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Letter to the Editor: Complete ^{1}H, ^{13}C, and ^{15}N assignments of the N-terminal DNA binding domain of the TraR protein

Takeshi Tanaka[a], Mariko Sugai[a], Kuniko Kobayashi[a], Masakazu Kataoka[b] & Toshiyuki Kohno[a,*]

[a] *Mitsubishi Kagaku Institute of Life Sciences, Minamiooya 11, Machida-shi, Tokyo 194-8511, Japan*
[b] *Department of Environmental Science and Technology, Faculty of Engineering, Shinshu University, Wakasato 4-17-11, Nagano-shi, Nagano 380-8533, Japan*

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Biological context

Horizontal gene transfer is a main force for increasing bacterial genetic diversity, and thus plasmid mediated conjugation systems, which can mobilize the chromosome, have a principle role in bacterial evolution. Most of the *Streptomyces* plasmids are self-transmissible and are able to mobilize chromosomal markers. Evidence suggests that the conjugation process in *Streptomyces* is novel, such as the fact that relatively few plasmid encoding genetic loci are required for as compared to other bacteria (Hopwood and Kieser, 1993). For example, there are only five transfer related loci on the *Streptomyces* plasmid pSN22: *traA, traB, traR, spdA,* and *spdB* (Kataoka et al., 1991a). Genetic analyses suggested that only *traB* and *traR* are essential for intermycelial, the principle process in horizontal transfer (Kataoka et al., 1991a). In the pSN22 system, the *traB* gene product is a membrane associated protein, and the expression is regulated by *traR* (Kataoka et al., 1994; Kosono et al., 1996). The *traR* gene product, TraR, is a 27 kDa protein that functions as a transcriptional repressor for the *tra* operon (*traA-traB-spdBs*), and *traR* itself (Kataoka et al., 1991b). TraR has a helix-turn-helix (HTH) motif and binds to a 12 bp consensus DNA sequence, TRE box. This is located within the divergent promoter region for the *tra* operon and *traR*, and regulates their expression negatively (Kataoka et al., 1994). Similar central regulators for the *Streptomyces* conjugation system have also been identified and characterized in detail (Pettis et al., 1996; Sezonov et al., 2000).

Although all of these regulators for the *Streptomyces* conjugation systems have HTH motifs, their primary structures exhibit little similarity. From the viewpoint of the structure–function relationships, it is significant work to determine and to compare 3D structures of such regulators. As a first step, we now report the sequence specific assignment of the NMR resonances of TraR(1-100).

Methods and results

At first, we determined the N-terminal structural domain of TraR, by using limited proteolysis (data not shown). The results showed that the N-terminal domain of TraRs of about 100 amino acid residues. Then, we prepared a plasmid that expresses the N-terminal 100 amino acids domain of TraR, TraR(1-100), as a fusion protein with glutathione-S-transferase (GST). The GST-TraR(1-100) fusion protein expressed in *E. coli* was immobilized onto a Glutathione Sepharose 4B column. The TraR(1-100) was cleaved by PreScission Protease and was eluted. The cleaved TraR(1-100) was further purified by chromatography on Resource S and Superdex 75pg columns. The uniformly $^{15}N/^{13}C$ enriched protein was obtained from the *E. coli* cells grown in the medium described previously (Kohno et al., 1998) or in the CHL medium-^{13}C, ^{15}N (Shoko Co., Ltd). For NMR spectroscopy, about 1 mM protein was prepared in 50 mM sodium phosphate buffer (pH 6.5) containing 0.1 M NaCl, 10% D_2O, and 1 mM DSS.

* To whom correspondence should be addressed. E-mail: tkohno@libra.ls.m-kagaku.co.jp

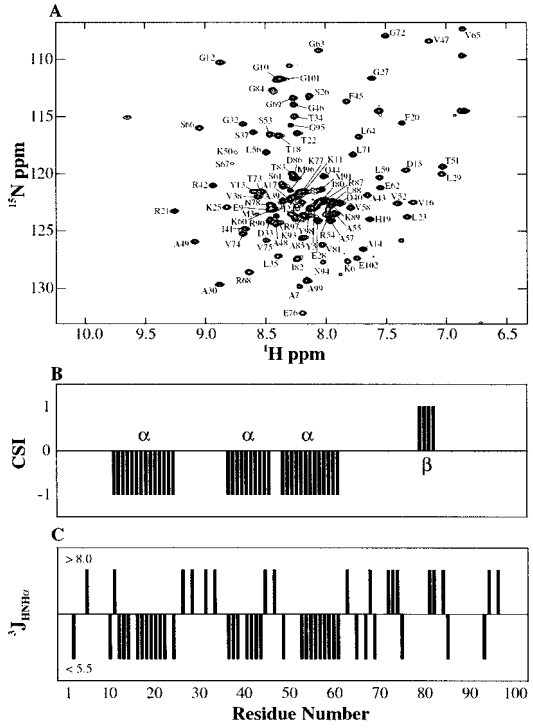

Figure 1. (A) ^{1}H–^{15}N HSQC spectrum of TraR(1-100) at a ^{1}H resonance frequency of 500 MHz (pH 6.5, 303 K). The resonance assignments are indicated with the one-letter amino acid code and residue number. (B) CSI consensus plot for TraR(1-100). Generated using ^{1}Hα, ^{1}Hβ, ^{13}Cα, ^{13}Cβ, and ^{13}CO chemical shifts. (C) The $^{3}J_{HN-Hα}$ values for TraR(1-100) obtained from the 2D intensity modulated ^{1}H–^{15}N HSQC.

NMR spectra were acquired on a Bruker Avance-500 spectrometer equipped with pulse field gradient accessories at 303 K. The sequence specific assignments were obtained from the triple resonance 3D NMR spectroscopy, HNCACB, CBCA(CO)NNH, HNCA, HN(CO)CA, HNCO, HN(CA)CO, HCC (CO)NH, CC(CO)NH, and HCCH-TOCSY (Sattler et al., 1999). The $^{3}J_{HN-Hα}$ values were obtained from the 2D intensity modulated ^{1}H-^{15}N HSQC. ^{1}H chemical shifts were referenced to DSS at 0 ppm, and ^{13}C and ^{15}N chemical shifts were calculated from the ^{1}H frequency. All spectra were processed using Azara v2.5 (provided by Wayne Boucher) and were analyzed using ANSIG v3.3 (Kraulis et al., 1994) on a Silicon Graphics O2 workstation or on a Linux workstation.

Extent of assignments and data deposition

Since the TraR(1-100) structure reported here includes two additional residues at the N-terminus, the assignments include these two residues, Gly1 and Pro2. On the ^{1}H–^{15}N HSQC spectrum, the ^{1}H and ^{15}N resonance assignments of the backbone amides for 92 out of 95 non-Pro residues have been achieved (97% and 97% completed, respectively). The unassigned residues were Gly1, Ala70 and Glu92. The extents of the assignments are: 99% of ^{13}Cα, 100% of ^{13}Cβ, 99% of ^{1}Hα, 100% of ^{1}Hβ, and 99% of ^{13}CO resonances. An example of an NMR spectrum of TraR(1-100) is shown in Figure 1A.

The secondary structure predictions based on CSI (Figure 1B) and on the $^{3}J_{HN-Hα}$ values (Figure 1C) both show the existence of three α-helices and one or two β-strands characteristic of TraR(1-100). The chemical shift values of ^{1}H, ^{15}N, and ^{13}C resonances of TraR(1-100) have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu/) under accession number 5187.

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