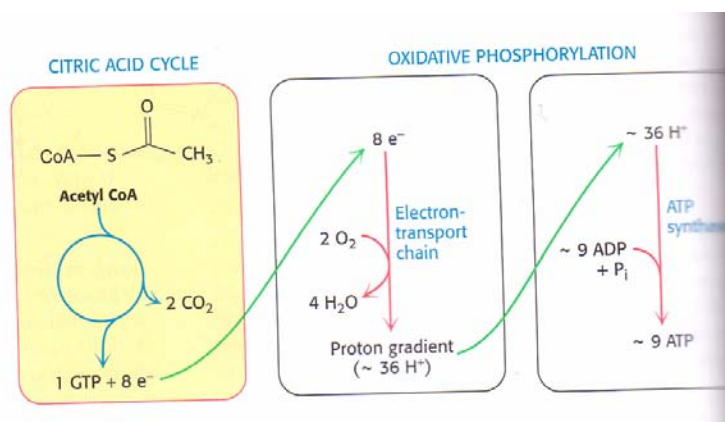


The Aerobic Fate of Pyruvate

February 12, 2003

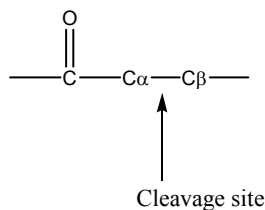
Bryant Miles

I could tell that some of you were not impressed by the mere 2 ATPs produced per glucose by glycolysis. The 2 ATP's produced are only a small fraction of the potential energy available from glucose. Under anaerobic conditions, animals convert glucose into 2 molecules of lactate. Much of the potential energy of the glucose molecule remains untapped. Under Aerobic conditions a much more dynamic pyruvate metabolism occurs. The 2 moles of NADH produced by glyceraldehyde-3-phosphate dehydrogenase are oxidized in the electron transport chain back to NAD^+ . The electron transport chain generates a proton gradient that drives the synthesis of 5 ATP molecules from ADP and P_i . Further more, the pyruvate formed by glycolysis is converted to acetyl-CoA by pyruvate dehydrogenase (generating another 2 moles of NADH per glucose and another 5 ATPs by oxidative phosphorylation). The acetyl-CoA formed enters into the citric acid cycle where it is completely oxidized into CO_2 . The electrons liberated by oxidation are captured by NAD^+ or FAD which are then transferred via the electron transport chain ultimately to O_2 , the final electron acceptor. The electron transport chain is coupled to generating a proton gradient which

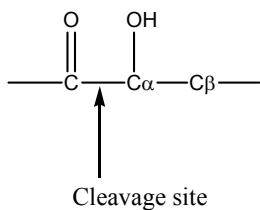


produces a proton motive force that drives the synthesis of ATP. This allows the net production of 32 molecules of ATP to be formed per glucose molecule. The function of the citric acid cycle is to harvest high energy electrons from carbon fuels. The citric acid cycle is the central metabolic hub of the cell, the gateway of aerobic metabolism. The citric acid cycle produces intermediates which are precursors for fatty acids, amino acids, nucleotide bases, cholesterol and porphyrins.

The citric acid cycle is shown on the next page. The citric acid cycle occurs in the mitochondria of eukaryotes. New carbons enter the citric acid cycle through acetyl-CoA. The acetyl group may come from pyruvate, fatty acids, ketobodies, ethanol or alanine. The two carbons of acetyl-CoA are transferred to oxaloacetate to yield the first tricarboxylic acid citrate in a reaction catalyzed by citrate synthase. A dehydration followed by a rehydration rearranges citrate into isocitrate. Two successive decarboxylations coupled to the generation of 2 NADH produce succinyl-CoA. Four steps later oxaloacetate is regenerated along with a GTP, FADH_2 and NADH.

