XXIII RD ICMRBS

International Conference on Magnetic Resonance in Biological Systems

August 24 – 29, 2008

Sheraton San Diego
Hotel & Marina
San Diego, California

Poster 292 (Wednesday)

Residual Structure within the Disordered C-terminal Segment of p21$^{Waf1/Cip1/Sdi1}$ and Its Implications for Molecular Recognition

Mi-Kyung Yoon[1]; Veena Venkatachalam[3]; Austin Huang[3]; Byong-Seok Choi[1]; Collin M. Stultz[3]; James J. Chou[2]
[1]*Korea Advanced Institute of Science and Technology, Daejeon, S. Korea;* [2]*Harvard Medical School, Boston, MA;* [3]*Massachusetts Institute of Technology, Cambridge, MA*

Probably the most unusual class of proteins in nature is the intrinsically unstructured proteins (IUPs), because they are not structured yet play essential roles in protein-protein signaling. Many IUPs can bind different proteins, and in many cases, adopt different bound conformations. The p21 protein is a small IUP (164 residues) that is ubiquitous in cellular signaling, e.g., cell cycle control, apoptosis, transcription, differentiation, etc; it binds to ~ 25 targets. How does this small, unstructured protein recognize each of these targets with high affinity? Here, we characterize residual structural elements of the C-terminal segment of p21 encompassing residues 145 – 164 using a combination of NMR measurements and molecular dynamics simulations. The N-terminal half of the peptide, p21(147-154), has a significant helical propensity which is recognized by calmodulin while the C-terminal half of the peptide, p21(155-160), prefers extended conformations that facilitate binding to the proliferating cell nuclear antigen (PCNA). Our results suggest that the final bound conformations of p21(145-164) pre-exist in the free peptide even without its binding partners. While the conformational flexibility of the p21 peptide is essential for adapting to diverse binding environments, the intrinsic structural preferences of the free peptide enables promiscuous yet high affinity binding to a diverse array of molecular targets.

Poster 293 (Thursday)

NMR Structure of the Sea Anemone Cytolisin Sticholysin I

Ines Castrillo; Jorge Santoro; Manuel Rico; Marta Bruix
IQFR, CSIC, Madrid, Spain

Sticholysin I (StnI) is an actinoporin, a pore forming toxin, produced by the sea anemone *Stichodactyla heliantus*. Together with Stn II it is the most potent cytolisin produced by this anemone. These proteins have the singularity of being produced as water soluble forms that are able to interact with membranes. Upon biding to the membrane they change their conformation, produce oligomeric pores in the membranes and cause cell lisation.

Here, Stn I soluble structure has been determined by NMR spectroscopy. Doubly labelled ^{13}C ^{15}N Stn I was produced using an *E. coli* expression system. NMR spectra were recorded on a Bruker AV-800 spectrometer at 25ºC. Samples were prepared both in 90% H_2O/ 10% D_2O and in D_2O at pH 4.0. Nearly complete assignment of ^{1}H, ^{13}C and ^{15}N resonances was achieved. Inter proton distances were derived from 3D ^{15}N- and ^{13}C-NOESY, and 2D NOESY spectra. Angle restraints were obtained using the programs TALOS and PREDITOR. Distance restraints and structure calculation were carried out in a semiautomated iterative manner using CYANA.

The Stn I structure consists of a β-barrel sandwich composed by 10 β-strands, flanked by two short α-helices on each side. The structure is in general well defined. The regions with higher RMSD values correspond to the loops between β4-β5 and β6-β7 strands. Dynamic studies, based on heteronuclear relaxation, are in progress to understand the possible intrinsic flexibility of these regions.

The StI structure will be compared with other actinoprines of known structures from the same family, like Stn II or EqtII. These studies would contribute to our understanding of the molecular processes directing pore formation.

Poster 294 (Monday)

Functional AND Structural Study of Budding Defective Mutants of Mason-Pfizer Monkey Virus Matrix Protein

Jan Prchal[1]; Michal Dolezal[1]; Jiri Vlach[1]; Jan Lipov[1]; Michaela Rumlova[2]; Richard Hrabal[1]; Eric Hunter[3]; Tomas Ruml[1]
[1]*Institute of Chemical Technology in Prague, Prague, Czech Republic;* [2]*Institute of Organic chemistry and Biochemistry, Prague, Czech Republic;* [3]*Emory Vaccine Center at Yerkes Nat. Primate Res.C., Atlanta, GA*

Polyprotein Gag as a precursor of structural proteins plays a key role in formation and budding of retroviral particles. The N-terminal domain of Gag, the matrix protein (MA) interacts with the cytoplasmic membrane of infected cell through the bipartite signal that involves a cluster of basic residues and myristic acid which is covalently attached to the amino-terminal glycine. Several mutations of MA have been described that alter various stages of the Mason-Pfizer monkey virus (M-PMV) life cycle.

In this work we focus on the determination of molecular basis of the phenotypic changes of M-PMV double-mutants T41I/T78I and Y28F/Y67F, which are unable to bud through the cytoplasmic membrane and rather accumulate on it. In contrast, they do not affect assembly and transport of immature virus particles. However, these particles are arrested in budding.

We determined the three-dimensional structures of both mutants using isotopically aided NMR spectroscopy. Comparison of the double-mutants T41I/T78I and Y28F/Y67F structures with the structure of the wild type MA shows that the mutation caused only marginal changes of the structural motif. In both cases the mutations increased the hydrophobicity of the