Journal of Biomolecular NMR, **22:** 381–382, 2002.
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Letter to the Editor: Backbone resonance assignment of the ^{2}H, ^{13}C, ^{15}N labelled 32kDa central domain of *Escherichia coli* TyrR

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Received 24 December 2001; Accepted 29 January 2002

Key words: enhancer-binding protein, NMR assignment, TyrR

Biological context

The bacterial enhancer-binding proteins are important gene regulators controlling a broad range of metabolic processes (Buck et al., 2000). Promoters at which transcription initiation is activated by enhancer-binding proteins respond only to RNA polymerase holoenzyme (Eσ^{54}), which has an ancillary subunit σ^{54} bound, whereas the majority of *E. coli* promoters respond to RNA polymerase with σ^{70} bound (Eσ^{70}). Enhancer-binding protein-mediated transcription activation requires oligomerization of DNA-bound enhancer-binding protein, the hydrolysis of ATP by the enhancer-binding protein, and the interaction of the enhancer-binding protein with promoter-bound RNA polymerase (Eσ^{54}) (Flashner et al., 1995). Most enhancer-binding proteins consist of three domains: An N-terminal signal input domain; a C-terminal domain which binds DNA through a helix-turn-helix motif and a central domain which contains an ATP binding and cleavage site and determinants for oligomerization (Morret and Segovi, 1993). The amino acid sequence of the central domain is highly conserved across the enhancer-binding protein family suggesting similar structure and mechanism of action and that specificity arises from differences in the N- and C-terminal domains.

The transcription factor TyrR regulates the biosynthesis and transport of aromatic amino acids by both activation and repression (Pittard and Davidson, 1991). During the course of the evolutionary process, TyrR retained, in common with enhancer-binding proteins, the ability to undergo a signal-induced self-association and the capacity to bind and hydrolyse ATP, but lost its ability to interact with Eσ^{54}. It activates genes transcribed by RNA polymerase associated with σ^{70}. As observed with several enhancer-binding proteins, the self-association enhances the affinity of TyrR for DNA. This self-association of TyrR is involved in tyrosine-mediated repression, but the role of the ATPase activity in TyrR function is unknown. TyrR is also a three-domain protein with a central domain that is homologous to other members of the enhancer-binding protein family. Here we report the HN, ^{15}N, ^{13}C′, ^{13}Cα, ^{13}Cβ assignments of the central domain of TyrR from *E. coli*.

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Methods and results

The gene encoding residues of 188 to 466 of TyrR (TyrR$_{188–466}$) was cloned into the pET22b vector and expressed in *E. coli* BL21(DE3) in 1 l of minimal media in a 2 l fermentor at 37 °C, following the method of Cai et al. (1998) except that $^{2}H_2O$ was used. Cell pellets were lysed by sonication in 25 mM potassium phosphate, 100 mM KCl, 10% glycerol, 5 mM imidazole, pH 8. Cell debris was removed by centrifugation and the supernatant passed through a 15 ml Talon column (Clontech). The protein was eluted with 100 and 200 mM imidazole and further purified on a Superose-12 column. From 1000 ml of cell culture, supplemented with 2.5 gm $^{15}NH_4Cl$ and 3 gm ^{13}C-glucose, 22 mg of His$_6$-tagged ^{2}H, ^{13}C,^{15}N TyrR$_{188–466}$ were purified. The His$_6$ tag was not removed.