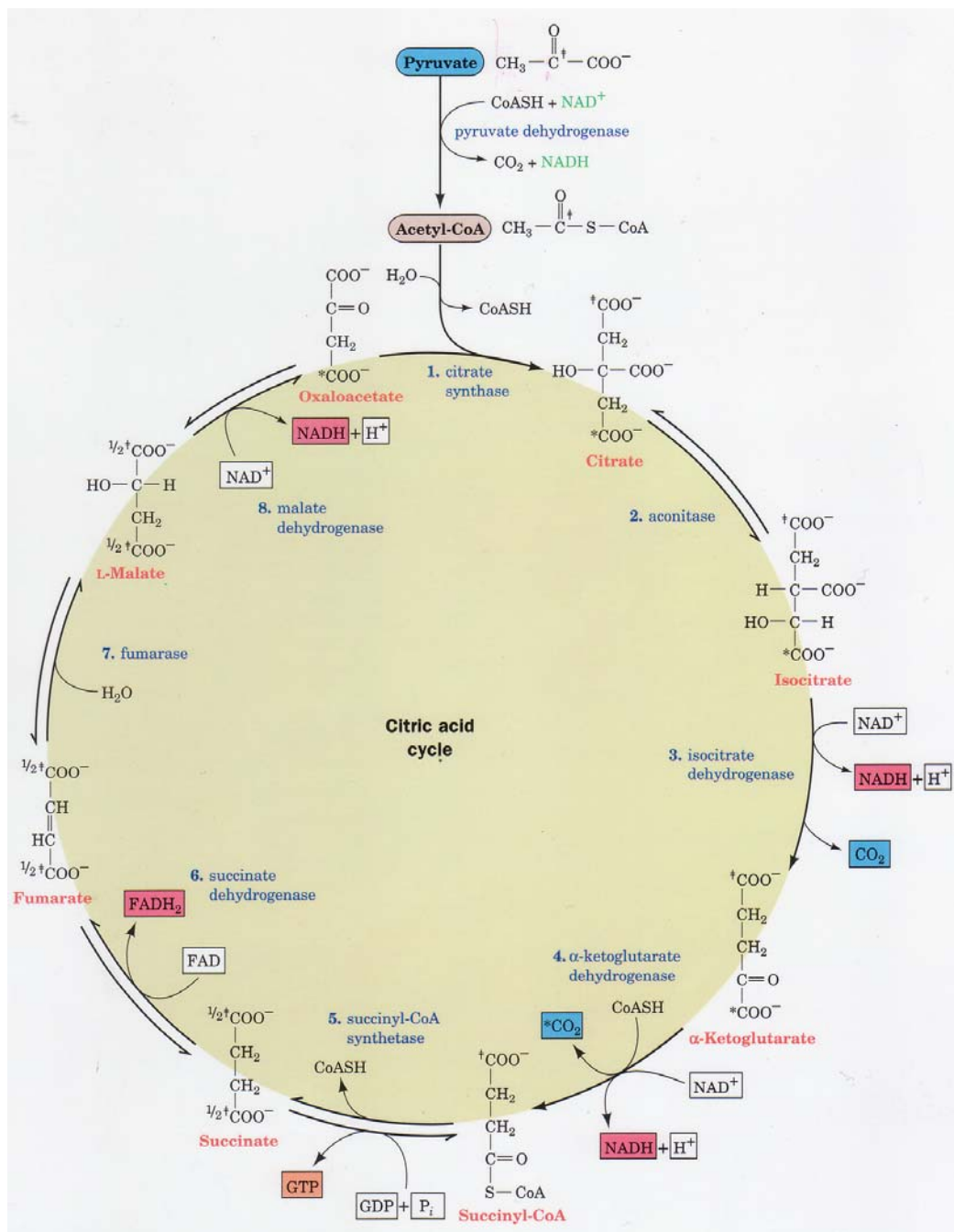


The citric acid cycle may seem like an elaborate way to oxidize acetate into carbon dioxide, but there is chemical logic to the cycle. In order to directly oxidize acetate into two molecules of CO_2 a C—C bond must be broken. Under the mild conditions found in cells, there is insufficient energy to break the bond. Biological systems often break C—C bonds between carbon atoms α and β to a carbonyl group. Examples are aldolase and transaldolase.

