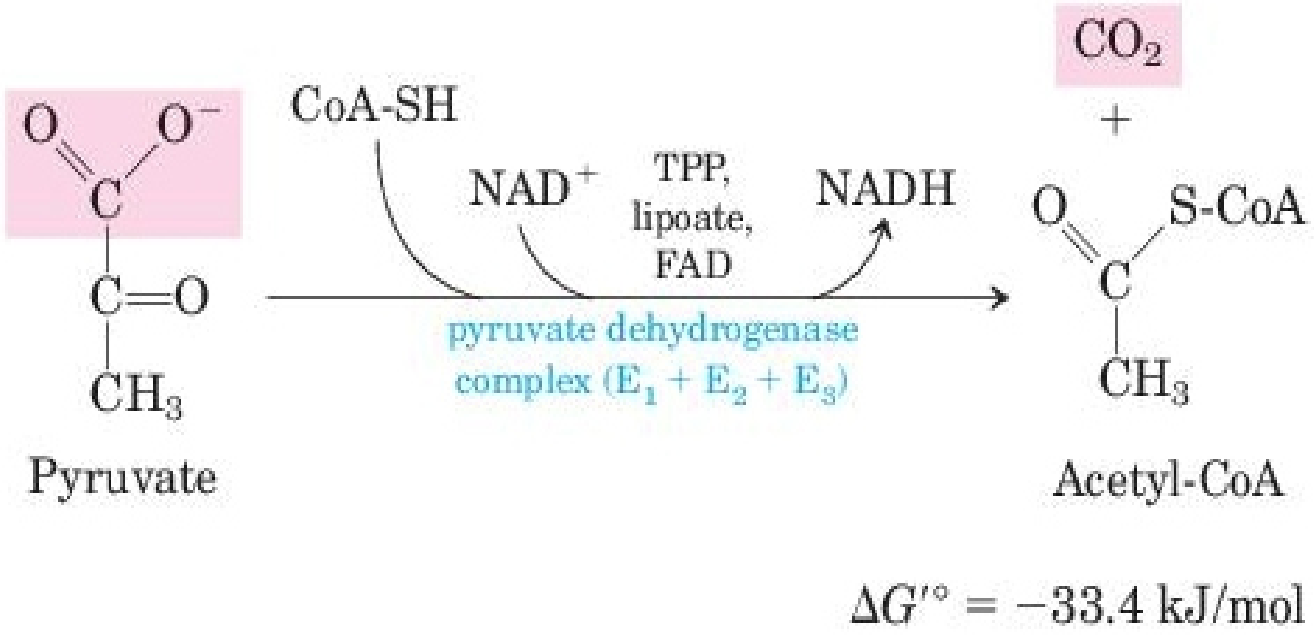
Formation of Acetyl CoA

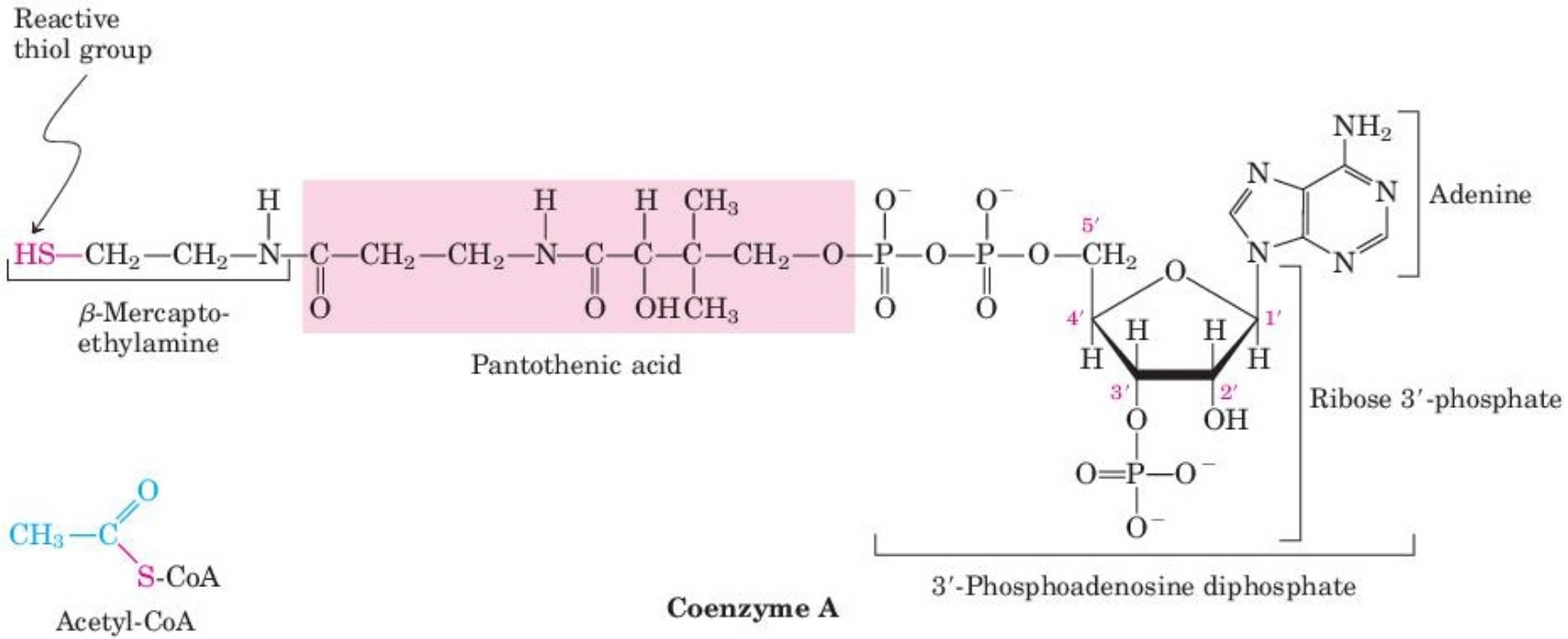
Source – Lehninger
(Principle of Biochemistry)

