

## W0018

**YbdK is a Carboxylate-amine Ligase with  $\gamma$ -glutamyl:cysteine Ligase Activity.** Christopher Lehmann<sup>1,4</sup>, V. Doseeva<sup>2</sup>, S. Pullalarevu<sup>2</sup>, W. Krajewski<sup>3</sup>, A. Howard<sup>4,5</sup>, O. Herzberg<sup>2</sup>, <sup>1</sup>Dept. of Chem & Chemical Biology, Cornell U., Ithaca, NY <sup>2</sup>CARB, U. of Maryland Biotechnology Inst., Rockville, MD <sup>3</sup>Inst. of Cell & Molecular Biology, Uppsala U., Uppsala, Sweden <sup>4</sup>APS, Argonne Nat'l Lab, Argonne, IL <sup>5</sup>Illinois Inst. of Technology, Chicago, IL.

The *Escherichia coli* open reading frame *ybdK* encodes a 372 amino acid residue protein, which is a member of a large bacterial protein family of unknown biological function. The sequences within this family are remotely related to the sequence of  $\gamma$ -glutamyl:cysteine ligase ( $\gamma$ -GCS, EC:6.3.2.2), an enzyme in the glutathione biosynthetic pathway. A gene coding for *E. coli*  $\gamma$ -GCS is already known. The 2.15 Å resolution crystal structure of YbdK from *E. coli* reveals a fold similar to that of glutamine synthetase (GS), a nitrogen metabolism enzyme that ligates glutamate and ammonia to yield glutamine (EC:6.3.1.2). GS and  $\gamma$ -GCS perform related chemical reactions, and require ATP and Mg<sup>2+</sup> for their activity. By analogy, we propose that YbdK requires the same cofactors. The Mg<sup>2+</sup>-dependent ATP binding to YbdK was confirmed using the fluorescence-enhanced compound, TNP-ATP. A deep cavity in YbdK corresponds to the cavity where ATP, Mg<sup>2+</sup>, and glutamate bind in GS. Many of the GS residues that coordinate the metal ions, interact with the glutamic acid, and with the phosphoryl and ribosyl groups of ATP are also present in YbdK. The amino acids implicated in ammonia binding in GS have no obvious counterpart in YbdK, consistent with a substrate specificity that is different than that of GS. Ligase activity between glutamic acid and each of the twenty amino acid residues was tested on HPLC by following the hydrolysis of ATP to ADP. Catalysis was observed only with cysteine. The pyruvate kinase/lactate dehydrogenase assay was used to determine a  $k_{cat}$  value of 0.007 s<sup>-1</sup> for the ligase reaction of Glutamate, ABA ( $\gamma$ -aminobutyric acid), and ATP.

<sup>1</sup>Yamashita MM, Almasy RJ, Janson CA, Cascio D, Eisenberg D. Refined atomic model of glutamine synthetase at 3.5 Å resolution. *J Biol Chem* 1989;264(30):17681-17690.

<sup>2</sup>Abbott JJ, Pei J, Ford JL, Qi Y, Grishin VN, Pitcher LA, Phillips MA, Grishin NV. Structure prediction and active site analysis of the metal binding determinants in gamma-glutamylcysteine synthetase. *J Biol Chem* 2001;276(45):42099-42107.