NMR Analyses of Structures, Dynamics, and Interactions of Biological Macromolecules

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Our biomolecular studies are based on detailed analyses of structures and dynamics of various biological macromolecules and their complexes at atomic level, primarily using nuclear magnetic resonance (NMR) spectroscopy. In particular, we conducted studies aimed at elucidating dynamic structures of glycoconjugates and proteins for integrative understanding of the mechanisms underlying their biological functions. For this purpose, we use multidisciplinary approaches integrating the methodologies of molecular and cellular biology and nanoscience along with molecular spectroscopy. Here we report NMR studies of Fbs1, α-synuclein, group II chaperonin and prefoldin.

1. Molecular Recognition by Fbs1, an Intracellular Lectin Contributing to Quality Control of Glycoproteins[1)]

Fbs1 is a cytosolic lectin putatively operating as a chaperone as well as a substrate-recognition subunit of the SCF^{Fbs1} ubiquitin ligase complex. To provide structural and functional basis of preferential binding of Fbs1 to unfolded glycoproteins, we herein characterize the interaction of Fbs1 with a heptapeptide carrying $Man_3GlcNAc_2$ by NMR spectroscopy and other biochemical methods. Inspection of the NMR data obtained by use of the isotopically labeled glycopeptide indicated that Fbs1 interacts with sugar-peptide junctions (Figure 1), which are shielded in native glycoprotein, in many cases, but become accessible to Fbs1 in unfolded glycoproteins. Furthermore, Fbs1 was shown to inhibit deglycosylation of denatured ribonuclease B by a cytosolic peptide:*N*-glycanase (PNGase). On the basis of these data, we suggest that Fbs1 captures malfolded glycoproteins, protecting them from the attack of PNGase, during the chaperoning or ubiquitinating operation in the cytosol.

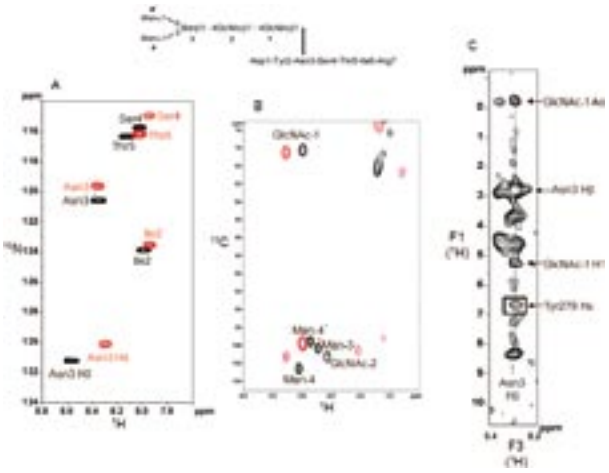

Figure 1. NMR spectra of the isotopically labeled heptapeptide carrying $Man_3GlcNAc_2$. (A) The anomeric region of ^{1}H-^{13}C HSQC spectrum of the glycopeptides labeled with [u-$^{13}C_6$]glucose and (B) amide region of ^{1}H-^{15}N HSQC spectrum of the ^{15}N-labeled glycopeptide in the presence (red) or absence (black) of equimolar amount of Fbs1-SBD. (C) Part of ^{15}N-edited NOESY spectrum of the ^{15}N-labeled glycopeptides bound to Fbs1-SBD (F2(^{15}N) = 129.6 ppm). The intermolecular NOE peak between Tyr279 Hε (Fbs1-SBD) and Asn3 Hδ (glycopeptide) is boxed.

2. Antibody Binding and Site-Specific Phosphorylation of α-Synuclein, an Intrinsically Disordered Protein[2)]

Although biological importance of intrinsically disordered proteins is becoming recognized, NMR analyses of this class of proteins remain as tasks with more challenge because of poor chemical shift dispersion. It is expected that ultra-high field NMR spectroscopy offers improved resolution to cope with this difficulty. α-synuclein, an intrinsically disordered protein, is identified as the major component of the Lewy bodies. Epitope mapping of an anti-α-synuclein monoclonal antibody and characterization of conformational effects of phosphorylation at Ser129 of α-synuclein were conducted

based on NMR spectral data collected at a 920 MHz proton frequency (Figure 2). These studies demonstrated that the employment of ultra-high field spectrometers is obviously advantageous for obtaining detailed information on conformations, modifications, and interactions of intrinsically disordered proteins in solution.

