

## E0016

**Importance of Glu28 in the Protection of Thiamine Diphosphate in Benzoylformate Decarboxylase.** Asim Kumar Bera, Natalie Anderson and Miriam S. Hasson, Dept. of Biological Sciences, Purdue Univ., West Lafayette, IN.

Thiamin diphosphate (ThDP)-dependent enzymes play important roles in the metabolic pathways of cells. The function of ThDP in one of these enzymes, benzoylformate decarboxylase, has been explored in our laboratory using enzymological assays and crystallographic studies at high resolution. The variety of crystal forms and the high-resolution structures have provided close views of various steps of the reaction mechanism. Here we concentrate only on one important active-site residue, Glu28. A 1.1Å crystal structure of the Glu28Ala mutant may explain its pH dependence by showing that a water molecule replaces the side chain of Glu28 to form a hydrogen bond with His70. This observation explains some of the catalytic properties of the mutant; the water molecule is ready to provide a proton in a similar way the carboxylate group of Glu28. Unexpectedly, an atom is bound to the C2 of ThDP in the Glu28Ala mutant. In order to identify this atom, a structure of Glu28Ala mutant with thiamin thiazolone diphosphate was solved to 1.0 Å resolution. The similarity of the two structures, the distance of the peak of the map from C2, and the strength of the density imply that the ThDP has been oxidized in the Glu28Ala mutant. We have also solved the structure of Glu28Gln structure to 1.2 Å. In this structure, the movement of His70 provides a water-mediated interaction with the side-chain nitrogen of the Gln residue. Therefore, our results suggest that Glu28 not only enhances the ability of His70 to transfer protons, but also inhibits a destructive side reaction.