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Effects of Mutations on a Pre-decarboxylation Reaction Intermediate Analogue-Pyruvate Dehydrogenase E1 Component Complex. P. Arjunan^{1, 2}, K. Chandrasekhar^{1, 2}, N. Nemeria³, F. Jordan³, W. Furey^{1, 2}, ¹Biocrystallography Laboratory, VA Pittsburgh Healthcare System, University Dr. C, Pittsburgh, PA 15240, ²Dept. of Pharmacology, Univ. of Pittsburgh, School of Medicine, Pittsburgh, PA 15261, ³Dept. of Chemistry, Rutgers Univ., Newark, NJ 07102.

The thiamin diphosphate (ThDP) dependent E1 component of the pyruvate dehydrogenase multienzyme complex (PDHc) catalyzes the rate-limiting step of the overall PDHc reaction and subsequent acetyl transfer to a lipoyl-lysine residue from the E2 component. It therefore provides an ideal target for mechanistic structural investigation. In an effort to obtain structural information on the first ThDP-bound intermediate in the presence of the enzyme, we had previously reported the crystal structure of the reaction intermediate analogue α -phosphonolactylthiamin diphosphate (PLThDP) in complex with the native E1. We have now studied the same complex with the active site variants H407A and E571A. While there are general similarities between the native and these two structures, there are significant differences in the active site. Regarding the PDHc specific reaction, the presence of PLThDP induces large-scale conformational changes in the enzyme. Comparison of these structures with catalytic activity will be presented.