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ARTICLE

^{1}H, ^{13}C, and ^{15}N resonance assignments of the dsRBDs of mouse RNA helicase A

Takashi Nagata · Kengo Tsuda · Naohiro Kobayashi · Peter Güntert · Shigeyuki Yokoyama · Yutaka Muto

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Abstract RNA helicase A (RHA) is a multifunctional protein that regulates gene expression. RHA has two double-stranded RNA-binding domains (dsRBDs) that serve as modules for highly structured RNA binding and protein–protein interactions. Using the dsRBDs, RHA binds to cellular and viral mRNAs, exports them from the nucleus, and regulates splicing as well as translational initiation. The RHA dsRBDs also reportedly mediate interactions with small RNAs and other dsRBD-containing proteins, and altogether form a processing complex involved in RNA silencing pathways. In addition, the RHA dsRBDs bridge RNA polymerase II with several transcription factors. Here we report the ^{1}H, ^{13}C, and ^{15}N chemical shift assignments of the dsRBDs of RHA. The resonance assignments obtained in this work will contribute to the elucidation of the interactions between RHA and transcriptional or post-transcriptional gene regulators.

Keywords RHA · dsRBD · dsRNA

Biological context

RHA (or DHX9/NDHII) is a ubiquitous and highly conserved RNA helicase that regulates the gene expression of cellular and viral mRNAs, from transcription through translation (Tettweiler and Lasko 2006; Hartman et al. 2006). From the N- to C-terminus, RHA comprises two double-stranded RNA-binding domains (dsRBDs: dsRBD1 and dsRBD2), the DExD/H-box RNA helicase domain, a domain of unknown function, the helicase-associated domain 2, a nuclear transport domain, and the C-terminal RG domain. The RNA helicase domain plays important roles in the remodeling of structured nucleotides and/or ribonucleoproteins (RNPs). The two dsRBDs allow RHA to recruit target macromolecules by interacting with double-stranded RNA (dsRNA), as well as dsRBD-containing and/or non-dsRBD-containing proteins, thus providing a scaffold to form regulatory complexes in catalytic processes.

T. Nagata
Institute of Advanced Energy and Graduate School of Energy Science, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan

T. Nagata
Department of Supramolecular Biology, Graduate School of Nanobioscience, Yokohama City University, 1-7-29 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan

T. Nagata · K. Tsuda · S. Yokoyama (✉) · Y. Muto (✉)
Systems and Structural Biology Center, Yokohama Institute, RIKEN, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan
e-mail: yokoyama@riken.jp

Y. Muto
e-mail: ymuto@gsc.riken.jp

N. Kobayashi
Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565-0871, Japan

P. Güntert
Institute of Biophysical Chemistry, Center for Biomolecular Magnetic Resonance, and Frankfurt Institute for Advanced Studies, Goethe University, Max-von-Laue-Str. 9, 60438 Frankfurt/Main, Germany

P. Güntert
Graduate School of Science and Technology, Tokyo Metropolitan University, 1-1 Minami-ohsawa, Hachioji, Tokyo 192-0397, Japan

In the nucleus, RHA mediates the complex formation between the co-activator CBP/p300 (CREB binding protein) and the holo-RNA polymerase II (Pol II), leading to the transcriptional activation of the target genes (Nakajima et al. 1997). RHA also recruits Pol II to the breast cancer-specific tumor suppressor protein, BRCA1. Perturbations of the RHA and BRCA1 interactions reportedly promote the development of breast cancer (Anderson et al. 1998). RHA also functions in the nuclear export of unspliced mRNAs. RHA binds to the highly structured CTE (constitutive transport element), which resides in the 3′ UTR (untranslated region) of viral RNA (Tang et al. 1997; Gruter et al. 1998), and mediates its association with the nuclear export receptor, Tap (Tang and Wong-Staal 2000). RHA also targets PCE (post-transcriptional control element), a highly structured RNA element found in the 5′ UTR of several cellular and retroviral mRNAs (Hartman et al. 2006), such as in the *junD* proto-oncogene and the HIV-1 *gag* gene (Hartman et al. 2006; Bolinger et al. 2010). RHA reportedly remodels the CTE and PCE structures, thus facilitating nuclear export, splicing, and the following translation initiation processes (ribosome scanning and polyribosome association; Hull and Boris-Lawrie 2003; Hartman et al. 2006). All of the above interactions between RHA and various macromolecules occur via the RHA dsRBDs.

