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SAXS and crystal structural analysis of *Helicobacter pylori* GroES

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Helicobacter pylori is a major risk factor of gastric cancer. Although many *H. pylori* virulence factors have been reported, the pathogenic mechanism by which *H. pylori* infection causes gastric cancer remains unclear. Previous studies identified the gastric cancer-related antigens: GroES from *H. pylori* and characterize their roles in the development of gastric cancer. In proteomics study, *H. pylori* GroES was shown as a dominant gastric cancer-related antigen, with a much higher seropositivity of gastric cancer samples compared to gastritis and duodenal ulcer. GroES seropositivity was more commonly associated with antral gastric cancer than with non-antral gastric cancer. Since GroES of *H. pylori* is a novel gastric cancer-associated virulence factor and may contribute to gastric carcinogenesis via induction of inflammation and promotion of cell proliferation. The structure of GroES is important to realize its role in pathogenic mechanism pathway. We try to get the GroES and target molecule complex structure. Until present, we got some SAXS structures and low resolution crystal structures of *H. pylori* GroES. The credo data show that the high similarity between *H. pylori* and *Thermus Thermophilus* GroES. It may tell us the role of H. pylori GroES in pathogenic mechanism pathway which can causes gastric cancer. Hopefully we will get into the structural and functional details of GroES molecular mechanism in the near future.

Keywords: GroES, *Helicobacter pylori*, SAXS

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Crystallographic study of the bacterial prolipoprotein posttranslational lipid modification system

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More than 130 bacterial lipoproteins of diverse structures and functions have been identified to date, and they all contain N-acyldiacylglyceryl-cysteine as their N-terminal amino acid. Posttranslational lipid modification of prolipoproteins in bacteria involves three sequential reactions catalyzed by cytoplasmic membrane enzymes, i.e., prolipoprotein: phosphatidylglycerol diacylglyceryl transferase (LGT), prolipoprotein signal peptidase (LSP), and apolipoprotein N-acyl transferase (LNT), resulting in the formation of N-acyl diacylglycerylcysteine as the N-terminal amino acid of these lipid-modified proteins. This pathway appears to be essential since mutants defective in the activity of any of these three enzymes are temperature sensitive in growth, suggesting that one or more lipoproteins are required for normal growth, division, and viability of bacterial cells . The third and last step of the pathway is the acylation of the N-terminal glyceride-cysteine residue by apolipoprotein N-acyl transferase (Lnt), resulting in mature lipoprotein. Defective mutant study suggests that apolipoprotein N-acyltransferase is an essential enzyme in *S. typhimurium* and *E. coli*, and lack of this enzyme results in cell death .Moreover lnt is necessary for efficient recognition of outer membrane lipoproteins by the Lol system, which transports them from the plasma to the outer membrane .The important roles of Lnt mentioned above have hastened us to investigate its structure and its structure-function relationship. In this study, we have initiated crystallographic studies on LGT, LSP and LNT. We have successfully purified these three membrane proteins and obtained the crystals of LGT and LNT.

Keywords: lipid modification, membrane proteins, apolipoprotein N-acyl transferase

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Preliminary crystallographic studies on ACAP1 BAR-PH and GAP-ANK domains

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Endocytic recycling is critical for many cellular events, including cell polarity, cell mobility, signal transduction and phagocytosis. ACAP1, a GTPase-activating protein (GAP) for ADP-ribosylation factor (ARF) 6, is part of a novel clathrin coat complex that is regulated by ARF6 for endocytic recycling in two key physiological settings, stimulation-dependent recycling of integrin that is critical for cell migration and insulin-stimulated recycling of glucose transporter type 4 (Glut4), which is required for glucose homeostasis. However, in contrast to TfR recycling that undergoes constitutive recycling, the role of ACAP1 in integrin beta1 recycling requires its phosphorylation by Akt, which is, in turn, regulated by a canonical signaling pathway. ACAP1 and ACAP2, together with ASAP1 and PAP, can be grouped into a protein family defined by several common structural motifs including coiled coil(BAR), pleckstrin homology(PH), Arf GAP, and ankyrin-repeat domains(ANK). Crystals of BAR-PH and GAP-ANK suitable for X-ray crystallography have been obtained and diffraction data have been collected in Photon Factory of KEK separately to 2.6Å and 3.0 Å resolution. BAR-PH crystal belongs to space group $P2_1$, with unit-cell parameters *a*=42.4, *b*=59.8, *c*=167.8 Å, *alpha*=90º, *beta*=91º, *gamma*=90º. There are two molecules per asymmetric unit. GAP-ANK crystal belongs to space group $P2_12_12_1$, with unit-cell parameters *a*=40.4, *b*=107.4, *c*=162.0Å, alpha=beta=gamma=90º. There are two molecules per asymmetric unit. And crystals of two mutants of GAP-ANK and their complexes with interaction partners of integrin beta1 have been obtained.

Keywords: endocytic recycling, coiled coil, integrin

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A comparative study on substrate specificity, activity and thermal stability of some plant proteases

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The papain-like cysteine proteases constitute an important family of