Protein NMR Spectroscopy

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Site-Specific Solid-State NMR Studies of "Trigger Factor" in Complex with the Large Ribosomal Subunit 50S**

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Abstract: *Co-translational protein folding is not yet well understood despite the availability of high-resolution ribosome crystal structures. We present first solid-state NMR data on non-mobile regions of a prokaryotic ribosomal complex. Localized chemical shift perturbations and line broadening are observed for the backbone amide resonances corresponding to the regions in the trigger factor ribosome-binding domain that are involved in direct contact with the ribosome or undergo conformational changes upon ribosome binding. This large asymmetric protein complex (1.4 MDa) becomes accessible for NMR investigations by the combined use of proton detection and high MAS frequencies (60 kHz). The presented results open new perspectives for the understanding of the mechanism of large molecular machineries.*

In the past few years, nuclear magnetic resonance (NMR) spectroscopy has become a key technique in structural biology, complementary to X-Ray crystallography and electron microscopy (EM). Indeed, NMR spectroscopy has the unique ability to study both structural and dynamical aspects in biomolecules at the atomic level, in vitro or in vivo. While substantial progress has been made in the structural analysis of large protein complexes in solution,[1] solid-state NMR has recently emerged as a powerful alternative to characterize biological systems, such as fibrils,[2] membrane-associated systems,[3] or viral capsids,[4] that cannot be studied otherwise with atomic resolution. In particular, the development of ultra-fast magic-angle spinning (MAS) and proton-detection techniques for biological solid-state NMR applications has revolutionized the field by making the assignment procedure and calculation of structural restraints easier, faster, and more robust.[5] Additionally, direct sedimentation of large biomolecular systems into solid-state NMR rotors yields hydrated samples at extremely high concentrations without the need for crystallization or precipitation.[6] Altogether, sedimentation, ultra-fast MAS, and proton detection provide a way of characterizing more and more complex systems at high resolution using solid-state NMR.

Ribosomes constitute one of the most complex biological machineries which are responsible for protein synthesis in all kingdoms. In the past, electron microscopy and X-ray crystallography yielded the overall structures of prokaryotic, yeast and eukaryotic ribosomes.[7] At the same time, structures in complex with messenger RNA, transfer RNA, and ribosome-associated factors have been determined, providing better understanding of the mechanisms involving this highly elaborate molecular machine.[8] However, the atomistic details of the involved interactions are often elusive in the respective structures. NMR spectroscopy appears to be the method of choice to address the atomic-level structural and dynamic aspects of protein synthesis and regulation.

The trigger-factor chaperone (TF) is a 48 kDa modular protein which binds to ribosomes by its N-terminal ribosome-binding domain (RBD, residues 1–118) near the polypeptide exit site in the large subunit of bacterial ribosomes (50S).[9] TF stabilizes nascent chains (NCs) emerging from the ribosomal tunnel and plays a crucial role in co-translational folding. The X-ray structure of the N-terminal ribosome-binding domain of TF (referred to as TF-RBD hereafter) from *D. radiodurans* in a homologous complex with the large ribosome subunit suggested that the second α helix of TF-RBD opens upon binding, creating a hydrophobic channel in which nascent chains can potentially be incorporated.[10] This conformational change is however under debate since other crystal structures have provided somewhat distinct models of ribosome-bound TF-RBD.[9] Additionally, the structure of ribosome-bound full-length TF in the presence of nascent chains have been determined by cryo-electron microscopy reconstruction but only at a resolution of 19 Å.[11]

Herein, we use proton-detected ultra-fast solid-state NMR spectroscopy to study TF-RBD in a homologous complex with the 50S subunit of *E. coli* ribosomes. A