Recently, many dsRBD-containing proteins were demonstrated to interact with each other through their dsRBDs. Various components of RNA-induced silencing complexes (RISC), which direct RNAi/miRNA pathways, contain dsRBD(s). For example, Dicer, an RNase III enzyme that generates small dsRNA, has one dsRBD. TRBP (transactivation-responsive RNA binding protein) and PACT (protein activator of the interferon-induced kinase), which both stabilize Dicer and/or up-regulate its activity, have three dsRBDs (Chendrimada et al. 2005; Haase et al. 2005; Lee and Hurwitz 1992). Together with RHA, these RISC components further promote the assembly with Ago2 (Argonaute2), as well as with either siRNA (small interfering RNA) or miRNA (micro RNA). Correspondingly, the siRNA (or miRNA) is processed into a single-stranded guide RNA and associates with Ago2. This effector RISC uses the guide RNA originating from the siRNA (or miRNA) for cleavage (or translational suppression) of the target mRNAs, resulting in gene silencing (Siomi and Siomi 2009; Hammond et al. 2001). Another protein, PKR (Protein kinase R), comprises two dsRBDs and a kinase domain. PACT and TRBP, which bind to the two dsRBDs of PKR, activate and inhibit the kinase activity of PKR, respectively. Importantly, PKR interacts with RHA through the dsRBDs and phosphorylates RHA, thus inhibiting the capacity of RHA to enhance the expression of HIV-1 gene elements (Sadler et al. 2009).

Here, we describe the NMR assignments of dsRBD1 and dsRBD2 of RHA. The resonance assignments represent a substantial contribution to studies of the interactions between RHA dsRBD1 and dsRBD2 with their target dsRNAs, and with dsRBD-containing and non-dsRBD-containing proteins, which all play pivotal roles in gene regulation.

Methods and experiments

Sample preparation

The portions of the mouse RHA gene (SwissProt ID O70133) encoding dsRBD1 (residues 4–89) and dsRBD2 (residues 163–262) were each cloned into pCR2.1 (Invitrogen). Both proteins contained a His6-tag, a TEV protease cleavage site, and a $(Gly\text{-}Ser\text{-}Ser)_2$-Gly sequence at the N-terminus; and a Ser-Gly-Pro-Ser-Ser-Gly sequence at the C-terminus. The dsRBD1 and dsRBD2 proteins were synthesized and purified as described below. Each dsRBD was synthesized by the cell-free protein expression system (Kigawa et al. 2004). After the reaction, the protein was dialyzed against dialysis buffer (20 mM Tris–HCl, pH 8.0, 1 M NaCl), and then purified by Ni affinity chromatography. The His_6-tag-fused protein was isolated, and the His_6-tag was removed by proteolysis. Subsequently, the dsRBD was dialyzed against dialysis buffer, and then passed through the Ni affinity column. The flow-through fraction was collected, and the buffer was exchanged to 20 mM Tris–HCl (pH 7.0) with 1 mM EDTA, by desalting chromatography. The dsRBD was then passed through an anion-exchange column, and the collected flow-through fraction was further purified by cation-exchange chromatography. Using this purification protocol, uniformly ^{13}C, ^{15}N-doubly labeled dsRBDs were prepared. The yield of each dsRBD was more than 0.5 mg from a 1 ml volume reaction. The RHA dsRBD1 and dsRBD2 samples for NMR experiments were concentrated to 1.30 and 1.16 mM, respectively, in 20 mM sodium phosphate (pH 6.0), containing 100 mM NaCl, 1 mM DTT, and 0.02 % NaN_3, in 90 % H_2O–10 % ^2H_2O.

