Journal of Biomolecular NMR, **15:** 341–342, 1999.
KLUWER/ESCOM

Letter to the Editor: Backbone NMR assignment and secondary structure of Ribosome Recycling Factor (RRF) from *Pseudomonas aeruginosa*

Hiromasa Kashimori[a], Takuya Yoshida[a], Hiroyuki Kijima[a], Hideto Shimahara[b], Susumu Uchiyama[b], Tetsuya Ishino[b], Masahiro Shuda[c], Hiroaki Nakano[a], Yasutaka Shibata[a], Yukari Saihara[a], Tadayasu Ohkubo[a], Toshio Yoshida[b], Akira Kaji[c] & Yuji Kobayashi[a,*]
[a]*Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565-0871, Japan;* [b]*RRF Research Inc., Kannondai, Tsukuba, Ibaragi 305-0856, Japan;* [c]*Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.*

Received 16 September 1999; Accepted 14 October 1999

Key words: backbone assignment, ribosome recycling factor, RRF, secondary structure

Biological context

Protein biosynthesis has three distinct steps: initiation, peptide chain elongation and termination. The group led by Kaji established that there is an additional essential fourth step in protein synthesis, namely, disassembly of the post-termination complex. Their studies on the fate of 70S ribosome of the post-termination complex led to the discovery of a ribosome recycling factor (RRF). They found that, in the presence of GTP and elongation factor G (EF-G), RRF catalyzes the disassembly of the post-termination complex, which consists of the ribosome, bound mRNA containing the termination codon and the ribosome bound deacylated tRNA, to allow recycling of components of the termination complex for the next round of protein synthesis (for review see Janosi et al., 1996). RRF-encoding genes (*frr*) have been found in every bacterial genome so far examined (for example, Redenbach et al., 1996). Furthermore, RRF is shown to be essential for bacterial growth (Janosi et al., 1994). RRF-like proteins are found in eukaryotes as well, although their functions are presumably for protein synthesis in the organelles (Rolland et al., 1999).

Despite intensive biochemical studies on this ubiquitous small protein (ca. 185 amino acids), its molecular characterization is not well established. To begin to understand the fourth step of protein biosynthesis at the molecular level, we have been studying the structure of this protein by NMR and we report here the backbone ^{1}H, ^{13}C and ^{15}N NMR assignments and the secondary structure of RRF from *Pseudomonas aeruginosa.* This report on RRF from an opportunistic pathogen is a step toward designing a new type of therapeutic antimicrobial agents targeted against RRF.

*To whom correspondence should be addressed.

Methods and results

The DNA fragment encoding RRF sequence of *Pseudomonas aeruginosa* (Ohnishi et al., 1999) was cloned into *Nde*I/*Bam*HI sites of the pET22b plasmid vector (Novagen). The recombinant plasmid was introduced into *E. coli* strain BL21(DE3). The bacteria were grown at 37 °C in M9 minimal medium and protein expression was induced by addition of IPTG. Incubation after induction was continued for 3 h. The bacteria were harvested by centrifugation. The cell pellets were sonicated and centrifuged. RRF was isolated and purified from the supernatant using DEAE-sepharose. Yield was about 100 mg per liter culture. ^{15}N labeled RRF and $^{15}N/^{13}C$ doubly labeled RRF were prepared with ^{15}N ammonium chloride and/or ^{13}C glucose. $^{2}H/^{13}C/^{15}N$ triply labeled RRF was expressed in the same medium prepared in D_2O. The yield in a D_2O medium was about half of that in H_2O media. Selective ^{15}N labeling was performed for the following seven amino acids: Lys, Val, Met, Ile, Leu, His and Arg, according to the method described by Lee et al. (1995). For the selective incorporation of Met and His residues, auxotrophic strains of *E. coli* for the corresponding amino acids were used. No isotopic dilution or incorporation of label at undesired sites was

detected. The final NMR sample contained RRF at a concentration of ca. 1.5 mM in 10 mM potassium acetate buffer of 90% H_2O/10% D_2O at pH 5.0 with 50 mM NH_4Cl, 10 mM $MgSO_4$ and 1 M glycine. For the H-D exchange experiment, a buffer of 100% D_2O was used.

