MS11-P4 **Crystal Structure of Enzyme-Substrate Complex of Protein-Glutaminase Obtained by the Mutant of Pro-Enzyme** Bunzo Mikami[a], Yukiko Maki, Ryota Hashizume[a], Kimihiko Mizutani[a], Nobuyuki Takahashi[a], Hiroyuki Matsubara[b], Akiko Sugita[b], Kimihiko Sato[b], Shotaro, Yamaguchi[b] [a]*Laboratory of Applied Structural Science, , Graduate School of Agriculture, Kyoto University, Japan,* [b] *Gifu R&D Center, Amano Enzyme Inc.Japan*
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Protein-glutaminase, which converts glutamine residues in proteins or peptides to glutamic acid residues, is expected to see wide use as a new food processing enzyme. Deamidation of proteins can improve their solubility, emulsifying activity, forming activity, and other functional properties by increasing the number of negative charges. We have reported the crystal structures of a mature protein-glutaminase with 185 amino acid residues refined at 1.15 Å resolution and a recombinant pro-enzyme with 299 amino acid residues refined at 1.5 Å resolution [1]. The enzyme has a catalytic triad, Cys-His-Asp conserved in transglutaminases and cysteine proteases. We found that a short loop around Ala 47 in the pro-region covers and interacts with the active site. In order to elucidate the catalytic mechanism of this enzyme, crystal structures of A47Q mutant were determined in a various conditions. We concluded that the side chain of Gln 47 forms a covalent bond with catalytic Cys156/S in the absence of ammonium ion but it forms a non-covalent ES complex in the presence of ammonium ion. We also found an oxidized Cys156 forming a covalent bond with Arg 159 in the presence of pottasium ion, which indicate the inactivation of this enzyme by oxidation. The structure of A47Q mutant provides insights into the catalytic mechanism of the enzyme which forms a covalent S-acyl intermediate before release of ammonia.

[1] Hashizume R, Maki Y, Mizutani K, Takahashi N, Matsubara H, Sugita A, Sato K, Yamaguchi S, Mikami B. (2011). *J Biol Chem.* **286**, 38691-38702.

Keywords: protein-glutaminase, pro-enzyme, enzyme mechanisms

MS11-P5 **Structural basis for the cytidylyltransferase reaction catalyzed by yeast ECT.** Jun Ohtsuka,[a] Ryouichi Fukuda,[b] Shipeng Wang,[a] Yusuke Ono,[b] Woo Cheol Lee,[a] Kosuke Ito,[a] Koji Nagata,[a] Akinori Ohta,[b] and Masaru Tanokura.[a] *Deparments of* [a]*Applied Biological Chemistry and* [b]*Biotechnology, Graduate Schrool of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan.*
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Cytidine-5'-diphosphoethanolamine (CDP-Etn) is a key intermediate compound in the synthesis of phosphatidylethanolamine (PtdEtn) *via* the eukaryote-specific biosynthetic pathway of PtdEtn, and is produced by CTP:*O*-phosphoethanolamine cytidylyltransferase (ECT). ECT requires Mg^{2+} for the conversion of CTP and *O*-phosphoethanolamine (P-Etn) to CDP-Etn and pyrophosphate (PPi). Although some crystal structures of cytiylyltransferases and nucleotidyltransferases were reported so far, the role of the essential divalent cation have not been revealed. Here we report three kinds of crystal structures of *Saccharomyces cerevisiae* ECT (yECT) that correspond to the three stages of its ordered Bi Bi reaction: yECT·CTP, yECT·CDP-Etn·PPi, and yECT·CDP-Etn. All these crystals belonged to $P2_12_12_1$ with the unit cell parameters of approximately a = 65 Å, b = 66 Å, and c = 150 Å. The crystal structure of yECT·CTP was determined by an Se-SAD phasing method and the others were determined by molecular replecement using the atomic coordinate of yECT·CTP as a search model. The final models contained CTP, CDP-Etn–pyrophosphate–Mg^{2+} complex, or CDP-Etn with good electron dencities. These crystal structures and activity measurements have revealed that 1) yECT is composed of N- and C-terminal domains and an extended C-terminal helix domain, 2) the ECT reaction is catalyzed by the N-terminal domain and the C-terminal helix, 3) Mg^{2+} reduces the repulsion force between the negatively charged substrates and products, and 4) the cytidylyltransferase reaction was promoted by a histidine residue of HxGH nucleotide-binding motif. These results suggested how yECT overcomes the electrostatic repulsion between the negatively charged substrates by utilizing Mg^{2+} and how yECT transfers the CMP moiety of CTP to the phosphate group of P-Etn to form a high-energy phosphoanhydride bond of CDP-Etn.

[1] Ohtsuka, J., et al. (2006). *Acta Crystallogr. Sec. F Struct. Biol. Cryst. Commun.*, **62**, 1003–1005.

Keywords: protein; enzyme; reaction mechanism