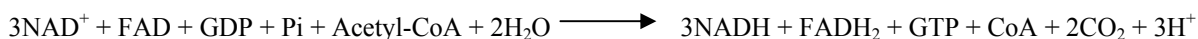


Another common type of C—C cleavage is α -cleavage of an α -hydroxy-ketone which we have seen before in transketolase and pyruvate decarboxylase, two TPP dependent enzymes.

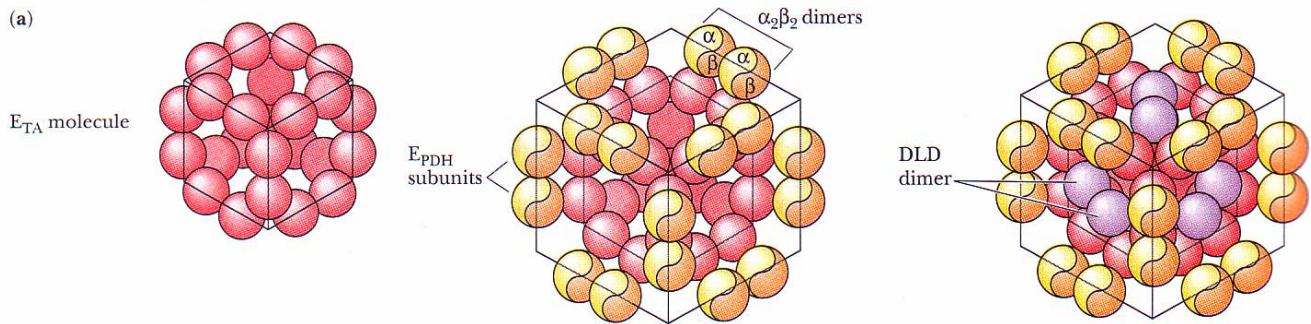
Neither of these common strategies for cleavage of C—C bonds is available to acetate. Instead the acetate is activated in the form acetyl-CoA, condensed with oxaloacetate to form citrate and then carrying out a β -cleavage in subsequent steps.



The Net reaction of the citric acid cycle is:

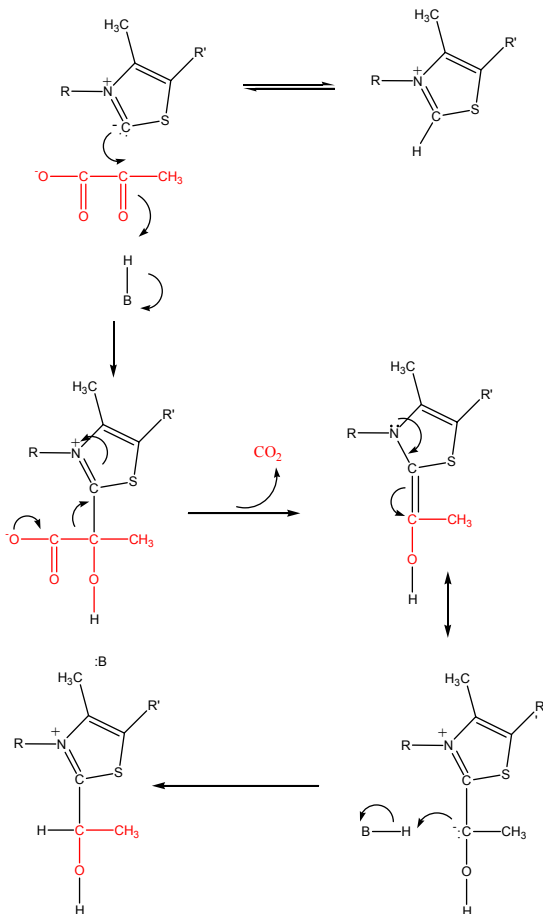


pyrophosphate coenzyme. In E_2 there is a lipoic acid coenzyme. In E_3 there is a FAD coenzyme. The molecular weight of pyruvate dehydrogenase isolated from *E. coli* is 4.6 million Daltons, slightly larger than a ribosome. In mammals, pyruvate dehydrogenase is twice that size and has two additional regulatory subunits. One is a protein kinase which phosphorylates three serine residues in E_1 , the other is a phosphatase which hydrolytically removes the same phosphoryl groups from the serines of E_1 .



