

## **Pyruvate Oxidation**

### **Overview of pyruvate metabolism**

Pyruvate can be produced in a variety of ways. It is an end product of glycolysis, and can be derived from lactate taken up from the environment (or, in multicellular organisms, from other cells). It can also be produced from a variety of amino acids.

In animals, pyruvate has a few main fates. Pyruvate can be converted to alanine. Alternatively, pyruvate can be converted to oxaloacetate, either as part of gluconeogenesis or for other biosynthetic purposes, or it can be converted to acetyl-CoA. **In animals, the conversion of pyruvate to acetyl-CoA is irreversible**, and produces a compound that has fewer physiological uses.

Acetyl-CoA is used for lipid synthesis or for a few other, relatively minor, pathways, or as the substrate for the TCA cycle. In animals, acetyl-CoA cannot be used to synthesize amino acids or carbohydrates. This means that the conversion of pyruvate to acetyl-CoA is an important step, and must be tightly controlled.

On the other hand, the conversion of pyruvate to acetyl-CoA is a necessary step. The glycolytic pathway extracts a relatively small amount of energy from glucose; most of the energy originally present in glucose is still present in the pyruvate product of glycolysis. In order to extract the remaining energy from pyruvate, the compound must enter the TCA cycle. The conversion of pyruvate to acetyl-CoA removes the fully oxidized carbon while extracting some energy, and prepares the molecule for the remaining process.

### **Pyruvate import into mitochondrion**

Molecules cannot cross membranes freely. This was already mentioned in the context of glucose transport; the transport of pyruvate into the mitochondria is another example. Pyruvate is actually pumped into the mitochondria, so it is possible for the pyruvate concentration inside the mitochondria to be higher than outside. The energy for the pump comes from a proton gradient, in which the proton concentration outside the mitochondria is higher than it is inside.

Many other molecules are present only on one side of the membrane, or have separate cytoplasmic and mitochondrial pools. Relatively few compounds can move freely; movement across the mitochondrial membrane can be an important regulatory step for metabolic processes.

### **Reactions of the pyruvate dehydrogenase complex**

The first step in the oxidation of pyruvate is an oxidative decarboxylation reaction. This reaction is carried out by a very large enzyme complex, the **pyruvate dehydrogenase complex**, which is located in the mitochondrial matrix. The reaction catalyzed by the pyruvate dehydrogenase complex is irreversible, and is tightly regulated.

Pyruvate dehydrogenase carries out the reaction:



This seems simple. However, in humans, the complex contains well over one hundred subunits. The complex is comprised of three separate enzymes involved in the actual catalytic process, and uses a total of five different cofactors. The large size of the complex allows the complicated reaction to proceed without dissociation of the reaction intermediates, and also allows regulation of the complex.

The pyruvate dehydrogenase complex is closely related to the  $\alpha$ -ketoglutarate dehydrogenase complex (an important TCA cycle enzyme) and to the branched-chain  $\alpha$ -ketoacid dehydrogenase complex (an important enzyme in the metabolism of leucine, valine, and isoleucine). It is therefore worth spending some time examining the features of this complex protein.

The protein complexes of this family contain three main enzymes: E<sub>1</sub>, E<sub>2</sub>, and E<sub>3</sub>. The same E<sub>3</sub> gene is used for each of enzyme complexes, while the E<sub>1</sub> and E<sub>2</sub> genes are specific for the different types of substrates.

In eukaryotes and gram-positive bacteria, 60 E<sub>2</sub> polypeptides form a symmetrical icosahedral complex. The E<sub>1</sub> ( $\alpha\alpha$ )<sub>2</sub> tetramers and E<sub>3</sub> dimers then associate with the E<sub>2</sub> complex. Three other proteins are also present within the complex in mammals. These are Protein X (which is required for the binding of the E<sub>3</sub> to the E<sub>2</sub> complex), and two regulatory proteins, E<sub>1</sub>-kinase and Phospho-E<sub>1</sub>-phosphatase.

