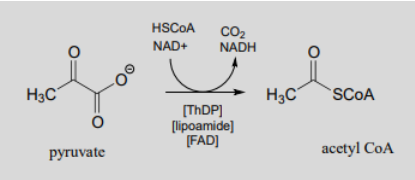
17.4: Thiamine Diphosphate, Lipoamide and the Pyruvate Dehydrogenase Reaction

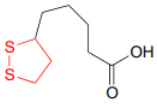
The enzyme pyruvate dehydrogenase is one of the most central of all the enzymes of central metabolism: by converting pyruvate to acetyl-CoA, it links glycolysis (where glucose is broken down into pyruvate) to the citric acid cycle, into which carbons enter in the form of acetyl-CoA. Five ceonzymes are involved: coenzyme A, nicotinamide, thiamine diphosphate, FAD, and finally lipoamide, one which is new to us at this point.

Reaction catalyzed by pyruvate dehydrogenase:

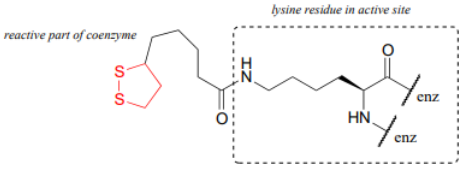


You will learn more about the structure and metabolic role of this complex and remarkable enzyme in a biochemistry course. Here, we will focus on the multi-step organic reaction it catalyzes, which we are at long last equipped to understand.

Looking at the reaction, you should recognize that, first of all, the pyruvate substrate is being oxidized - it starts out as a ketone, and ends up as a thioester, losing carbon dioxide in the process. Ultimately, the oxidizing agent in this reaction is NAD^+, but the reduction of NAD^+ is linked to the oxidative decarboxylation of pyruvate by FAD and a disulfide-containing coenzyme called lipoamide, which is lipoic acid attached by an amide linkage to a lysine residue on the enzyme.



lipoic acid

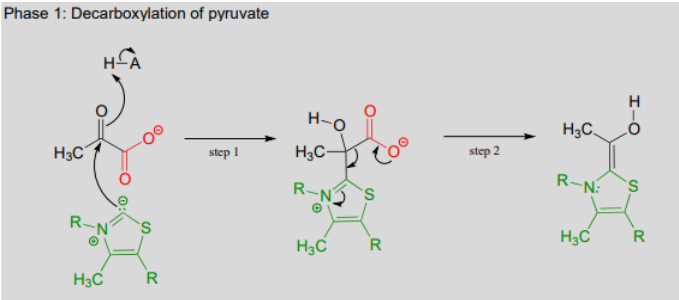


lipoamide
(linked to lysine residue in enzyme)

The second thing to notice is that, because the reaction involves breaking the bond between the ketone carbon and an adjacent carbon, thiamine diphosphate ($ThDP$) coenzyme is required. In fact, the first phase of the reaction (steps 1 and 2 below) is identical to that of pyruvate decarboxylase, an enzyme we discussed a few pages ago.

The pyruvate decarboxylase reaction mechanism

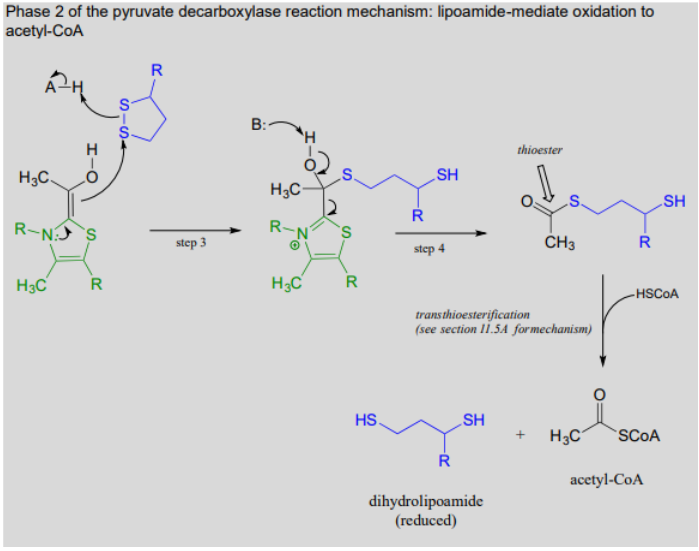
Phase 1: Decarboxylation of pyruvate



The $ThDP$-stabilized carbanion then acts as a nucleophile, cleaving the disulfide bridge of lipoamide (step 3 below). It is in this step that oxidation of the substrate is actually occurring. After the resulting thioester product is released from $ThDP$ (step 4

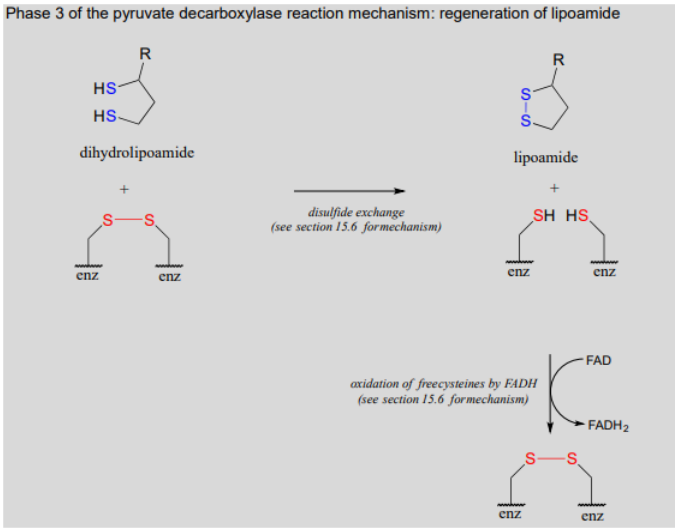
below), it undergoes transesterification form acetyl-CoA, the product of the reaction.

Phase 2 of the pyruvate decarboxylase reaction mechanism: lipoamide-mediate oxidation to acetyl-CoA



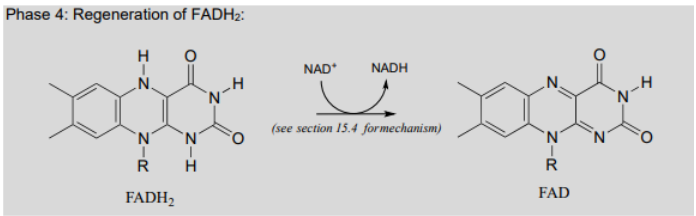
We are not done yet! In order for the catalytic cycle to be complete, the reduced dihydrolipoamide must be regenerated back to its oxidized state through disulfide exchange with a disulfide bond on the enzyme. The pair of enzymatic cysteines is then oxidized back to disulfide form by an FAD-dependent reaction.

Phase 3 of the pyruvate decarboxylase reaction mechanism: regeneration of lipoamide



Finally, FAD is regenerated with concurrent reduction of NAD^+:

Phase 4: Regeneration of $FADH_2$:



platform.