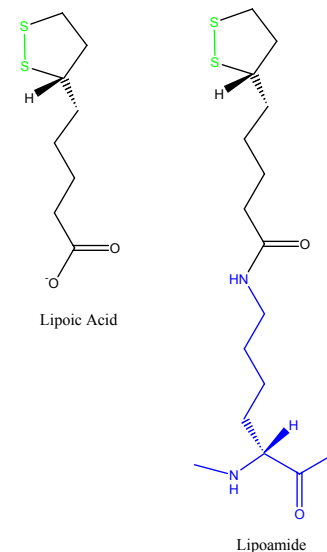
The structure of the pyruvate dehydrogenase complex is shown above. The E_2 , dihydrolipoamide transacetylase are shown as red balls. These 24 subunits create the core upon which the pyruvate dehydrogenase complex is built. The E_1 , pyruvate dehydrogenase enzyme is an $\alpha_2\beta_2$ dimer shown as the orange and yellow balls. There are 24 of the $\alpha\beta$ dimers in the complex. There are 12 subunits of the E_3 , dihydrolipoamide dehydrogenase complex shown in purple. The E_3 subunits associate into dimers, so there are six E_3 dimers in the complex.

Lets look at the chemistry going on in this complex.

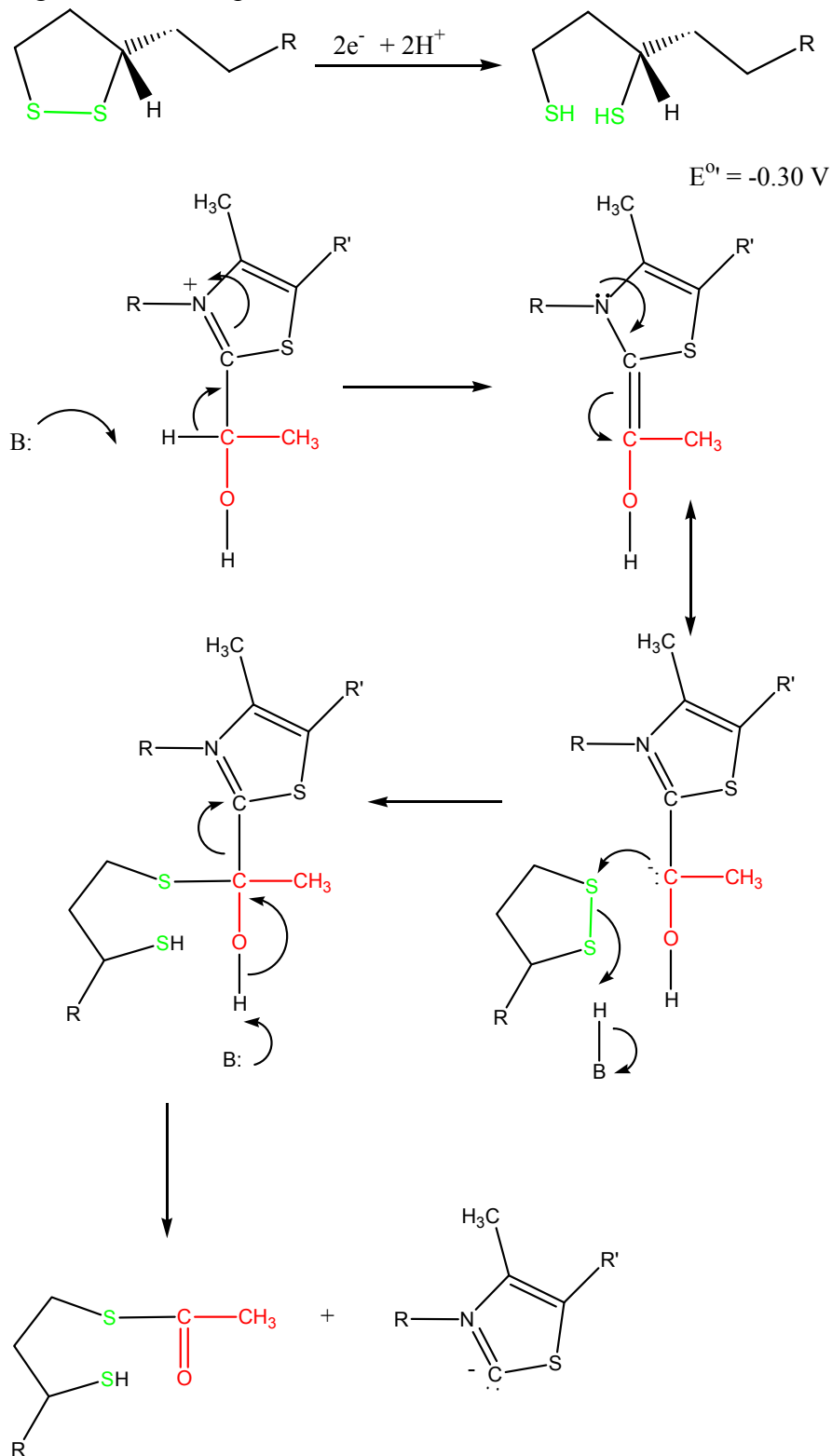


In E_1 , pyruvate dehydrogenase enzyme, we have a tightly bound thiamine pyrophosphate coenzyme or prosthetic group. We have encountered this cofactor before, pyruvate decarboxylase and transketolase. The reaction catalyzed in this subunit is similar to that of pyruvate decarboxylase.

