

W0145

Structural Effects of a Catalytically Important E571A Mutation in the *E. coli* Pyruvate Dehydrogenase Multienzyme Complex E1 Component. P. Arjunan^{1,2}, K. Chandrasekhar^{1,2}, N. Nemeria³, F. Jordan³, W. Furey^{1,2}, ¹Biocrystallography Laboratory, VA Pittsburgh Healthcare System, Univ. Dr. C, Pittsburgh, PA 15240; ²Dept. of Pharmacology, Univ. of Pittsburgh, School of Medicine, Pittsburgh, PA 15261 and ³Dept. of Chemistry, Rutgers Univ., Newark, NJ 07102.

In thiamin (ThDP)-dependent enzymes dual active sites are formed at the interface between subunits comprising a tightly packed dimer, with conserved cofactor binding interactions involving both subunits. In the pyruvate dehydrogenase multienzyme complex (PDHc) E1 component and related enzymes, these interactions include a catalytically important conserved hydrogen bond between the N1' nitrogen and the side chain of a conserved glutamic acid residue. Mutation of the conserved Glu571 in PDHc E1 to alanine has a profound effect on catalytic activity, retaining only 1% of the wild-type activity. Crystal structures of the E571A variant of *E. coli* PDHc E1 with and without the ThDP cofactor present have been determined to resolutions of 2.1 and 1.9 Å, respectively. While there are general similarities between the native and these two structures, there are significant differences in the active site. Details and implications of ThDP binding on catalytic activity will be presented.