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Extended Abstract

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Combination of NMR methods to solve key structures of the pRN1 primase in complex with its substrates

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Primases are single-stranded DNA dependent polymerases that synthesize RNA/DNA primers during replication. A primase, a DNA polymerase and an helicase compose the replication machinery of the archaeal plasmid pRN11. The structure of the archaeal functional primase domain has been solved by X-ray crystallography2,3 and it revealed an heteromeric structure with a catalytic prim/ pol domain tethered to a novel helix bundle domain. We investigated the NMR structure of the functional pRN1 primase domain in complex with a single-stranded DNA template containing the GTG motif4. We showed that the catalytic prim/pol domain of this 38 kDa enzyme is not required for template binding. Intermolecular contacts detected exclusively between the helix bundle domain and the DNA led us to isolate specifically this structurally independent unit. Our results are compatible with a conformational switch between a template-bound open state and a closed active complex3,5,6. We used multiple NMR dataset to solve the solution structures of the helix bundle domain in complex with the single-stranded DNA template alone and upon cofactors addition. Affinity measurements validated our structural data demonstrating the importance of residues located in helices 10 and 12 for the interaction with the GTG motif and confirmed the specificity improvement observed upon cofactors binding. In association with functional assays, these novel transient structures bring new perspectives and will help us to characterize the molecular steps required for priming. Structural biology provides essential information for elucidating molecular mechanisms that underlie biological function. Advances in hardware, sample preparation, experimental methods, and computational approaches now enable structural analysis of protein complexes with increasing complexity that more closely represent biologically entities in the cellular environment. Integrated multidisciplinary approaches are required to overcome limitations of individual methods and take advantage of complementary aspects provided by different structural biology techniques. Although X-ray crystallography remains the method of choice for structural analysis of large complexes, crystallization of flexible systems is often difficult and does typically not provide insights into conformational dynamics present in solution. Nuclear magnetic resonance spectroscopy (NMR) is well-suited to study dynamics at picosecond to second time scales, and to map binding interfaces even of large systems at residue resolution but suffers from poor sensitivity with increasing molecular weight. Small angle scattering (SAS) methods provide low resolution information in solution and can characterize dynamics and conformational equilibria complementary to crystallography and NMR. The combination of NMR, crystallography, and SAS is, thus, very useful for analysis of the structure and conformational dynamics of (large) protein complexes in solution. In high molecular weight systems, where NMR data are often sparse, SAS provides additional structural information and can differentiate between NMR-derived models. Scattering data can also validate the solution conformation of a crystal structure and indicate the presence of conformational equilibria. Here, we review current state-of-the-art approaches for combining NMR, crystallography, and SAS data to characterize protein complexes in solution. Structural analysis of multi-domain protein complexes is a key challenge in current biology and a prerequisite for understanding the molecular basis of essential cellular processes. The use of solution techniques is important for characterizing the quaternary arrangements and dynamics of domains and subunits of these complexes. In this respect solution NMR is the only technique that allows atomic- or residue-resolution structure determination and investigation of dynamic properties of multi-domain proteins and their complexes. As experimental NMR data for large protein complexes are sparse, it is advantageous to combine these data with additional information from other solution techniques. Here, the utility and computational approaches of combining solution state NMR with small-angle X-ray and Neutron scattering (SAXS/SANS) experiments for structural analysis of large protein complexes is reviewed. Recent progress in experimental and computational approaches of combining NMR and SAS are discussed and illustrated with recent examples from the literature. The complementary aspects of combining NMR and SAS data for studying multi-domain proteins, i.e. where weakly interacting domains are connected by flexible linkers, are illustrated with the structural analysis of the tandem RNA recognition motif (RRM) domains (RRM1-RRM2) of the human splicing factor U2AF65 bound to a nine-uridine (U9) RNA oligonucleotide. NMR spectroscopy is a key method for studying the structure and dynamics of (large) multidomain proteins and complexes in solution. It plays a unique role in integrated structural biology approaches as especially information about conformational dynamics can be readily obtained at residue resolution. Here, we review NMR techniques for such studies focusing on state-of-the-art tools and practical aspects. An efficient approach for determining the quaternary structure of multidomain complexes starts from the structures of individual domains or subunits. The arrangement of the domains/subunits within the complex is then defined based on NMR measurements that provide information about the domain interfaces combined with (long-range) distance and orientational restraints. Aspects discussed include sample preparation, specific isotope labeling and spin labeling; determination of binding interfaces and domain/subunit arrangements from chemical shift perturbations (CSP), nuclear Overhauser effects (NOEs), isotope editing/filtering, cross-saturation, and differential line broadening; and based on paramagnetic relaxation enhancements (PRE) using covalent and soluble spin labels.

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