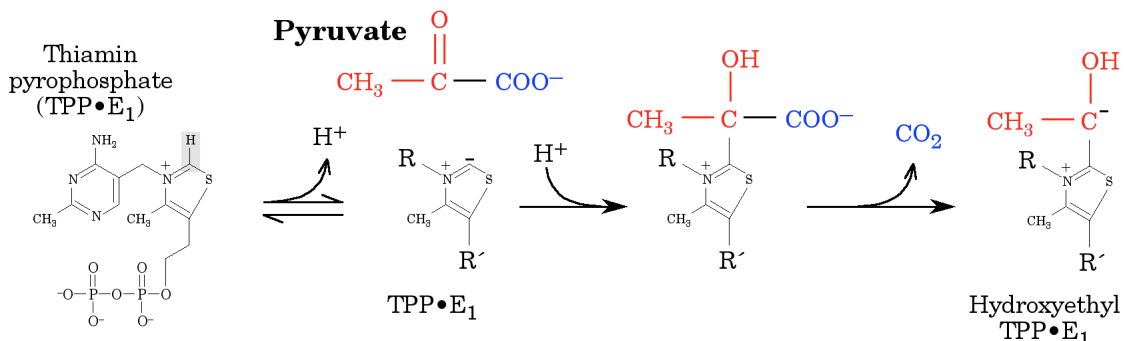
*E. coli* and other gram-negative bacteria use somewhat smaller complexes, comprised of 24 E<sub>2</sub>, 24 E<sub>1</sub>, and 12 E<sub>3</sub> polypeptides. Although smaller than the eukaryotic complex, the *E. coli* pyruvate dehydrogenase is larger than a ribosome, and is visible in electron micrographs.

For each of the types of  $\alpha$ -ketoacid dehydrogenase, the E<sub>1</sub> requires the specific corresponding E<sub>2</sub> to allow activity. The E<sub>1</sub> cannot donate acetyl groups to free lipoic acid. The E<sub>1</sub> enzyme therefore appears to recognize both the lipoyl-lysine and the protein to which the lipoyl-lysine is attached. This prevents loss of the acetyl group, and is important in regulation of the complex.

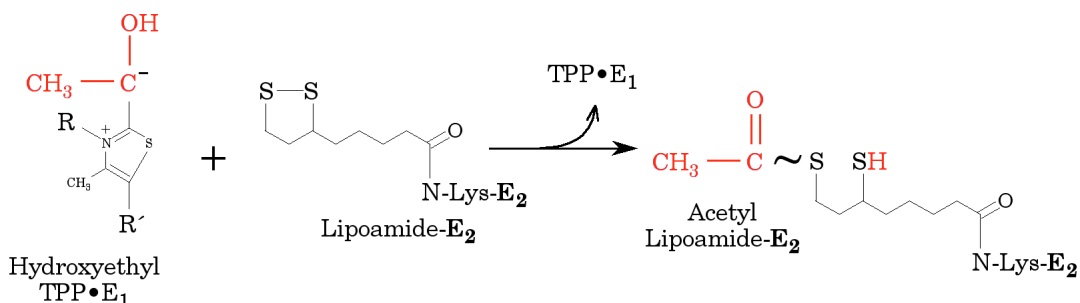
Each E<sub>2</sub> polypeptide has one (in eukaryotes) to three (in *E. coli*) 80-residue lipoyl domains. These are attached to the core of the complex by flexible domains within the E<sub>2</sub> peptide. The combination of the flexible E<sub>2</sub> and the lipoyl-lysine group (which in itself is 14Å long), allow each E<sub>2</sub> prosthetic group to react with all three types of active sites present in the complex.

The E<sub>1</sub> (sometimes called pyruvate dehydrogenase) binds pyruvate, and then forms a covalent bond between the thiamin pyrophosphate cofactor and the two carbon hydroxyethyl group remaining after decarboxylation of the pyruvate. (Note: the name of E<sub>1</sub> is slightly confusing, because it is also the name of the complex, and because it is not a dehydrogenase. Although the better name, pyruvate decarboxylase, is used for the completely independent enzyme that forms

acetaldehyde from pyruvate in microorganisms, the term pyruvate decarboxylase is also used in some literature for the E<sub>1</sub> enzyme of the pyruvate dehydrogenase complex.)