Conversion of Pyruvate to Acetyl CoA



$\Delta G'^{\circ} = -33.4$ kJ/mol

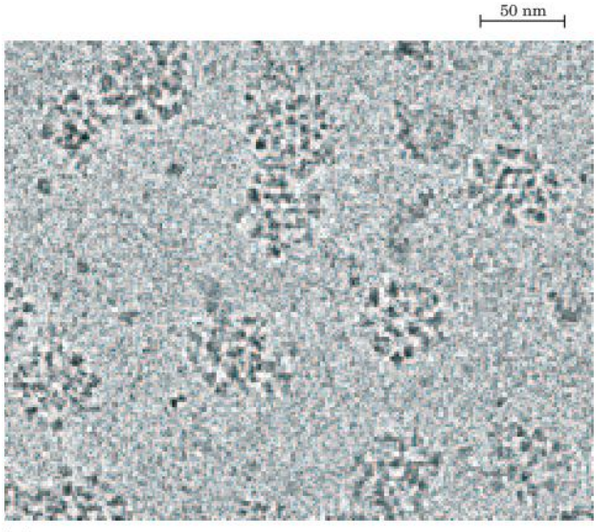
Addition of CoA to Acetyl Grp



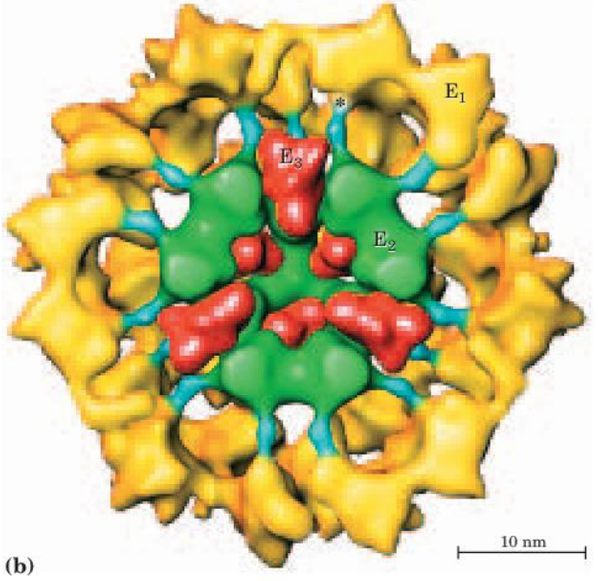
The Pyruvate Dehydrogenase Complex Consists of Three Distinct Enzymes

