S1f2-1

NMR studies of periplasmic binding proteins

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Recent developments on protein stable isotope labeling and TROSY-based NMR measurements were applied to a 56kDa (502 a.a.) Escherichia coli NikA and a 59kDa (517 a.a.) Salmonella typhimurium OppA.NikA is the periplasmic nickel-binding protein required for nickel transport and chemotactic response by E. coli. Assignments for more than 86% of non-proline residues were obtained by the analysis of TROSY-based triple-resonance experiments. For around 10% of non-proline residues, 1H-15N correlation cross peaks were not detected in 1H-15N TROSY-HSQC spectra. These "unobservable" residues were found to be located around the Ni-binding pocket, identified by mapping the residues onto the recently determined crystal structures of apo- and Ni-bound NikA. This result suggested a local conformational flexibility around the Ni-binding pocket in Ni-free state. OppA has a remarkably broad substrate specificity, binding peptides of two or five amino-acid residues with high affinity, but little regard to sequence. It is therefore an ideal system for studying how different chemical groups can be accommodated in a protein interior. By applying a similar TROSY-based NMR approach, more than 90% of non-proline residues have been assigned. As in the case of NikA, the 10% "unobservable" residues were located around the ligand-binding pocket, suggesting a common ligand-recognition mechanism of periplasmic binding proteins.

S1f2-2

High field NMR studies for the structure and interaction of disease-related proteins

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Due to the discovery of functional genomics and proteomics many proteins related with disease process are identified with tremendously high speed. These disease-related proteins are now changing the way of drug discovery and the new technologies speed up the discovery process. Rational drug designs based on protein structures are emerging as a promising technology for faster drug discovery because they can help researchers design drug leads efficiently. NMR can provide valuable information for the structure-based drug discovery, including 3D structures of active sites, protein-ligand interactions, and possible conformational changes upon binding of ligands. Here we present some examples of NMR based structural studies for disease-related proteins. The results include 3D structural features of biotinyl carboxyl carrier protein (BCCP) domain of Acetyl-coenzyme A carboxylase (ACC), a ubiquitin-like protein, and DNA replication licensing related protein. We also report our new results of MR microimaging and MR animal imaging. With an advancement of magnetic resonance technology, we could follow developmental events from as early as the first cleavage stage to the early tailbud stage sequentially. Temporal and spatial resolutions have been improved, and consequently subcellular dynamic events inside an embryo during development could be revealed.

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In vitro and in cell NMR studies of modifier proteins

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Modifications of intracellular proteins by ubiquitin or its-related modifier proteins, such as SUMO, exerts signaling functions that mediate a wide range of cellular processes.Chains or single molecules of ubiquitin can be attached to proteins. Of various forms of polyubiquitin chains so far reported, Lys 48- and Lys 63-linked polyubiquitin have been functionally well characterized, which are involved in distinct functions. Attachment of Lys 48-linked chain generally target modified proteins for degradation by the proteasome, while Lys 63-linked chain is involved in various cellular events, such as DNA repair, activation of IkB kinase and ribosome function, not relying on degradative signaling via the proteasome. To characterize their conformations, we analyzed intersubunit interfaces of Lys 63- and Lys 48-linked di- and tetraubiquitin chains by isotope-aided NMR. In cell NMR studies of ubiquitin derivatives using Xenopus laevis oocytes will also be presented..In order to characterize the functional regulation of a protein by SUMO modification, we have determined the crystal structures of the central region of thymine DNA glycosylase (TDG), which contains the catalytic domain and the SUMOylation site K330, conjugated to SUMO-1, and the central region of TDG conjugated to SUMO-3.

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Structural insights into packaging of the helical nucleocapsid from the structure of SARS coronavirus

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Severe acute respiratory syndrome (SARS) is the first emerging infectious disease in the 21st century and is caused by a novel SARS-associated coronavirus (SARS-CoV) The nucleocapsid protein (NP) of SARS-CoV is of the most abundant proteins in the virus and a major antigen in severe acute respiratory syndrome. It also affects a number of processes within the host cell, resulting in adverse effects on the patient. It binds to the viral RNA genome and forms the ribonucleoprotein core. Our NMR, X-ray crystallography and other physico-chemical studies have found that the nucleocapsid protein of the severe acute respiratory syndrome (SARS) coronavirus contains two structural domains: the N-terminal putative RNA-binding domain (RBD) and the C-terminal dimerization domain (DD), flanked by disordered regions. We further showed that both DD and RBD binds to ssRNA or ssDNA synergistically. We have solved the structure of the dimerization domain by both NMR and crystal crystallography methods. Sequence alignment suggests that other coronavirus may share the same structural topology. In the crystal, the DD exists as dimers and four dimer molecules form a ring-like octameric structure of 90 Å in diameter with a central cavity of 30 Å in diameter in an asymmetric unit. Packaging of the octamers in the crystal forms a helical core with two parallel, positively charged grooves wound around each other as two left-handed helices. The crystal packing suggests a novel mechanism for fast and efficient helical packaging of viral RNA.

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