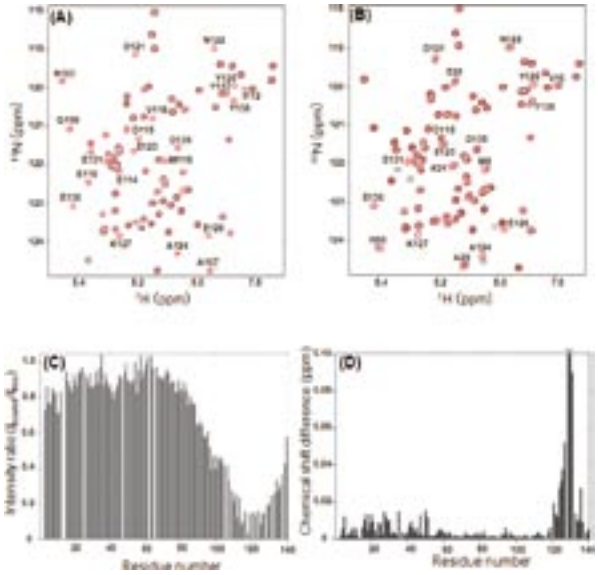

Figure 2. NMR analyses of antibody binding and phosphorylation of α-synuclein ^{1}H-^{15}N HSQC spectra of (A) [^{15}N]α-synuclein in the presence (black) and absence (red) of LB509 and (B) phosphorylated (black) and non-phosphorylated (red) [^{15}N]α-synuclein recorded at a proton frequency of 920 MHz. (C) Plot of the relative peak intensities, I_{bound}/I_{free}, of the HSQC cross peaks in the α-synuclein/LB509 complex and free α-synuclein versus the amino acid sequence of α-synuclein. (D) Profiles of chemical shift changes $[(\delta_N/5)^2 + \delta_H^2]^{1/2}$] upon phosphorylation at Ser129. Asterisks indicate the chemical shift differences are larger than 0.1 ppm for Ser129 (0.48 ppm) and E130 (0.20 ppm).

3. Dynamics of Group II Chaperonin and Prefoldin Probed by ^{13}C NMR Spectroscopy[3)]

Group II chaperonin (CPN) cooperates with prefoldin (PFD), which forms a jellyfish-shaped heterohexameric complex with a molecular mass of 87 kDa. PFD captures an unfolded protein with the tentacles and transfers it to the cavity of CPN. Although X-ray crystal structures of CPN and PFD have been reported, no structural information has been so far available for the terminal regions of the PFD tentacles nor for the C-terminal segments of CPNs, which were regarded to be functionally significant in the previous studies. We conducted ^{13}C NMR analyses on archaeal PFD, CPN and their complex, focusing on those structurally uncharacterized regions. The PFD and CPN complexes selectively labeled with ^{13}C at methionyl carbonyl carbons were separately and jointly subjected to NMR measurements. ^{13}C NMR spectral data demonstrated that the N-terminal segment of the α and β subunits of PFD as well as the C-terminal segments of the CPN hexadecamer retain significant degrees of freedom in internal motion even in the complex with a molecular mass of 1.1 MDa (Figure 3).

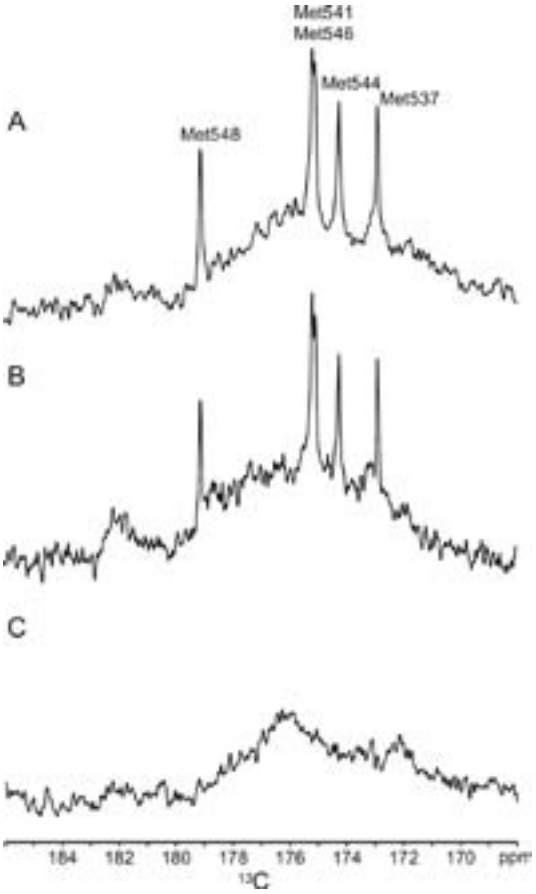

Figure 3. Hundred mega hertz ^{13}C NMR spectra of wild-type *Thermococcus* sp. strain KS-1 CPN (TKSαCPN) (A and B) and the truncated mutant of TKSαCPN that lacks the C-terminal 17 amino acid residues (C) labeled with [1-^{13}C]methionine in the absence (A and C) or presence (B) of 2 molar equivalent PFD. The proteins were dissolved in 50 mM Tris-HCl buffer, pH 8.0, containing 150 mM NaCl, 25 mM $MgCl_2$, 0.02% NaN_3, and 10% $^{2}H_2O$. The probe temperature was 45 °C.

References

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