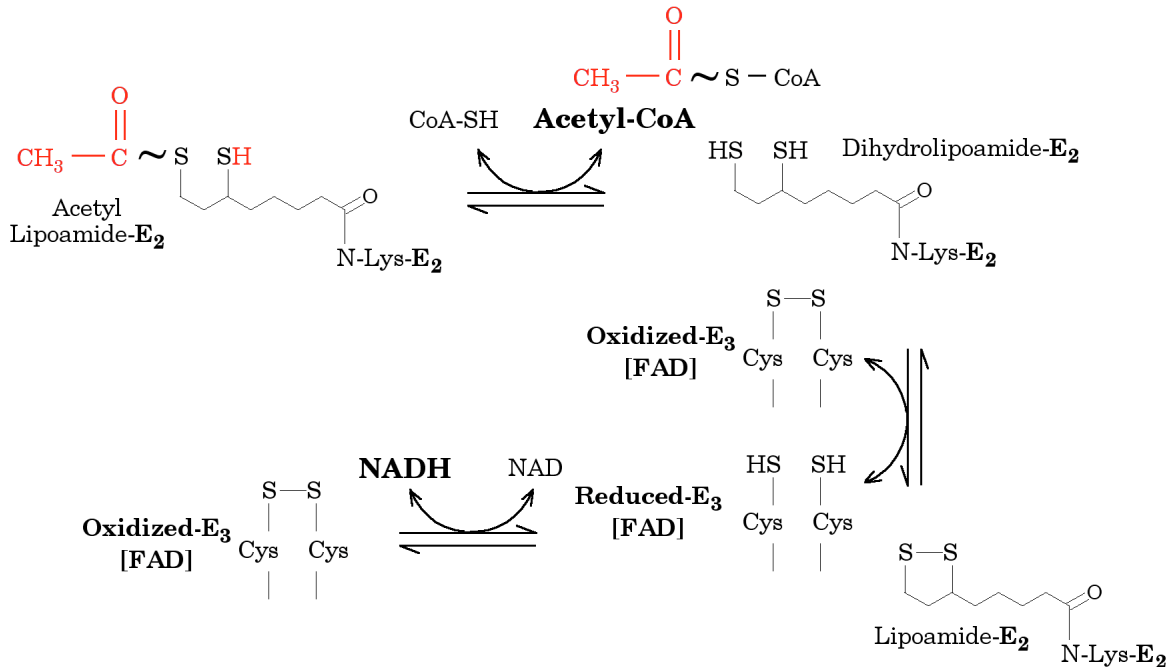


The E<sub>1</sub> reaction (and therefore the entire pyruvate dehydrogenase reaction) is irreversible because the E<sub>1</sub> enzyme has a very low affinity for carbon dioxide. As a result, reversal of the decarboxylation is very unlikely; the carbon dioxide release therefore drives the entire process.



The second enzyme, dihydrolipoyl transacetylase or dihydrolipoyl acetyltransferase (E<sub>2</sub>), then transfers the hydroxyethyl group from the thiamin pyrophosphate of E<sub>1</sub> to the E<sub>2</sub> lipoamide prosthetic group, forming the acetyl-lipoamide bound to the enzyme. The E<sub>2</sub> then transfers the acetyl group to Coenzyme A, which is released as acetyl-CoA. Note the **high-energy bond between the acetyl group and the CoA**; in this case the energy to form this bond comes from the pyruvate. In contrast, acetyl-CoA formation from acetate requires the use of ATP.

The result of the acetyl-CoA formation is a reduced lipoamide (dihydrolipoamide). The third enzyme, dihydrolipoyl dehydrogenase (E<sub>3</sub>), oxidizes the dihydrolipoamide to form oxidized lipoamide, the starting form of the E<sub>2</sub>. The reducing equivalents obtained from the oxidation of E<sub>2</sub> are given first to a cysteine disulfide bond in the enzyme, to FAD, and from there, transferred to NAD.