The PDH complex contains three enzymes—**pyruvate dehydrogenase** (E_1), **dihydrolipoyl transacetylase** (E_2), and **dihydrolipoyl dehydrogenase** (E_3)—each present in multiple copies. The number of copies of each enzyme and therefore the size of the complex varies among species. The PDH complex isolated from mammals is about 50 nm in diameter—more than five times the size of an entire ribosome and big enough to be visualized with the electron microscope (Fig. 16–5a). In the bovine enzyme, 60 identical copies of E_2 form a pentagonal dodecahedron (the core) with a diameter of about 25 nm (Fig. 16–5b). (The core of the *Escherichia coli* enzyme contains 24 copies of E_2.) E_2 is the point of connection for the prosthetic group lipoate, attached through an amide bond to the ε-amino group of a Lys residue (Fig. 16–4). E_2 has three functionally distinct domains (Fig. 16–5c): the amino-terminal *lipoyl domain*, containing the lipoyl-Lys residue(s); the central E_1- and E_3-*binding domain;* and the inner-core *acyltransferase domain,* which contains the acyltransferase active site. The yeast PDH complex has a single lipoyl domain with a lipoate attached, but the mammalian complex has two, and *E. coli* has three (Fig. 16–5c). The domains of E_2 are separated by linkers, sequences of 20 to 30 amino acid residues, rich in Ala and Pro and interspersed with charged residues; these linkers tend to assume their extended forms, holding the three domains apart.

The active site of E_1 has bound TPP, and that of E_3 has bound FAD. Also part of the complex are two reg-

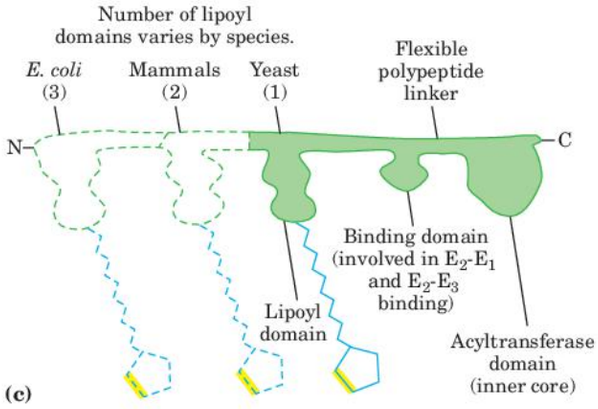


(a)



(b)

FIGURE 16–5 Structure of the pyruvate dehydrogenase complex **(a)** Cryoelectron micrograph of PDH complexes isolated from bovine kidney. In cryoelectron microscopy, biological samples are viewed at extremely low temperatures; this avoids potential artifacts introduced by the usual process of dehydrating, fixing, and staining. **(b)** Three-dimensional image of PDH complex, showing the subunit structure: E_1, pyruvate dehydrogenase; E_2, dihydrolipoyl transacetylase; and E_3, dihydrolipoyl dehydrogenase. This image is reconstructed by analysis of a large number of images such as those in **(a)**, combined with crystallographic studies of individual subunits. The core (green) consists of 60 molecules of E_2, arranged in 20 trimers to form a pentagonal dodecahedron. The lipoyl domain of E_2 (blue) reaches outward to touch the active sites of E_1 molecules (yellow) arranged on the E_2 core. A number of E_3 subunits (red) are also bound to the core, where the swinging arm on E_2 can reach their active sites. An asterisk marks the site where a lipoyl group is attached to the lipoyl domain of E_2. To make the structure clearer, about half of the complex has been cut away from the front. This model was prepared by Z. H. Zhou et al. (2001); in another model, proposed by J. L. S. Milne et al. (2002), the E_3 subunits are located more toward the periphery (see Further Reading). **(c)** E_2 consists of three types of domains linked by short polypeptide linkers: a catalytic acyltransferase domain; a binding domain, involved in the binding of E_2 to E_1 and E_3; and one or more (depending on the species) lipoyl domains.



(c)

ulatory proteins, a protein kinase and a phosphoprotein phosphatase, discussed below. This basic E_1-E_2-E_3 structure has been conserved during evolution and used in a number of similar metabolic reactions, including the oxidation of α-ketoglutarate in the citric acid cycle (described below) and the oxidation of α-keto acids derived from the breakdown of the branched-chain amino acids valine, isoleucine, and leucine (see Fig. 18–28). Within a given species, E_3 of PDH is identical to E_3 of the other two enzyme complexes. The attachment of lipoate to the end of a Lys side chain in E_2 produces a long, flexible arm that can move from the active site of E_1 to the active sites of E_2 and E_3, a distance of perhaps 5 nm or more.