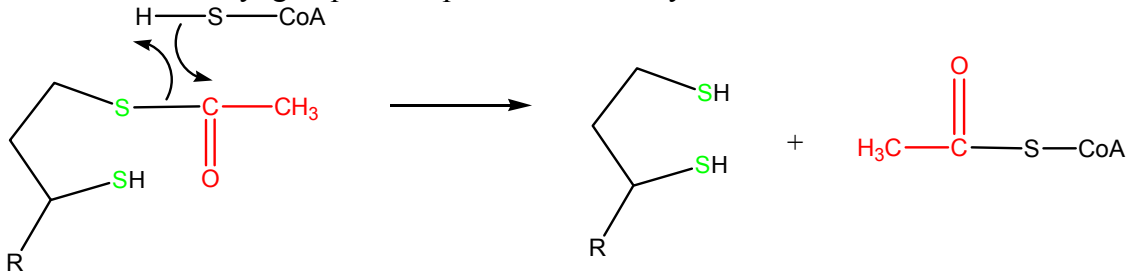
The next step in the enzyme catalyzed reaction involves a new cofactor we haven't encountered yet. It is lipoic acid.



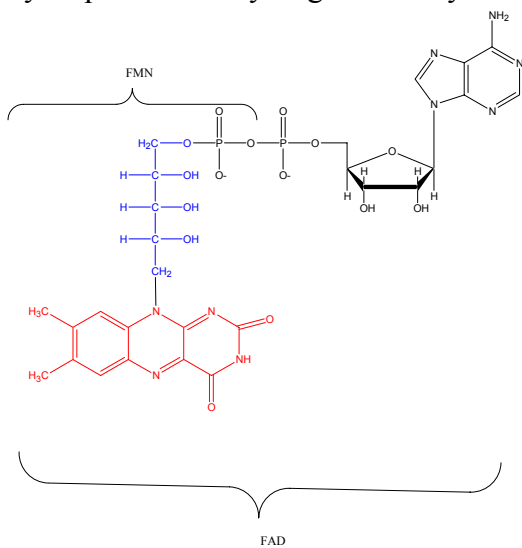
Lipoic acid is not found free in nature. Instead lipoic acid is covalently attached to a lysine residue through an amide linkage. The enzyme that catalyzes the formation of the lipoamide linkage uses ATP to generate AMP and pyrophosphate. Lipoic acid is an important cofactor because it couples acyl-group transfers with electron transfers during the oxidation and decarboxylation of α -keto acids. Because the ring strain inherent in the disulfide cyclic structure is relieved upon reduction, lipoic acid has a strong negative reduction potential, $E^{0'} = -0.30$ V.



