

remains poorly understood. The enzyme used in this study is papain, a member of the C1 family and the archetypal CP. Papain is obtained from papaya fruits; its utility in tenderizing meat has been known for thousands of years. Papain finds immunological utility in the cleavage of immunoglobulins into Fc and FAB fragments, and medical use in the treatment of stings and chronic wounds. Earlier work reported the crystallization of papain from ethanol/methanol solutions, revealing that papain comprises 2 major structural domains. Papain activation is believed to depend on the formation of a thiolate-imidazolium pair between residues Cys25 and His159 at the cleft between domains. In this study we have obtained crystals from a new aqueous condition containing PEG, buffer, and sodium thiosulfate. In this condition, papain crystallizes in a low-solvent-content unit cell. A 1.60 Å X-ray data set was collected at 300 K in 4 h on a copper-source diffractometer. Results show that a thiosulfate moiety is bound to the active site cysteine, Cys25. Efforts to optimize crystal size for ultra-high resolution X-ray diffraction and neutron diffraction data collection are ongoing. By locating the hydrogen atoms at the active site, we hope to determine the protonation state of His159 and obtain a clearer picture of papain activation and substrate hydrolysis.

Keywords: X-ray and neutron diffraction, proteases proteinases, agricultural natural products

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#### Crystal structures of *Streptococcus pneumoniae* penicillin-binding proteins acyl-enzyme complexes

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Penicillin-binding proteins (PBPs) are enzymes that catalyze the polymerization and cross-linking of peptidoglycan precursors during bacterial cell wall biosynthesis.  $\beta$ -lactam antibiotics inhibit transpeptidase and DD-carboxypeptidase activities of PBPs by acylating their active-site Ser. To envisage the binding of  $\beta$ -lactam antibiotics to PBPs, we determined a crystal structure of a trypsin-digested form of PBP 2X from *Streptococcus pneumoniae* strain R6 complexed with a cephalosporin antibiotic, cefditoren [1]. We also determined crystal structures of the trypsin-digested form of both PBPs 2X and 1A, each complexed with a carbapenem antibiotic, biapenem or tebipenem [2]. The structures of the acyl-enzyme complexes showed that the cephalosporin C3 side chain and the carbapenem C2 side chains form hydrophobic interactions with Trp374 and Thr526 of PBP 2X and with Trp411 and Thr543 of PBP 1A, however a conformational change of the Trp374 side chain of PBP 2X occurred only upon cefditoren binding. Although the structures studied here were products of inactivation reactions by  $\beta$ -lactams, these hydrophobic interactions are likely to play a role in drug binding upon acylation. There may be similar interactions in PBP 2B from *S. pneumoniae* because a crystal structure of PBP 2B showed that Trp429 and Thr605 occupy positions similar to those of the Trp and Thr residues in the active sites of PBPs 2X and 1A [3].

[1] Yamada, M., Watanabe, T., Miyara, T., Baba, N., Saito, J., Takeuchi, Y. & Ohsawa, F. (2007). *Antimicrob. Agents Chemother.* 51, 3902-3907.

[2] Yamada, M., Watanabe, T., Baba, N., Takeuchi, Y., Ohsawa, F & Shuichi Gomi. (2008). *Antimicrob. Agents Chemother. in press.*

[3] Yamada, M., Watanabe, T., Baba, N., Miyara, T., Saito, J. &

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Keywords: antibiotics, peptidoglycan biosynthesis, inhibitor interactions

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#### Crystal structure of a serine protease defines a novel family of secreted bacterial proteases

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Recently, a serine protease secreted by an alkaliphilic and moderately halophilic microbe AL20 belonging to the Nesterenkonia abyssinica family, has been purified and characterized. AL20 protease is optimally active at pH 10, 1.0 M NaCl and 343 K and it shows good stability at 323 K in the presence of EDTA and detergents. From sequence similarity search, AL20 protease has defined a novel protein family of bacterial secreted proteases. Combined with biochemical analyses and high resolution crystal structure determination, we have unambiguously characterized the AL20 protease and its sequence related family as a trypsin-like serine protease.

Keywords: AL20 protease, bacterial secreted proteases, trypsin-like

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#### Crystal structures of *Esheria coli* $\gamma$ -glutamyltranspeptidase in complex with glutamine antagonists

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$\gamma$ -Glutamyltranspeptidase (GGT) is a heterodimeric enzyme that catalyzes the transfer of the  $\gamma$ -glutamyl group in  $\gamma$ -glutamyl compounds such as glutathione and its S-conjugates either to water or to other amino acids and peptides. GGT is involved in a number of biological events such as drug resistance and metastasis of cancer cells by detoxification of xenobiotics. Azaserine and acivicin are classical and irreversible inhibitors of GGT, but their binding sites and the inhibition mechanisms remain to be defined. We have determined the crystal structures of GGT from *Esheria coli* in complex with glutamine antagonists, azaserine and acivicin, at 1.65 Å resolution. Both inhibitors are bound to GGT at its substrate-binding pocket in a similar manner as observed previously with the  $\gamma$ -glutamyl-enzyme intermediate: they form a covalent bond with