NMR spectroscopy

All NMR spectra were recorded at 298 K on Bruker AVANCE 600, 700, and 800 MHz NMR spectrometers, each equipped with a cryogenic probe. For the assignment of the backbone ^1H, ^{13}C and ^{15}N resonances, ^1H–^{15}N HSQC, HNCO, HN(CA)CO, HNCA, HN(CO)CA, HNCACB, and CBCA(CO)NH spectra were recorded. Assignments of side chain resonances were obtained from ^1H–^{13}C HSQC, HBHA(CO)NH, C(CCO)NH, HC(CCO)NH, H(C)CH-COSY,

H(C)CH-TOCSY, and (H)CCH-TOCSY for nonaromatic residues, and from ^{1}H–^{13}C HSQC, H(C)CH-COSY and H(C)CH-TOCSY spectra for aromatic residues. The backbone and side chain resonance assignments were aided by ^{15}N-separated and ^{13}C-separated NOESY-HSQC spectra, which were both recorded with a mixing time of 80 ms. All NMR spectra were processed with NMRPipe/NMRDraw (Delaglio et al. 1995). Spectral analysis was performed with KUJIRA (Kobayashi et al. 2007), a program suite for interactive NMR analysis working with NMRView (Johnson 2004), according to the methods described previously (Nagata et al. 2008).

Assignments and data deposition

A standard sequential assignment procedure was performed for both dsRBD1 and dsRBD2. The 2D ^{1}H–^{15}N HSQC spectra of RHA dsRBD1 and dsRBD2, with the assignments indicated, are shown in Fig. 1. The extents of the assignments for the $^{1}H^{N}$, ^{15}N resonances of the backbone amide groups of dsRBD1 and dsRBD2 were 100 % (81 out of 81 non-Pro residues) and 99 % (98 out of 99 non-Pro residues), respectively. In addition, 96 % of the $^{1}H^{\alpha}$ and $^{13}C^{\alpha}$ (83 out of 86 residues), 83 % of the $^{13}C'$ (80 out of 86 residues), and 95 % of the $^{13}C^{\beta}$ (76 out of 80 non-Gly residues) resonances were assigned for dsRBD1. For dsRBD2, 100 % of $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, and $^{13}C'$ (all 100 residues), and $^{13}C^{\beta}$ (93 out of 93 non-Gly residues) were assigned. The sequential correlations of the backbone resonances for H209 in dsRBD2 were missing, most likely due to solvent exchange. This histidine, which is highly conserved among the canonical dsRBDs and is often involved in dsRNA binding, is located at the tip of the loop between β1 and β2. Substantial numbers of the side chain ^{1}H, ^{13}C, and ^{15}N atoms of most of the residues were assigned. In total, among the assignable side chain resonances, 83 % (669 out of 809) for dsRBD1 and 86 % (813 out of 948) for dsRBD2 were assigned. The secondary structural elements of the dsRBD1 and RBD2 of RHA were identified using the TALOS software (Cornilescu et al. 1999), utilizing $^{13}C\alpha$, $^{13}C\beta$, $^{13}C'$ and ^{15}N chemical shifts and the backbone NOE data (data not shown). Both dsRBD1 and