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comparison with solution-state NMR spectroscopic data of free TF-RBD allowed us to identify the residues which are affected by binding to the 50S large subunit. We show by chemical shifts perturbations mapping that the ribosome-binding-induced conformational changes in TF-RBD are consistent with the crystal structure of the homologue TF-RBD-50S complex from *D. radiodurans*.[10a] Uniformly [^{2}H,^{13}C,^{15}N]-labeled TF-RBD with full back re-protonation at exchangeable sites, was titrated to highly deuterated (ca. 80%) *E. coli* 50S large ribosomal subunit. The resulting complex was sedimented into a 1.3 mm rotor. Figure 1 shows the solid-state ^{15}N-^{1}H CP-HSQC spectrum (MAS rotation frequency of 60 kHz) along with the solution-state HSQC spectrum of free TF-RBD.

The solid-state NMR spectrum yields very good sensitivity within a reasonable experimental time (signal-to-noise ratio of 3–5:1 in 8.5 h). We would like to point out that TF-RBD constitutes only about 1% of the total sample volume. This high sensitivity is only achievable by exploiting the combination of ultra-fast MAS and proton detection to push the current limits of the technique. In principle, the sensitivity of the experiments can be increased employing dynamic nuclear polarization (DNP).[12] However, the required cryogenic conditions induce an increase in sample heterogeneity which potentially decreases spectral resolution. The conditions used herein yield highly resolved spectra for such a complex system (full width at half height (FWHH) ca. 100 Hz in ^{1}H and 60 Hz in ^{15}N). In the non-overlapping regions of the spectrum, the assignment can easily be transferred from the solution-state spectrum of TF-RBD.[13] Comparison of the solution- and solid-state NMR spectra reveals clear peak shifts and disappearance upon complexation. These shifts are not due to temperature (see Supporting Information, Figure S3) but rather likely correspond to the residues that are in proximity to the ribosome and for which the local environment and/or dynamics are changed upon binding.

Figure 2 shows the superposition of the crystal structure of TF-RBD in complex with the 50S large ribosomal subunit from *D. radiodurans* (protein data bank pdb: 2d3o) with that of *E. coli* TF-RBD (pdb: 1w26), on which we mapped the residues that show chemical shift perturbations. As expected, the most affected residues cluster at the binding interface,

