Active Site Coupling in Multienzyme Complexes

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We present here the first crystal structure of a complex between pyruvate decarboxylase (E1) and the peripheral subunit-binding domain (PSBD) of the acetyltransferase (E2), which interact within the pyruvate dehydrogenase (PDH) multienzyme complex.

Remarkably, the PSBD uses essentially the same surface to recognize alternately the third component of the PDH assembly, namelyE3. The PSBD achieves this dual recognition largely through the addition of a network of interfacial water molecules unique to the E1-PSBD complex. These structural comparisons illuminate our observations that the formation of the water-rich interface in the E1-E2 complex is largely enthalpy-driven, whereas that of the E3-PSBD complex (from which bound water is excluded) is entropy-driven.

E1 is a thiamine diphosphate (ThDP)-dependent enzyme composed of a dimer of active sites. We present evidence that the ThDPs in the two active sites of the E1 communicate over a distance of 20 Å by reversibly shuttling a proton through an acidic tunnel in the protein [1]. This ‘‘proton wire’’ permits the cofactors to serve reciprocally as general acid/base in catalysis, which synchronizes the progression of chemical events and can account for the oligomeric organization, conformational asymmetry, and ‘‘ping-pong’’ kinetic properties of E1 and other ThDP-dependent enzymes.

[1] Frank R. A. W., Titman C. M., Pratap V., Luisi B. F., Perham R. N., *Science*, 2004, **306**, 872.

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