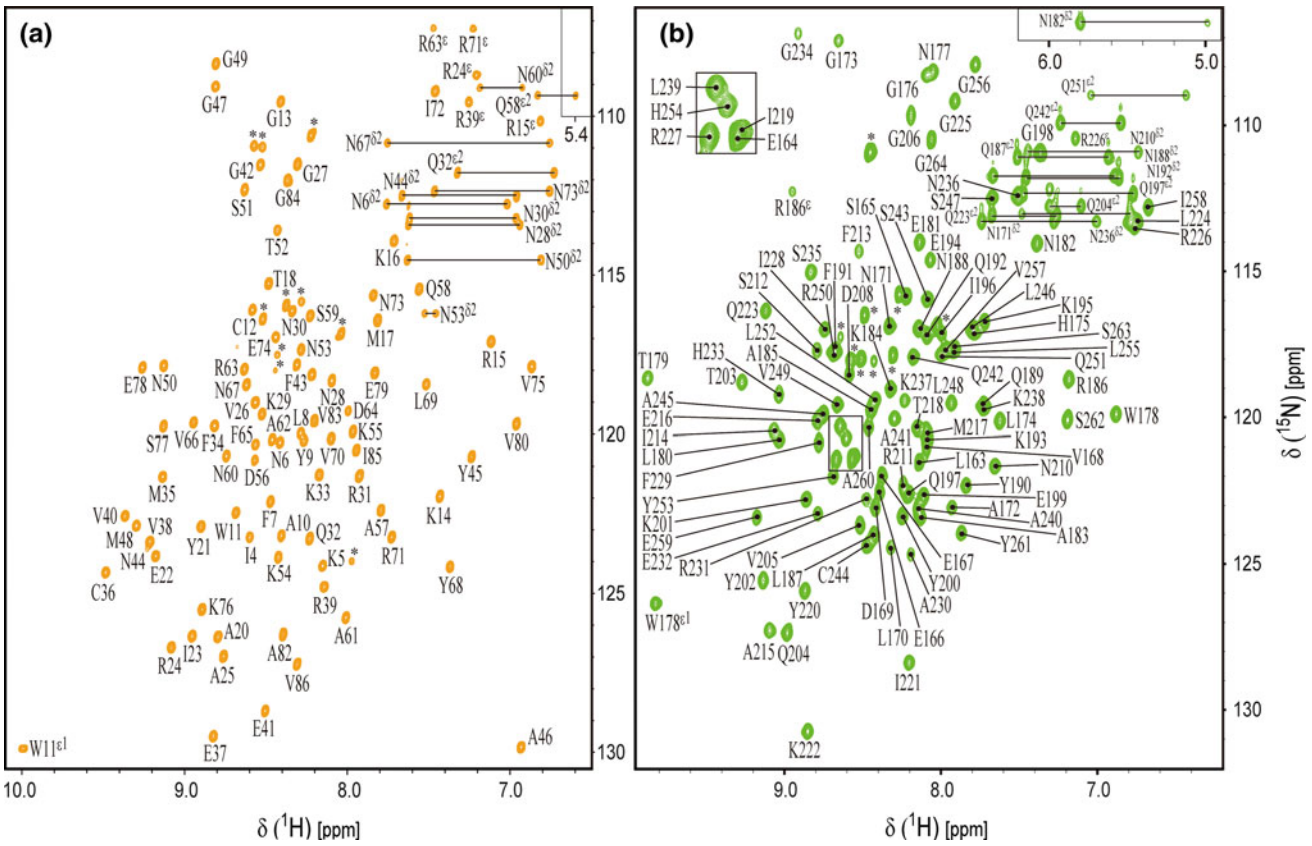

Fig. 1 Amide resonance assignments of ^{13}C, ^{15}N-labeled RHA dsRBDs. **a** 2D ^{1}H–^{15}N HSQC spectrum of RHA dsRBD1 (residues 4–89) at pH 6.0, recorded at 700 MHz ^{1}H frequency. **b** 2D ^{1}H–^{15}N HSQC spectrum of RHA dsRBD2 (residues 163–262) at pH 6.0, recorded at 600 MHz ^{1}H frequency. Assignments are shown with the one-letter amino acid code and the sequence number. The central region indicated by the *rectangle* is expanded and shown in the *inset* for clarity. The peaks corresponding to the side chain NH_2 groups of the asparagine and glutamine residues are connected by *horizontal lines*. *Asterisks* indicate unassigned peaks that probably originated from extra amino acid residues from the plasmid

dsRBD2 contain secondary structure elements that are common to dsRBD family proteins: αA(K5-R15), β1(A20-G27), β2(Q32-R39), β3(G47-S51), and αB(K54-R71) for dsRBD1; and αA′(L180-E194), β1′(K201-P207), β2′(N210-I221), β3′(R226-G234), and αB′(K237-L255) for dsRBD2. Interestingly, dsRBD1 has the extra helix-like segment, h-like C (S77-E79), at the C-terminal region, while dsRBD2 begins with the extra helix-like segment, h-like o′ (N171-H175), at the N-terminal region. A detailed analysis of the structural features of dsRBD1 and dsRBD2 of RHA and their interactions with dsRNA will be discussed elsewhere (Nagata et al. in preparation). The assigned chemical shifts of the dsRBD1 and dsRBD2 of RHA have been deposited in the BioMagResBank (http://bmrb.protein.osaka-u.ac.jp), under the accession numbers 11456 and 11457.

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