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Sequence specific ^{1}HN,^{15}N, ^{1}H$^{\alpha}$, ^{13}C$^{\alpha}$, and ^{13}C$^{\beta}$ assignments for RNA-1 modulator protein ROM

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Biological context

RNA–protein complexes mediate many processes in gene regulation. RNA-one modulator protein (ROM) regulates *Escherichia coli* ColE1 plasmid replication by modulating the initiation of transcription of the primer RNA precursor. Processing of the precursor of the primer, RNA II, is inhibited by base pairing of RNA II to its complementary sequence in RNA I. ROM binds to a hairpin loop–hairpin loop complex formed by RNA I and RNA II. This facilitates the formation of the base-paired duplex and decreases the rate of replication initiation events (Tomizawa and Eguchi, 1990). Eberle et al. (1990) reported ^{1}H assignments for ROM, which exists as a homodimer, at pH 2.3. We have obtained nearly complete ^{1}HN, ^{15}N, ^{1}H$^{\alpha}$, ^{13}C$^{\alpha}$, and ^{13}C$^{\beta}$ assignments for the ROM homodimer (a 63 amino acid monomer) at pH 6.3. These data provide a basis for investigations of the RNA–protein complex at physiological pH.

Materials and Methods

E. coli strain BL21(DE3), transformed with the plasmid p2R that encodes ROM, was a kind gift of Professor L. Regan (Yale University). ROM produced by plasmid p2R differs from the wild type by a Gly insertion after the N-terminal Met (Predki et al., 1995). Expression and purification were conducted as described (Predki et al., 1995). Approximately 30 mg of ^{15}N- or ^{15}N/^{13}C-labeled protein was obtained per liter of M9 minimal medium. Electrospray ionization mass spectrometry was used to confirm the ROM sequence and to check the purity of the samples. Two species which differed by the presence or absence of an N-terminal Met were found. The purity of the ROM samples was at least 95%, with the ratios of these two species varying according to sample preparation. Purified ROM was exchanged into a solution containing 10 mM NaCl, 10 mM sodium phosphate (pH 6.3), 0.1 mM ethylenediaminetetra acetic acid and 1 mM dithiothreitol by ultracentrifugation through a 3 kDa molecular weight cutoff membrane. Sample concentrations ranged from 1 to 4 mM.

Spectra were recorded at 30 °C on a Bruker AMX spectrometer operating at 600 MHz. Quadrature detection in indirectly detected dimensions was achieved by the States-TPPI method. Data were processed with the NMRPipe suite of programs (Delaglio et al., 1995). Typically, data in indirectly detected dimensions were apodized with phase-shifted sine-bell window functions followed by zero-filling to twice the original data size. For constant-time evolution periods linear prediction was used to double the number of points before apodization. Spectra were analyzed using CAPP/PIPP software (Garrett et al., 1991).

NMR and X-ray data show that the ROM homodimer folds into a 4-helix bundle (Banner et al., 1987; Eberle et al., 1991). Strong NOEs between adjacent amide protons (d_{NN} NOEs), characteristic of helical secondary structure (Wüthrich, 1986), were observed for virtually all residues. These data, along with C_i^{α}, C_{i-1}^{α} linkages observed in a 3D HNCA spectrum formed the basis for the backbone ^{1}H, ^{15}N, and ^{13}C$^{\alpha}$ assignments (Figure 1). $^{13}C_i^{\beta}/^{13}C_{i-1}^{\beta}$ linkages obtained from CBCANH and CBCA(CO)NH spectra and $H_i^{\alpha}/H_{i-1}^{\alpha}$ linkages obtained from ^{15}N-TOCSY-

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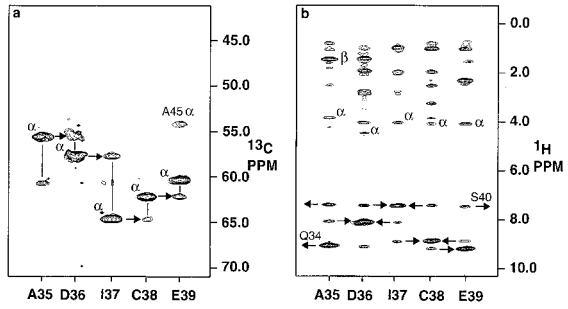

Figure 1. Representative strips for residues Ala[35] to Glu[39] taken from (a) a 3D HNCA spectrum ($\omega_1(^{13}C^{\alpha})$) and (b) a 3D ^{15}N-NOESY-HSQC spectrum (100 ms mixing time, $\omega_1(^1H)$) (30 °C, pH 6.3). Amino acids are identified by one-letter amino acid code and residue number. In the HNCA spectrum Greek letters denote intra-residue H-N_i-C_i^{α} correlations. Arrows connect each intra-residue correlation with the two-bond H-N_i-C_{i-1}^{α} correlation of the preceding residue. In the NOESY-HSQC spectrum Greek letters denote intra-residue HN to H^{α} and H^{β} NOEs. Arrows denote NOEs between adjacent amide protons.

HSQC and HA(CO)NH spectra were used to confirm the backbone assignments and assign the $^{13}C^{\beta}$ signals (Bax and Grzesiek, 1993).

The aromatic ^{1}H resonances of the single Tyr and two Phe residues were identified in a 2D DQF-COSY spectrum. The Tyr ^{1}H-^{13}C signals were also identified in a 2D HCCH-TOCSY spectrum optimized for observation of these resonances (Damberger et al., 1994). The ring spin systems were correlated with the backbone assignments through HN-H^{δ} NOEs observed in a 3D ^{15}N NOESY-HSQC spectrum.

Side-chain NH resonances of Asn and Gln were assigned by comparison of ^{13}C shifts derived from backbone and side-chain NH groups in a CBCA(CO)NH experiment, characteristic ^{13}C shifts of Gln $^{13}C^{\gamma}$ signals, and NOEs between backbone and side-chain NH groups.

Extent of assignments and data deposition

^{1}HN, ^{15}N, $^1H^{\alpha}$, $^{13}C^{\alpha}$, and $^{13}C^{\beta}$ assignments were obtained for all but the first six residues (Gly[1], Thr[2], Lys[3], Gln[4], Asp[5], Lys[6]), His[42] H^{α} and Arg[13] $^{13}C^{\beta}$. The presence or absence of the N-terminal Met caused a doubling of the NH resonances for residues Thr[7], Ala[8], Asn[10] and Ala[54]. Complete aromatic ^{1}H assignments were obtained for one of the two Phe residues, Phe[54]. Only the H^{δ} resonance was identified for Phe[14] due to ^{1}H chemical shift degeneracy. Both aromatic ^{1}H-^{13}C signals were assigned for the single tyrosine (Tyr[49]). Finally, side-chain ^{1}H-^{15}N assignments were obtained for the three Asn and one of the three Gln residues (Gln[34]). Many of the aliphatic side-chain resonances were broader than expected for a 14.3 kDa protein. For this reason the H^{β} signals as well as the other side-chain ^{1}H-^{13}C resonances have not been assigned. The ^{1}H, ^{13}C, and ^{15}N chemical shifts described herein have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4072.

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