In Substrate Channeling, Intermediates Never Leave the Enzyme Surface

Figure 16–6 shows schematically how the pyruvate dehydrogenase complex carries out the five consecutive reactions in the decarboxylation and dehydrogenation of pyruvate. Step ① is essentially identical to the reaction catalyzed by pyruvate decarboxylase (see Fig. 14–13c); C-1 of pyruvate is released as CO_2, and C-2, which in pyruvate has the oxidation state of an aldehyde, is attached to TPP as a hydroxyethyl group. This first step is the slowest and therefore limits the rate of the overall reaction. It is also the point at which the PDH complex exercises its substrate specificity. In step ② the hydroxyethyl group is oxidized to the level of a carboxylic acid (acetate). The two electrons removed in this reaction reduce the —S—S— of a lipoyl group on E_2 to two thiol (—SH) groups. The acetyl moiety produced in this oxidation-reduction reaction is first esterified to one of the lipoyl —SH groups, then transesterified to CoA to form acetyl-CoA (step ③). Thus the energy of oxidation drives the formation of a high-energy thioester of acetate. The remaining reactions catalyzed by the PDH complex (by E_3, in steps ④ and ⑤) are electron transfers necessary to regenerate the oxidized (disulfide) form of the lipoyl group of E_2 to prepare the enzyme complex for another round of oxidation. The electrons removed from the hydroxyethyl group derived from pyruvate pass through FAD to NAD^+.

Central to the mechanism of the PDH complex are the swinging lipoyllysyl arms of E_2, which accept from E_1 the two electrons and the acetyl group derived from pyruvate, passing them to E_3. All these enzymes and coenzymes are clustered, allowing the intermediates to react quickly without diffusing away from the surface of the enzyme complex. The five-reaction sequence shown in Figure 16–6 is thus an example of **substrate channeling.** The intermediates of the multistep sequence never leave the complex, and the local concentration of the substrate of E_2 is kept very high. Channeling also prevents theft of the activated acetyl group by other enzymes that use this group as substrate. As we shall see, a similar tethering mechanism for the channeling of substrate between active

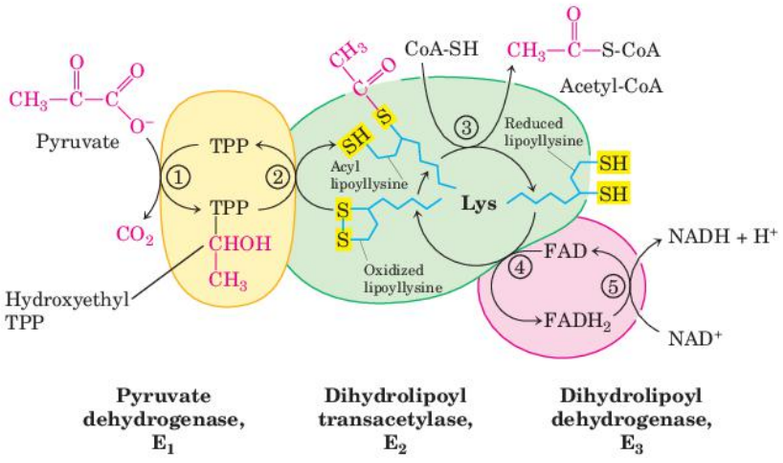


FIGURE 16–6 Oxidative decarboxylation of pyruvate to acetyl-CoA by the PDH complex. The fate of pyruvate is traced in red. In step ① pyruvate reacts with the bound thiamine pyrophosphate (TPP) of pyruvate dehydrogenase (E_1), undergoing decarboxylation to the hydroxyethyl derivative (see Fig. 14–13). Pyruvate dehydrogenase also carries out step ②, the transfer of two electrons and the acetyl group from TPP to the oxidized form of the lipoyllysyl group of the core enzyme, dihydrolipoyl transacetylase (E_2), to form the acetyl thioester of the reduced lipoyl group. Step ③ is a transesterification in which the —SH group of CoA replaces the —SH group of E_2 to yield acetyl-CoA and the fully reduced (dithiol) form of the lipoyl group. In step ④ dihydrolipoyl dehydrogenase (E_3) promotes transfer of two hydrogen atoms from the reduced lipoyl groups of E_2 to the FAD prosthetic group of E_3, restoring the oxidized form of the lipoyllysyl group of E_2. In step ⑤ the reduced $FADH_2$ of E_3 transfers a hydride ion to NAD^+, forming NADH. The enzyme complex is now ready for another catalytic cycle. (Subunit colors correspond to those in Fig. 16–5b.)