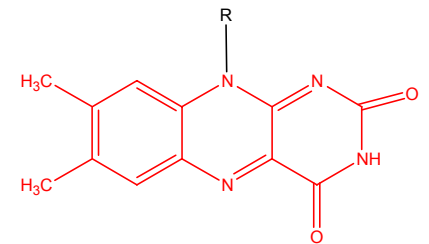
To summarize the chemistry up to this point, This enzyme has taken pyruvate, used TPP to catalyze the decarboxylation of an α -ketoacid to form the hydroxyethyl-TPP intermediate which is stable enough to isolate. The hydroxyethyl-TPP was then oxidized by and concomitantly transferred to lipoamide to form an acetylipoamide. The acetylipoamide is the final product of E₁. The lipoamide prosthetic group is part of the E₂ Dihydrolipoyl transacetylase enzyme. The next step in the course of the reaction mechanism occurs in the active site of the E₂ Dihydrolipoyl transacetylase enzyme, which catalyzes the transfer of the acetyl group from lipoamide to coenzyme A.



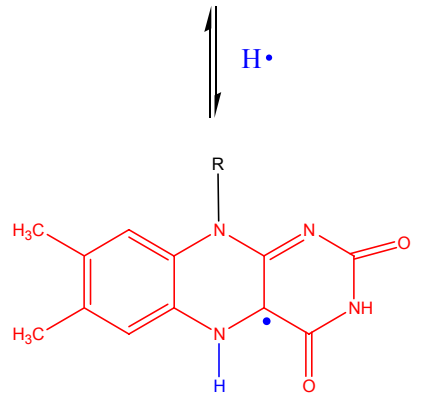
If you look back to the structure of lipoamide, you will see it has a long flexible tether linking the amide to the E₂ enzyme. The length of this tether is about 45 Å. This is long enough to reach the active site of E₁ to form the acetylipoamide and then carry this intermediate to the active site of the E₂ enzyme to form acetyl-CoA and the dihydrolipoamide which is then carried to yet the third active site, the E₃, dihydrolipoamide dehydrogenase enzyme.



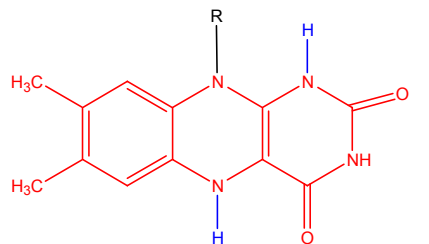
FAD
Quinone



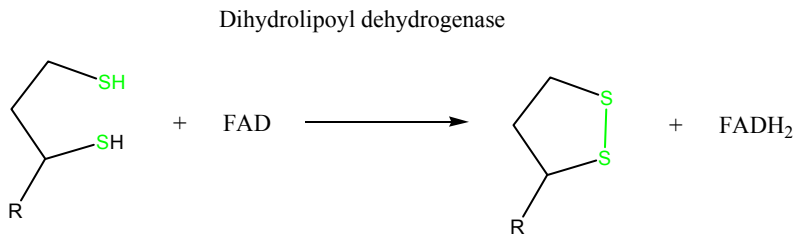
FADH
Semiquinone



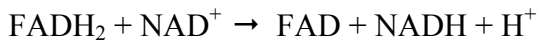
FADH₂
Hydroquinone



The E₃, dihydrolipoamide dehydrogenase enzyme regenerates lipoamide for another round of catalysis. This enzyme is a flavoprotein, meaning, it has a tightly bound FAD prosthetic group. Flavins undergo 2 electron reductions, but can accept electrons one at a time, unlike NAD⁺ which can only accept two electrons at a time in the form of a hydride. This gives flavoproteins more catalytic diversity than NAD coenzymes.



The final step of the reaction catalyzed by this E₃ enzyme is the transfer of the 2 electrons from the tightly bound FADH₂ to the transiently bound NAD⁺ to generate NADH + H⁺.



This is a usual electron transfer. The common role of FAD is to accept electrons from NADH. One thing about flavoproteins is that the protein bound flavins have a great variety of reduction potentials. In this enzyme the reduction potential is shifted such that FADH₂ is the electron donor and NAD⁺ is the acceptor.

Typical flavoprotein

