

E0032

**A Thiamin-Bound, Pre-decarboxylation, Reaction Intermediate Analogue for Pyruvate in the Pyruvate Dehydrogenase Multi-enzyme Complex E1 Component.** P. Arjunan, A. Brunskill, W. Furey, VA Medical Center, Pittsburgh, PA, Dept. of Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA, N. Nemeria & F. Jordan. Dept. of Chemistry, Rutgers Univ., Newark, NJ.

The thiamin diphosphate (ThDP) dependent E1 component of the pyruvate dehydrogenase multi-enzyme complex (PDHc) catalyzes the decarboxylation of pyruvate to 2-alpha-hydroxyethylidene-ThDP and subsequent acetyl transfer to a lipoyl-lysine residue from the E2 component. The crystal structure of the PDHc E1 component from *E.coli* has been determined with a thiamin-bound, pre-decarboxylation, reaction intermediate analogue in its active site and refined to a resolution of 2.1Å. The structure has been refined by a combination of simulated annealing and restrained least squares to an R factor of 0.185 for 90245 reflections. This represents the first structural example of a thiamin-bound, pre-decarboxylation, intermediate analogue in the active site of a ThDP dependent enzyme, and serves as a model for all such enzymes. The structure reveals movements of key active site residues that stabilize the intermediate, as well as conformational changes in more distant residues that may be involved in protein-protein interactions within the multi-enzyme complex.