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Letter to Editor: Backbone NMR assignments of Ribosome Recycling Factors (RRFs) from *Escherichia coli* and *Thermotoga maritima*

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Biological context

The termination step of protein biosynthesis and the release of the completed polypeptide chain are signaled by the presence of a stop codon in the A-site of the ribosome. In eubacteria, protein release factors, RF1, RF2, and RF3 mediate the process of termination. Following the termination step, the resulting post termination complex, which is composed of 70S ribosome, mRNA and deacylated tRNA, is dissociated for the recycling of ribosome. This process is a crucial step for an efficient protein biosynthesis. Hirashima and Kaji found a 21 kD protein, which catalyzes the dissociation process, and named it as ribosome recycling factor (RRF) (for review see Janosi et al., 1996). The activity of RRF requires the presence of elongation factor G (EF-G) and GTP. It was demonstrated that the *Escherichia coli* strain with nonfunctional RRF-encoding gene (*frr*) led to cell death in the absence of rescue by the plasmid carrying native *frr* gene. The importance of RRF is evident because no eubacteria genome without *frr* gene has been found. Therefore, RRF might be a target for new therapeutic antibiotics. Recently, the structures of RRF of several bacteria have been solved by X-ray crystallography (*Thermotoga maritima*, *E. coli*, *Thermus thermophilus*) and NMR spectroscopy (*Aquifex aeolicus*) (Selmer et al., 1999; Kim et al., 2000; Toyoda et al., 2000; Yoshida et al., 2001). RRF is shown to be composed of two domains. The overall shape and dimensions of RRF are quite similar to those of tRNA. This similarity suggests that RRF might be a mimic of tRNA and may bind to the A-site of ribosome. The interaction between RRF and the A-site is supported by the finding that RRF and RF1 have overlapping binding sites on the ribosome (Pavlov et al., 1997). Although the mechanism of ribosome recycling by RRF is still unclear, the importance of the fluctuation in inter-domain orientation was suggested from some genetic experiments (Toyoda et al., 2000; Atarashi and Kaji, 2000). While variations of the inter-domain orientation among reported structures of RRF have been observed, no direct evidence of domain motion in solution has been shown yet. To better understand the function of RRF, we have characterized RRFs of several bacteria by NMR spectroscopy. In this note, we report the backbone ^{1}H, ^{13}C, and ^{15}N NMR assignments of RRFs from *E. coli* and *T. maritima*. These data are essential for relaxation analyses to study dynamic properties of RRF molecule. Moreover, assignments of backbone amide resonances should be very useful for identifying interactions involving RRF with ribosomes, other translational factors, or drugs.

Methods and experiments

The DNA fragment encoding RRF sequence of *E. coli* was cloned into *Nde*I/*Bam*HI sites of pET22b plasmid vector. The construct, pET-ERRF, was introduced into *E. coli* strain BL21(DE3). Uniform ^{15}N, ^{13}C, and ^{2}H labeling was achieved using M9 medium prepared in 99% D2O containing [*U*-^{2}H/^{13}C] glucose (2 g/L) and [^{15}N] ammonium chloride (1 g/L) as the sole carbon and nitrogen sources. The cells were grown at 37 °C to A600 = 0.5 and the protein expression was induced by addition of IPTG, followed by 3h incubation. Harvested cells were sonicated and centrifuged. According to the method described by Kim et al. (2000), the product was purified. The NMR sample of [*U*-

