decarboxylation of biotin does not release carbon dioxide but instead uses the carbon dioxide in a synthetic step to produce oxaloacetate. Have you seen any organic reactions that use the carbon dioxide lost from acetoacetic acid (or malonic acid) use in a coupled synthetic step?

16.5 Show the analogous mechanism between decarboxylation of a α -ketoacid and the thiamin decarboxylation of pyruvate.

16.6 Another biological reagent is vitamin B₆ which displays activity through pyridoxal phosphate as shown in chapter 15. Show the structure of pyridoxal phosphate and explain its function.