

STRUCTURAL BASIS FOR FLIP-FLOP ACTION OF THIAMIN PYROPHOSPHATE-DEPENDENT ENZYMES REVEALED BY HUMAN PYRUVATE DEHYDROGENASE

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Thiamin pyrophosphate (TPP), the biologically active form of vitamin B₁, is a cofactor of enzymes catalyzing reactions involving the cleavage of a carbon-carbon bond adjacent to an oxo group. TPP-dependent enzymes show a common mechanism of TPP activation by (i) forming the ionic N-H...O' hydrogen bonding between the N1' atom of the aminopyrimidine ring of the coenzyme and intrinsic γ -carboxylate group of glutamate and (ii) imposing an "active" V-conformation that brings the N4' atom of the aminopyrimidine to the distance required for the intramolecular C-H...N hydrogen bonding with the thiazolium C2 atom. Within these two hydrogen bonds that rapidly exchange protons, protonation of the N1' atom is strictly coordinated with the deprotonation of the 4'-amino group and eventually abstraction of the proton from C2..

The human pyruvate dehydrogenase E1p, component of human pyruvate dehydrogenase complex, catalyzes the irreversible decarboxylation of the pyruvate followed by the reductive acetylation of the lipoyl group of dihydrolipoyl acyltransferase. E1p is $\alpha_2\beta_2$ -heterotetrameric with a molecular mass of 154 kDa, which has two catalytic sites, each providing TPP and magnesium ion as cofactors and each formed on the interface between the PP and PYR domains. The dynamic nonequivalence of two otherwise chemically equivalent catalytic sites has been observed and the flip-flop mechanism was suggested, according to which two active sites affect each other and in which different steps of the catalytic reaction are performed in each of the sites at any given moment. Based on specific features of human pyruvate dehydrogenase including rigid and flexible connections between domains that bind the cofactor we propose a mechanistic model for the flip-flop action of this enzyme [1]. We postulate that the dynamic protein environment drives the exchange of tautomers in the 4'-aminopyrimidine ring of the cofactor through a concerted shuttle-like motion of tightly connected domains. The dynamic exchange of those tautomers, in turn, is required during the reactions of pyruvate decarboxylation and reductive acetylation of lipoamide. Thus the shuttle-like motion of the domains is coordinated with the reactions of decarboxylation and acetylation, which are carried out in each of the cofactor sites resulting in a flip-flop action of the enzyme.

The structure-derived mechanism of action of human pyruvate dehydrogenase may be likely common for other TPP-dependent enzymes.

[1] E. M. Ciszak, L. G. Korotchkina, P. M. Dominiak, S. Sidhu, and M. S. Patel, *J. Biol. Chem.*, 278 (2003) 21240