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ARTICLE

^{1}H, ^{13}C and ^{15}N resonance assignment of phage T4 endoribonuclease RegB

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Abstract RegB is involved in the control of the phage T4 life cycle. It inactivates the phage early mRNAs when their translation is no more required. We determined its structure and identified residues involved in substrate binding. For this, all backbone and 90% of side-chain resonance frequencies were assigned.

Keywords RegB · mRNA inactivation · Translation initiation · Toxin-antitoxin modules · Ribosomal S1 protein

Biological context

Inactivation and degradation of messenger RNAs constitute a key process in the regulation of gene expression. They involve two different classes of enzymes: the exoribonucleases and the endoribonucleases. While exoribonucleases are general regulators that processively degrade RNAs, most endoribonucleases act as specific regulators by selecting their targets and cleaving them at specific internal sites. The molecular mechanism of such enzymes remains poorly understood.

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The ribonuclease RegB (18 kDa) is encoded by the bacteriophage T4. It is involved in the timing of the phage life cycle through the inactivation of early messenger RNAs produced after viral infection (Sanson et al. 2000). RegB, indeed, preferentially cleaves in the middle of the GGAG sequence found in bacterial translation initiation sites (Jayasena et al. 1996; Uzan 2001). RNA inactivation by RegB may involved the ribosomal complex as suggested by the 10- to 100-fold enhancement observed *in vitro* in the presence of the ribosomal protein S1, whose role is precisely to allow the recognition of the translation initiation region by the ribosome (Ruckman et al. 1994). Stimulation of RegB activity by S1 depends strongly on the sequence located 3' to the GGAG motif, suggesting a crucial role in the selection of RegB substrates (Durand et al. 2006). An *in vitro* study has also indicated that the presence of a stem-loop structure around the GGAG motif could favor RNA cleavage (Lebars et al. 2001).

The absence of sequence similarities to any known protein and the particular properties of RegB have motivated us to decipher its three-dimensional structure and to identify residues involved in the substrate recognition and/or processing. Our results have shown that, despite the absence of any sequence homology, RegB shares structural and functional similarities with the bacterial sequence-specific endoribonucleases YoeB and RelE (Odaert et al. 2007), involved in the translated mRNA inactivation when a rapid change of the bacterial metabolism is required (Codon 2006). To achieve this goal, the ^{1}H, ^{13}C and ^{15}N resonances of the H48A point mutant of RegB have been assigned using multidimensional heteronuclear NMR experiments. The RegB H48A mutation was necessary to over-express the protein under an inactive, non-cytotoxic but correctly folded form (Saïda et al. 2004).

Methods and experiments

Expression and purification were previously described (Saïda et al. 2004). ^{15}N and ^{15}N,^{13}C-labeled RegB H48A proteins were expressed in the M9 minimal medium supplemented with ^{15}N ammonium chloride (1 $g.l^{-1}$) and ^{13}C glucose (2 $g.l^{-1}$). NMR samples contained 0.7–1 mM of labeled protein in 50 mM sodium citrate (pH 6.0), 300 mM NaCl, 0.1 mM AESBF protease inhibitor, 2 mM dithiothreitol and 5:95% D_2O/H_2O or 100% D_2O.

NMR data were recorded at 32 °C on a Bruker DRX600 spectrometer equipped with a triple resonance Z-gradient cryoprobe. Data were processed on the Bruker station with XWINNMR 3.0 and analyzed with the Sparky software (University of California San Francisco, Thomas L. Goddard). Backbone assignment was achieved with the 3D triple resonance experiments: HNCA/HN(CO)CA, HNCO/HN(CA)CO, HNHA. Side-chain resonance assignment was achieved with the following experiments: HNCACB, CBCA(CO)NH, HBHA(CBCACO)NH, aliphatic HCCH-TOCSY, ^{15}N-edited TOCSY-HSQC, aromatic ^{13}C-edited HSQC, aromatic ^{13}C-edited NOESY-HSQC and 2D TOCSY/NOESY (60 and 120 ms mixing time) in D_2O. The assignment was confirmed and completed with ^{15}N-edited NOESY-HSQC (60 and 120 ms mixing time) and aliphatic ^{13}C-edited HSQC-NOESY (60 ms mixing time) in H_2O and D_2O.

Extent of assignments and data deposition

The structure determination was achieved by using the INCA software (Savarin et al. 2001) that uses an iterative procedure to simultaneously build the structure and assign the nOe cross-peaks. A critical point of the method is to dispose of precise frequency assignments perfectly coherent with the NOESY experiments used for the reconstruction. We mainly used three NOESY spectra, a ^{15}N-NOESY-HSQC, a ^{13}C-NOESY-HSQC and a two-dimensional NOESY spectrum recorded in D_2O. Accordingly, we determined (and deposited; BMRB accession number 15127) three chemical shift lists corresponding to the frequencies observed in these three spectra. Several assignments derived from scalar experiments (such as HCCH-TOCSY) that could not be confirmed by the presence of unambiguous intra-residual cross-peaks in the NOESY spectra were not retained.

Nearly all N, H^N, C^α, H^α, C^β and H^β resonance frequencies were assigned in the ^{13}C- and ^{15}N-NOESY-HSQC experiments. All Ala, Cys, Asp, Glu, Gly, Leu, Asn, Gln, Ser, Thr, Val hydrogen frequencies were assigned in at least one of the two experiments. The missing resonances mainly concern the Lys (73 hydrogen assigned among 110), Arg (88 among 112), Pro (39 among 49) residues and the aromatic part of His17, Phe67, Phe93, Tyr99 and Phe124 that could not be observed.

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