surface identified by NMR was flat and included a strip of three solvent-exposed Trp residues flanked by acidic residues. The overall structure of the AD2 was a TIM-barrel fold, which is a common fold in family 18 chitinases. The active site of the AD2 was in the groove-like cleft and was open to the solvent due to the lack of an additional small domain, which is observed in other family 18 chitinases.

Keywords: catalysts, enzyme structure, domains

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Successful expression of archaeal STT3/AglB membrane protein in *E.coli* cells

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Oligosaccharyltransferase (OST) is an enzyme that catalyzes the transfer of the oligosaccharide from a lipid donor to the side chain of an Asn residue within a consensus sequence of Asn-X-Thr/Ser, where X can be any amino acid residue except for Pro. Asn-glycosylation is widespread not only in eukaryotes but also in archaea and some eubacteria. OST catalyzes the co-translational transfer of an oligosaccharide to Asn residues in nascent polypeptide chains. The OST enzyme is a membrane-associated multisubunit protein complex in eukaryotes. STT3 is the most conserved subunit in the OST complex in the three domains of life. The STT3 is called AglB for archaea and PglB for bacteria. All STT3/AglB/PglB proteins are characterized by 11-13 transmembrane helices in the N-terminal half of the amino acid sequence and a globular domain in the C-terminal half on the luminal/out side of the membranes. We reported that the OST of *Pyrococcus furiosus*, a thermophilic archaeon, is composed of the STT3 protein alone, and catalyzes the transfer of a heptasaccharide onto peptides in an Asn-X-Thr/Ser-motif-dependent manner[1]. We determined the crystal structure of the C-terminal soluble domain of *P. furiosus* STT3 (PfSTT3) [1,2]. Here, we expressed the full-length PfSTT3 in *E. coli*, and found that the membrane fraction of *E. coli* cells had the OST activity. We then optimized *E. coli* strains and culture conditions to minimize the degradation of the full-length PfSTT3, and succeeded to obtain homogenous PfSTT3 after several purification steps, including heat treatment and His-tag affinity chromatography. The PfSTT3 is estimated to be monomeric from the elution volume of gel filtration in the presence of various detergents.

1) EMBO J., **27**, 234-43 (2008)
2) Acta Cryst., **F63**, 798-801(2007)

Keywords: membrane proteins, purification, quaternary association of proteins

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X-ray structure of HIV-1 protease-product peptides complex: Insights into the reaction mechanism

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Inhibitors based on the correct mechanism are likely to provide an alternate set of drugs against AIDS. To investigate the mechanism of HIV-1 protease, we have undertaken crystal structure analyses of HIV-1 protease complexed with substrate oligo-peptides, corresponding in sequence to natural cleavage sites of this enzyme. The crystalline complexes were prepared by soaking hexagonal crystals of unliganded enzyme in solutions of substrate peptides under different conditions of pH. We have earlier reported structure of HIV-1 protease complexed with the oligopeptide HKARVL*nFEAnLS ('*' is the site of cleavage, nF-nitrophenylalanine and nL is nor-Leucine), which was found to be trapped as a tetrahedral intermediate. The present study has been carried out on a complex with the same oligopeptide, but at a pH value of 6.2 as against the value of 2.0 used in the earlier study. X-ray diffraction data to 1.8Å resolution has been collected on the FIP beamline at ESRF. The crystal structure, solved using difference Fourier methods, has been refined to crystallographic *Rw/Rf* values of 21.3% / 25.5%, to 1.8Å resolution. In the refined structure, the substrate peptide is found cleaved, and the product peptides are not yet fully dissociated from the enzyme active site. On the basis of comparison of structures of the present product complex with those of Michaelis complex and tetrahedral intermediate complex, a mechanism is proposed for the action of HIV-1 protease. An important and novel feature of this mechanism is invocation of bifurcated hydrogen bonds as a means to influence proton migration rates during different steps of the cleavage reaction.

Keywords: HIV-1 protease, X-ray crystallography, reaction mechanism

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Crystal structure of *Helicobacter pylori* spermidine synthase suggests a distinct active site

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Spermidine synthase (putrescine aminopropyltransferase, PAPT) catalyzes the transfer of the aminopropyl group from decarboxylated S-adenosylmethionine to putrescine during spermidine biosynthesis. *Helicobacter pylori* PAPT (HpPAPT) has a low sequence identity with other PAPTs and lacks the signature sequence found in other PAPTs. The crystal structure of HpPAPT, determined by multiwavelength anomalous dispersion, revealed an N-terminal β-stranded domain and a C-terminal Rossmann-like domain. Structural comparison with other PAPTs showed that HpPAPT has a unique binding pocket between two domains, numerous non-conserved residues, a less acidic electrostatic surface potential, and a large buried space within the structure. HpPAPT lacks the gatekeeping loop which facilitates substrate binding in other PAPTs. PAPTs are essential for bacterial cell viability; thus, HpPAPT may be a potential antimicrobial drug target for *H. pylori* due to its characteristic PAPT sequence and distinct conformation.

Keywords: *Helicobacter pylori*, spermidine synthase,