

W0269

Crystallization of Partially Trypsinized *E. coli* PEP Carboxykinase. K.C. Klemmer¹, H. Goldie², L. Prasad¹, L.T.J. Delbaere¹, Depts. of Biochemistry¹ and Microbiology & Immunology², Univ. of Saskatchewan. S7N 5E5, Canada.

Phosphoenolpyruvate carboxykinase [ATP dependent] (PCK) from *Escherichia coli* is activated by Ca²⁺ or by Mn²⁺ in the presence of saturating concentrations of MgATP. Partial digestion of PCK with trypsin abolishes activation by Ca²⁺ but not via Mn²⁺. A 120-minute trypsin digest of PCK produced PCKD, which crystallized in space group P2₁, a novel space group for PCK. The crystal structure revealed no electron density for amino acid residues 390-400 but the remainder of the C-terminal polypeptide was present. Amino acid residues from 442-455 were demonstrated to have a unique orientation. These latter residues form the P-loop, which is important for binding and stabilizing the phosphate backbone of ATP. SDS-PAGE verified the trypsin cleavage at Arg396, through the separation of PCK into two distinct fragments, 45 and 15 kDa in size. Strikingly, kinetic assays of PCKD demonstrated Ca²⁺ activation is partially regained. Ca²⁺ activation is restored through gel filtration and/or a freeze thaw cycle. A possible mechanism thought to result in the restoring of Ca²⁺ activation, is by a conformational change.