The E<sub>3</sub> is faster than the other enzymes, and fewer molecules of E<sub>3</sub> are present in the complex.

### Regulation of the pyruvate dehydrogenase complex

As mentioned above, pyruvate is a much more widely useful molecule than acetyl-CoA. Regulation of the irreversible pyruvate dehydrogenase reaction is therefore required to prevent unnecessary destruction of pyruvate.

Within the complex, only the E<sub>1</sub> reaction is irreversible. This means that the E<sub>1</sub> reaction is the focus of the control steps.

The pyruvate dehydrogenase complex is **inhibited by acetyl-CoA**. Acetyl-CoA is the product of the reaction, and can also be produced by fatty acid breakdown. If the levels of acetyl-CoA rise, oxidation of pyruvate is not necessary, and may use up NAD molecules needed elsewhere. The inhibition by acetyl-CoA occurs by two related mechanisms. The first is a competitive inhibition between acetyl-CoA and the substrate CoA-SH for the E<sub>2</sub> binding site. The second mechanism is due to the fact that the E<sub>2</sub> reaction is reversible; acetyl-CoA can acetylate the lipoyl-lysine group of E<sub>2</sub>, and thereby prevent the E<sub>2</sub> from accepting an acetyl group from E<sub>1</sub>.

Pyruvate dehydrogenase is also **inhibited by NADH**. The TCA cycle requires NAD; converting large amounts of NAD to NADH in the pyruvate dehydrogenase complex would inhibit the TCA cycle, and therefore, would inhibit acetyl-CoA utilization. NADH inhibition of E<sub>3</sub> occurs by both competition for the E<sub>3</sub> nicotinamide binding site and by NADH-mediated reduction of the E<sub>3</sub> cysteine disulfide, which prevents E<sub>3</sub> from oxidizing E<sub>2</sub>.

The mammalian pyruvate dehydrogenase complex is also **inhibited by**

**phosphorylation.** The kinase responsible for the phosphorylation is activated by acetyl-CoA and NADH, and inhibited by  $\text{Ca}^{2+}$ , ADP, and pyruvate. Insulin and  $\text{Ca}^{2+}$  stimulate acetyl-CoA formation (to support synthetic processes such as fatty acid synthesis, and to stimulate the TCA cycle, respectively) by stimulating the phosphatase that removes the phosphate from the pyruvate dehydrogenase complex.

The regulation of the enzyme complex by calcium illustrates some interesting and elegant features of cellular control. Calcium is a frequently used signal. In muscle cells, calcium is used to trigger muscle contraction. The regulation of pyruvate dehydrogenase by calcium allows the same signal to stimulate conversion of pyruvate to energy in order to support the muscular activity.

As mentioned above, both the kinase and phosphatase are associated with the complex. The kinase phosphorylates the  $\text{E}_1$  enzyme on serine residues. Active site arginines are thought to stabilize the interaction of pyruvate with the enzyme; the phosphoserines may bind to the arginines and indirectly prevent the pyruvate from binding.

Although in eukaryotes pyruvate dehydrogenase is found exclusively in the mitochondria, all of its proteins are expressed from nuclear genes. The  $\alpha$  subunit of pyruvate dehydrogenase  $\text{E}_1$  is located on the X-chromosome. Mutations in this gene that reduce enzymatic activity result in lactic acidosis of varying severity. One possible explanation for the variation in the disorder is the presence of another gene, normally only expressed in the testis and a few other tissues that may be able to substitute for the main  $\text{E}_1\alpha$  isozyme.

In contrast to the mammalian enzyme, the *E. coli* pyruvate dehydrogenase is not regulated by phosphorylation. Instead, binding of thiamin pyrophosphate is stimulated by pyruvate. High concentrations of pyruvate result in increased thiamin pyrophosphate binding, and therefore in increased activity of the enzyme complex. Low concentrations of pyruvate allow dissociation of the thiamin pyrophosphate, and therefore conserve the scarce pyruvate for other processes.