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Structural basis and DNA binding property of α domain from *Bacillus subtilis* Lon protease

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Lon protease belongs to the ATPase associated with diverse cellular activities (AAA+) superfamily and is mainly responsible for eliminating misfolded or damaged proteins as well as for controlling the cellular activities of short-lived regulatory proteins. It consists of three distinct functional domains, and the small α domain, a subdomain of the central ATPase domain, is suggested as the substrate-recognition and DNA binding sites. Therefore, knowing the tertiary structures of α domain is particularly intriguing for discerning its substrates-discriminating and DNA binding mechanisms on Lon proteases. CD experiments showed that there is a conformational change for α domain of *Bacillus subtilis* Lon protease (Bs-Lon α domain) at extreme acidic and basic pH values. Using multidimensional NMR techniques, 3D NMR solution structure of Bs-Lon α domain at pH 5.8 was determined to comprise of four α -helices and a two-stranded parallel β -sheet, similar to that of the *E. coli* Lon α domain. The ensemble of 15 NMR structures was well defined, with average root-mean-square deviations of 0.64 ± 0.05 Å for the backbone atoms and 1.58 ± 0.09 Å for heavy-atoms in secondary structure regions. The gel mobility shift assays (GMSA) have been applied to further confirm the DNA-binding activity of Bs-Lon α domain. In this poster presentation, the detailed structural study on Bs-Lon α domain and structure-function relationships on α domains of ATP-dependent proteases will be discussed.

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Structure of a Polymyxin B Resistant Protein (PmrD) and its complex formation when interacting with PmrA from *Klebsiella pneumoniae*

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Bacterial have many two-component signal-transduction systems (TCSs) that response to specific environmental signals by altering the phosphorylated state of a response regulator (RR). It is known that PmrD can mediate the activation of a PmrA-PmrB two-component system by another PhoP-PhoQ two-component system that responds to the magnesium levels in the environment in *Salmonella*. PmrD is the first example of a small protein that enables a TCSs to response to the signal governing a different TCS by protecting the phosphorylated state of RR. Therefore, PmrD provides a novel mechanism for connecting bacterial two-component signal-transduction systems. So far, no structural information was available for this mystic protein. A polymyxin b resistant protein of KP is composed of 81 amino acid residues. We present the first NMR solution structure of PmrD, which shows a beta-barrel like protein consisting of a six-stranded antiparallel beta-sheet, on the basis of 1939 restraints. The ensemble of 20 NMR structures was well defined, with average root-mean-square deviations of 0.46 ± 0.11 Å for the backbone atoms and 1.35 ± 0.26 Å for heavy atoms. Besides, the phosphorylated PmrA, which was produced by the use of acetyl-phosphate as a phosphoryl donor on PmrA, was titrated with the 15N-labeled PmrD protein (a 4-fold excess of PmrA). Compared to the 2D HSQC spectrum of the free PmrD, we found that a number of residues are disappeared or possess shift change in the mixture, indicating that a structural change occurs in PmrD upon complex formation.

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SOLUTION STRUCTURE OF FAMILY 21 CARBOHYDRATE-BINDING MODULE FROM *RHIZOPUS ORYZAE* GLUCOAMYLASE

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Carbohydrate-binding modules (CBMs) function independently to assist carbohydrate-active enzymes. Family 21 CBMs contain about 100 amino acid residues, and some members have starch-binding functions or glycogen-binding activities. We report here the first structure of a family 21 CBM from the starch binding domain (SBD) of *Rhizopus oryzae* glucoamylase (RoCBM21) determined by nuclear magnetic resonance spectroscopy. This CBM has a β -sandwich fold with an immunoglobulin-like structure. Ligand-binding properties of RoCBM21 were analyzed by chemical shift perturbations and automated docking. Structural comparisons with previously reported SBDs revealed two types of topologies, type I and type II, with CBM20, CBM25, CBM26, and CBM41 belonging to the type I topology group, whereas CBM21 and CBM34 belonging type II topology. According to the chemical shift perturbations, RoCBM21 contains two ligand-binding sites, as do most SBDs. Residues in site II are similar to those found in the family 20 CBM from *Aspergillus niger* glucoamylase (AnCBM20). Site I, however, is enclosed by two flexible asparagine-rich loops that are unique and only found in some members of CBM21s. Additionally, docking of a large amylose molecule (comprising 26 glucose residues) into RoCBM21 highlights possible mechanism that SBDs may interact with the structured portion of starch. The structure of RoCBM21 helps us characterize the structural features of SBDs and to understand the basis of protein-carbohydrate recognitions.

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The flexible and clustered lysine residues are critical for membrane permeability and antimicrobial activity of human RNase 7.

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RNases are found widely within living organisms and are thought to play important roles in RNA metabolism, angiogenesis or antimicrobial activity. The mechanism of antimicrobial activity of RNase remains unclear although they possess high isoelectric point and net positive charge. In this study we investigate the role of positive-charged residues of hRNase7 on the bactericidal activity. We found the hRNase 7 were bactericidal through membrane permeability, even at 4 degree. Mutational studies showed that the N-terminal lysine residues, K1 and K3, as well as K111 and K112 at C-terminus rather than conserved catalytic residues or other positive-charged residues are critical for the bactericidal activity. NMR studies showed that the 22 positive-charged residues of hRNase 7 are mainly distributed into three clusters on the surface. The critical K1/ K3, K111/K112 lysine cluster was located at the flexible loops, while non-critical positive clusters, K32/K35, K96/R97/K100, were located on the rigid secondary structures. The 16-mer oligopeptide from N-terminus of hRNase 7 is sufficient to exert pore-forming and antimicrobial activity. The solution structure of 16-mer oligopeptide was induced by SDS/TFE and similar to the corresponding region of hRNase 7. Based on sequence alignment and structural comparison among the RNase A superfamily, it is suggested that flexible and clustered positive-charged residues are critical for the antimicrobial activity through binding of negative-charged component(s) and disruption of bacterial membrane.