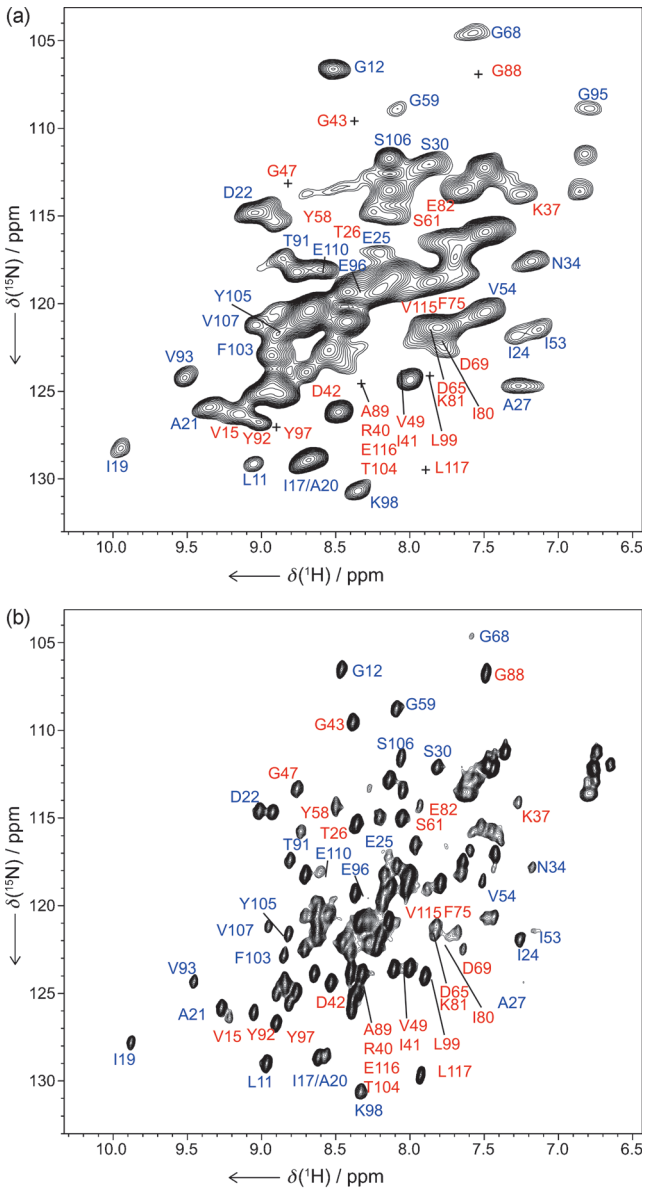

Figure 1. a) 2D solid-state ^{15}N–^{1}H correlation spectrum of *E. coli* [U-^{1}H^{N},^{2}H,^{13}C,^{15}N]-labeled TF-RBD in complex with the 50S *E. coli* ribosomal subunit recorded on a 850 MHz spectrometer under 60 kHz MAS. b) 2D solution-state ^{15}N–^{1}H correlation spectrum of free monomeric [U-^{1}H^{N},^{2}H,^{13}C,^{15}N]-labeled TF-RBD recorded on a 500 MHz spectrometer. Residues in the resolved parts of the solid-state spectrum are labeled in the two spectra; blue: residues for which chemical shifts do not change, red: resonances for which the signal either shifts or broadens below detection owing to binding to the ribosomes. More experimental details can be found in the Supporting Information.

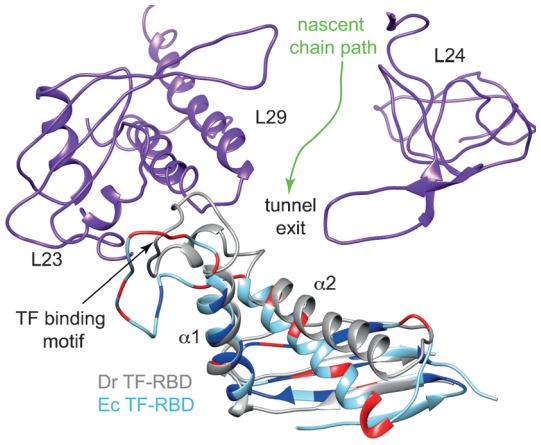

Figure 2. Structural mapping of the NMR spectral changes in TF-RBD. Alignment of the crystal structures of *D. radiodurans* TF-RBD in complex with the 50S ribosomal subunit (DrTF-RBD pdb: 2d3o) with that of *E. coli* TF-RBD (EcTF-RBD pdb: 1w26). The ribosomal proteins L23, L29, and L24 at the tunnel exit and close to the binding site are shown in purple. The residues for which resonance signals are not affected by ribosome binding are shown in blue, those that exhibit chemical shift changes or line broadening are shown in red, and those that could not be assigned because of spectral overlap are shown in gray.

while unaffected ones are in regions further from the ribosome. Interestingly, both α-helix 2 and the TF-binding motif (XGFRxGxxP in the loop L1 between α-helices 1 and 2) show perturbations upon binding. This is consistent with the reported biochemical[14] and crystallographic data.[10] Our solid-state NMR analysis therefore seems to confirm these hypotheses.

We have shown the first solid-state NMR spectroscopic data on a ribosomal complex using ultra-fast MAS and proton detection. With these data we could identify the residues in TF-RBD that are involved in the binding to the ribosome. Our results open new perspectives for studies of co-translational folding involving TF and ribosome nascent chains. In contrast to previous studies,[15] regions of the ribosome that are less dynamic become available to "standard" NMR spectroscopy, paving the way for the investigation of nascent chain signaling.

Keywords: magic-angle spinning (MAS) · NMR spectroscopy · protein–protein interactions · ribosome complex · trigger factor

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