All NMR spectra were acquired at 25 °C on a Varian INOVA 500 MHz spectrometer with a triple-resonance z-gradient probehead. The NMR experiments performed included sensitivity-enhanced 2D ^{1}H-^{15}N HSQC, 3D HNCA, HN(CO)CA, HA(CA)NH, HA(CACO)NH, HN(CA)CO, HNCO and 4D ^{15}N/^{15}N-NOESY (Kay, 1995). All the NMR data were processed with NMRPipe (Delaglio et al., 1995) and analyzed with the PIPP program (Garrett et al., 1991). The sequential resonance assignments were established by the combined analysis of the double- and triple-resonance NMR data of uniformly labeled RRF. The assignments were also facilitated and confirmed by seven selective ^{15}N-labeling experiments. Figure 1A shows the ^{1}H-^{15}N HSQC spectrum for RRF with assignments for the cross peaks indicated with residue number. Figure 1B shows the consensus CSI data calculated from ^{1}Hα, ^{13}Cα, ^{13}Cβ and ^{13}CO chemical shift data by Wishart and Sykes' method (1994), the NOE connectivity profile of NH_i-NH_{i+1} and NH_i-NH_{i+2}, and NH with slow HD exchange rates. The summery of the secondary structure elements identified in RRF is presented in the lower panel of Figure 1. Five α-helices and six β-strands are observed, of which three α-helices (α1, α4, α5) are characteristically long. These secondary structures are basically in agreement with a recently established tertiary structure of *T. maritima* RRF (M. Selmer, S. Al-Karadaghi, G. Hirokawa, A. Kaji and A. Liljas, in preparation).

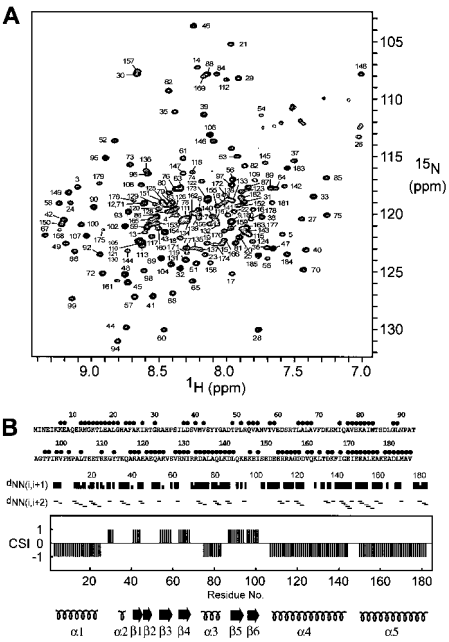

Figure 1. (A) 2D ^{1}H-^{15}N HSQC spectrum of 1.5 mM [U-^{15}N] ribosome recycling factor (RRF) from *Pseudomonas aeruginosa* with assignments. (B) Top: The amino acid sequence of RRF with circles indicating slowly exchanging NH protons that persist for 5 h in D_2O solution. Middle: Consensus CSI data, and observed short range dNN(*i, i+1*) and dNN(*i, i+2*). Bottom: Summary of the secondary structure elements (α-helix, spiral; β-strands, arrow).

Extent of assignments and data deposition

For RRF, 171 out of 178 backbone amide resonances (185 residues minus six prolines and N-terminal) in the HSQC spectrum were unambiguously assigned. Those unassigned were I2, Q10, E11, T114, S127, T164, and F167. A large percentage of the resonances was assigned for other nuclei: 95% for Cα, 97% for C′ and 89% for Hα. The low value for Hα comes from unassigned residues, 121, 125–129, 131, that are localized in the center of the α4-helix (see Figure 1B). These peaks were broad, presumably due to relaxation. The chemical shifts of ^{1}H, ^{15}N and ^{13}C resonances for RRF at 25 °C and pH 5.0 have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4389.

References

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Garrett, D.S., Powers, R., Gronenborn, A.M. and Clore, G.M. (1991) *J. Magn. Reson.*, **95**, 214–220.

Janosi, L., Shimizu, I. and Kaji, A. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 4249–4253.

Janosi, L., Hara, H., Zhang, S. and Kaji, A. (1996) *Adv. Biophys.*, **32**, 121–201.

Kay, L.E. (1995) *Prog. Biophys. Mol. Biol.*, **63**, 277–299.

Lee, K.M., Androphy, E.J. and Baleja, J.D. (1995) *J. Biomol. NMR*, **5**, 93–96.

Ohnishi, M., Janosi, L., Shuda, M., Matsumoto, H., Hayashi, T., Terawaki, Y. and Kaji, A. (1999) *J. Bacteriol.*, **181**, 1281–1291.

Redenbach, M., Kieser, H.M., Denapaite, D., Eichner, A., Cullum, J., Kinashi, H. and Hopwood, D.A. (1996) *Mol. Microbiol.*, **21**, 77–96.

Rolland, N., Janosi, L., Block, M.A., Shuda, M., Teyssier, E., Miege, C., Cheniclet, C., Carde, J.P., Kaji, A. and Joyard, J. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 5464–5469.

Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR*, **4**, 171–180.