Samples of TyrR$_{188-466}$ (0.5 ml, 0.7 mM) were prepared in a 25 mM potassium phosphate buffer, pH 7.5, 5 mM dithiothreitol, 1 mM EDTA, 0.02% sodium azide and 90% H_2O/10%$^{2}H_2O$. Using a

Varian 600 Inova operating at 20 °C and equipped with a 5 mm ^{1}H, ^{13}C, ^{15}N single z-axis gradient probe the following three-dimensional TROSY based spectra were acquired: HNCACB, HNCO, HN(CA)CB, HN(COCA)CB, HNCA, HN(CA)CO (Yang and Kay, 1999). 2D ^{15}N,^{1}H TROSY and 3D ^{15}N,^{1}H NOESY-HSQC spectra were also recorded. Data were processed with NMRPipe (Delaglio et al., 1995) and analyzed with XEASY (Bartels et al., 1995). ^{1}H chemical shifts were referenced to DSS at 0 ppm, and ^{13}C and ^{15}N chemical shifts calculated from the ^{1}H spectrometer frequency.

Extent of assignments and data deposition

Solution conditions for the analysis of $TyrR_{188–466}$ are restrictive. At a concentration greater than 0.7 mM, above 25 °C or below pH 7 precipitation of the protein is too rapid even in the presence of potential stabilizers, for example sorbitol, glycerol, and DMSO. However, light scattering analysis of 0.5 mM protein, pH 7.5, 20 °C suggest that the protein remains monomeric (Dixon, unpublished). The ^{1}H,^{15}N HSQC spectra acquired on undeuterated samples between 0.5 to 1 mM were not high quality, but clearly showed a central strip of $\sim$ 30 strong resonances characteristic of mobile regions. As the central domain is expected to span residues 208 to 433 attempts were made to truncate the protein and exclude mobile tails. Constructs spanning 204–466, 204–44 and 204–439 were prepared, but either expressed insolubly, precipitated during purification or on concentration.

Deuteration of $TyrR_{188–466}$ combined with TROSY methodology was essential for assignment progress. Resonances of the first seven N-terminal and the six His-tag C-terminal residues were not observed, presumably as they are in rapid exchange at pH 7.5. Of the remaining residues 96% of backbone (N, HN, C′, Cα, Cβ) resonances have been assigned (Figure 1). The level of deuteration (<85%) precluded the use of constant time methods in the ^{13}C-dimension of the HN(CA)CB and HN(COCA)CB experiment and therefore spin system typing was not trivial (Shan et al., 1996). As a consequence, helical regions were assigned first and confirmed by sequential NOE data. Chemical shift analysis suggests the protein consists of five β-strands spanning residues 230–234, 258–262, 293–298, 333–340 and 365–367, and nine helices spanning residues 208–226, 241–251, 268–278, 307–319, 343–353, 355–363, 382–394, 406–411 and 418–431. The positions of the strands and helices are consistent with this domain having a AAA+ (ATPases associated with various cellular activities) fold as suggested by PSI-BLAST analysis (Neuwald et al., 1999).

The ^{1}H, ^{13}C and ^{15}N assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 5234.

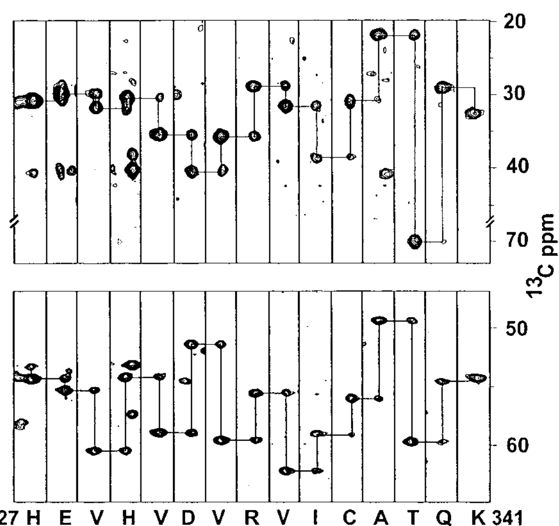

Figure 1. Selected strips from 3D HNCA-TROSY (lower panel) and HN(CA)CB-TROSY (upper panel) spectra of ^{2}H,^{13}C,^{15}N $TyrR_{188–466}$ showing the connectivities for the region His327 to Lys341.

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