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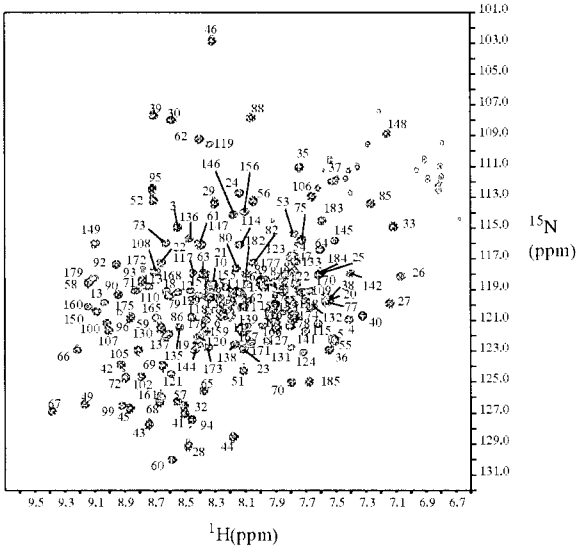

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum of *E. coli* RRF with assignments for the cross-peaks indicated by residue numbers.

^{2}H/^{13}C/^{15}N] *E. coli* RRF was prepared in 90% H_2O/ 10% D_2O acetate buffer of 50 mM at pH 5.0.

The DNA fragment encoding RRF sequence of *T. maritima* was cloned into *Nde*I/*Bam*HI sites of pET22b plasmid vector, giving rise to the construct, pET-TMRRF. In the case of *T. maritima* RRF, uniformly ^{15}N and ^{13}C labeled sample was prepared for assignments. Expression and purification procedures of *T. maritima* RRF were basically same as that of *E. coli* RRF. The NMR sample of [*U*-^{13}C/^{15}N] *T. maritima* RRF was prepared in 90% H_2O/ 10% D_2O phosphate buffer of 50 mM at pH 7.4.

All NMR spectra were measured at 25 °C for *E. coli* RRF or 40 °C for *T. maritima* RRF on a Varian INOVA 600 spectrometer equipped with a triple resonance z-gradient probe. ^{15}N-^{1}H HSQC, HNCO, HNCA, HN(CO)CA, HN(CA)CO, CBCANH, and CBCA(CO)NH were acquired for assignments (for experimental details, see Bax et al., 1994). Processing of the data was carried out using the NmrPipe software package (Delaglio et al., 1995). For analysis of multidimensional spectra, PIPP program (Garrett et al., 1991) was used. The sequential resonance assignments were obtained by analysis of HNCA and HN(CO)CA spectra. While the sensitivity of normal triple resonance experiments at 25 °C for *E. coli* RRF was not sufficiently high, resolution enhancements achieved by deuteration and ^{2}H decoupling permitted nearly complete assignments. These assignments were confirmed in the analysis of HNCO and HN(CA)CO spectra for *E. coli* RRF or CBCANH and CBCA(CO)NH spectra for *T. maritima* RRF. Figure 1 shows the ^{1}H-^{15}N HSQC spectrum of *E. coli* RRF with assignments for the cross-peaks indicated by residue number. The consensus CSI data for both RRFs were calculated from $^{13}C_\alpha$ and $^{13}C'$ chemical shifts to estimate the secondary structures. These secondary structures are basically consistent with previously reported structures of RRFs.

Extent of assignments and data deposition

For *E. coli* RRF, 175 out of 176 backbone resonances (185 residues minus 8 prolines and Met1) in the HSQC spectrum were unambiguously assigned. The amide resonance of Ile2 was not observed presumably due to conformational exchange or rapid exchange with solvent. The chemical shifts of ^{1}H, ^{15}N, and ^{13}C resonances for *E. coli* RRF at 25 °C and pH 5.0 have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under accession code 5190. For *T. maritima* RRF, 164 out of 174 backbone resonances (185 residues minus 10 prolines and Met1) in the HSQC spectrum were unambiguously assigned. Since Ser33, Thr49, and Ser104 lie between prolines, amide resonances of these residues were not assigned uniquely. The amide resonances of Val2, Asn3, Thr106, and Thr107 were not observed presumably due to conformational exchange or rapid exchange with solvent. The chemical shifts of ^{1}H, ^{15}N, and ^{13}C resonances for *T. maritima* RRF at 40 °C and pH 7.4 have been deposited in BioMagResBank under accession code 5191.

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