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Chemical shift assignments of a reduced N-terminal truncation mutant of the disulfide bond isomerase TrbB from plasmid F, TrbB 29

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Abstract

TrbB from the conjugative plasmid F is a 181-residue disulfide bond isomerase that plays a role in the correct folding and maintenance of disulfide bonds within F plasmid encoded proteins in the bacterial periplasm. As a member of the thioredoxin-like superfamily, TrbB has a predicted thioredoxin-like fold that contains a C-X-X-C active site required for performing specific redox chemistries on protein substrates. Here we report the sequence-specific assignments of the reduced form of the N-terminally truncated TrbB construct, TrbB 29.

Keywords

TrbB; bacterial conjugation; disulfide bond isomerase; NMR chemical assignments

Biological Context

In Gram-negative bacteria, disulfide bond isomerases are responsible for maintaining the integrity of disulfide bonds in the periplasm (Hiniker 2003, Ito 2008, Kadokura 2003, Nakamoto 2004). Disulfide bonds are incorporated into proteins as they are secreted into the periplasm by the catalytic activity of the thiol-oxidase DsbA (Darby 1995). As these bonds are typically incorporated between sequential cysteines in the proteins primary sequence, this mechanism can lead to incorrect linkages in those proteins that require disulfide bonds between non-consecutive cysteine residues. In these cases, disulfide bond isomerases are utilized to reduce these errant bonds and “shuffle” them into their native location (Hiniker 2003, Ito 2008, Kadokura 2003, Nakamoto 2004). In addition, disulfide bond isomerases are utilized under conditions of oxidative stress, where they function to resolve increased levels of errant disulfide bonds (Hiniker 2005). In the absence of a disulfide bond isomerase, oxidative stress leads to aggregation of periplasmic residents, and, eventually, cell death.

Proteins belonging to the thioredoxin-like superfamily have recently been identified in 89 different plasmid systems, including the protein TrbB from the conjugative plasmid F (Wu

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1987, Elton 2005, Hemmis 2013). Structural predictions, which here are confirmed by NMR and limited proteolysis experiments, reveal that the N-terminal 29 residues of the mature TrbB protein are largely unstructured. Further biochemical analysis revealed that TrbB functions as a disulfide bond isomerase and likely functions to mediate the proper folding and maintenance of disulfide networks in a subset of plasmid F encoded proteins (Hemmis 2011, Hemmis 2013). Interestingly, the TrbB redox active site is maintained by the host-encoded redox system rather than through a plasmid-mediated process, indicating a dependence of the plasmid on the redox system of its host (Hemmis 2011).

While structural predictions of TrbB suggest it adopts a thioredoxin-like fold, several deviations from other disulfide bond isomerases, such as the canonical disulfide bond isomerase DsbC from *E. coli*, are predicted. Included in these differences are the absence of an alpha helical subdomain and an N-terminal dimerization, the former which is characteristic of both DsbA-like and DsbC-like thioredoxin family members (Martin 1993, McCarthy 2000). Disulfide bond isomerase activity is completely abrogated in dimerization-deficient DsbC mutants (Bader 2001), making the lack of an N-terminal dimerization domain in TrbB quite striking. Reflecting these deviations, recent phylogenetic analyses place TrbB in a unique family of plasmid-based disulfide isomerases that are likely responsible for the correct folding and maintenance of a subset of F plasmid-encoded proteins including TraH, TraN, and TraU (Hemmis 2011, Hemmis 2013).

Methods and Experiments

Limited proteolysis was utilized to identify a stable fragment of the full-length mature TrbB protein (161 residues, 17.7 kDa) as previously described (Hemmis 2011). Briefly, TrbB was purified and subjected to limited trypsin digestion at a 1:250 trypsin to TrbB molar ratio. Aliquots of the digestion were removed at 1, 2, 5, 10, 20, and 30 minutes and quenched with 1 [proportional]L of 100 mM PMSF. Samples from these aliquots were analyzed using a 10% Tris-Tricine SDS-PAGE gel stained with Coomassie Blue. The molecular weight of the resulting stable band was identified by mass spectroscopy using a Voyager MALDI-TOF MS (Applied Biosystems) at the Johns Hopkins University Mass Spectroscopy facility, and indicated the stable construct to consist of residues 49-181.

The DNA sequence encoding residues 49-181 of *trbB* was PCR amplified from a full-length TrbB construct. This product was digested and ligated into the *NdeI* and *EcoRI* restriction sites of the pET24a vector (Novagen). BL21(DE3) *E. coli* containing the TrbB 29 vector were grown in 1 L of ^{15}N or ^{15}N , ^{13}C MOPS minimal media containing 30 [proportional]g/mL kanamycin (Wright 2005). $^{15}\text{NH}_4$ and ^{13}C glucose was obtained from Cambridge Isotopes, Andover, MA. Cells were grown to an OD_{600} of 0.4 and expression was induced with 1 mM IPTG for 3 h. Cells were then pelleted by centrifugation and stored at -80°C . For purification, cells were thawed and resuspended in 25 mL of 50 mM Tris-HCl (pH 7.5) with 3 M NaCl and 1 mM EDTA. Cells were lysed by sonication and cellular debris was removed by centrifugation. Purification of TrbB 29 was performed on an AKTA Prime Plus (GE Healthcare) using a 5 mL Hi-Trap Butyl Column (Amersham Biosciences) equilibrated with 50 mM Tris-HCl (pH 7.5) with 3 M NaCl and 1 mM EDTA. Using a 3000 mM to 25 mM gradient of NaCl, TrbB 29 eluted as a single peak beginning at 600 mM.

Fractions were analyzed by 10% Tris-Tricine SDS-PAGE gels stained with Coomassie Blue. Pooled fractions were then exhaustively dialyzed against 10 mM phosphate buffer (pH 6.9) with 20 mM NaCl at 4°C. After dialysis, DTT was added to 5 mM to reduce the TrbB redox active site and D₂O was added to 10%. The protein was then concentrated to >500 μM as quantified by absorbance ($\epsilon_{280} = 16,960 \text{ M}^{-1} \text{ cm}^{-1}$) using Amicon Ultra 10,000 MWCO concentrators.

All experiments were collected on a 600MHz Bruker AVANCE II spectrometer equipped with a 5 mm triple-resonance cryogenic probe and z-axis pulse field gradient coils. A 2D ¹⁵N-HSQC and standard triple resonance experiments, including the HNCACB, CBCA(CO)NH, HNCO, C(CO)NH-TOCSY, H(CCO)NH-TOCSY, and a ¹⁵N-edited NOESY were collected. NMR data was processed using NMRPipe (Delaglio 1995) and analyzed with Sparky (Goddard TD). All assignments were done manually, while MARS was used for verification (Jung 2004). The ¹H chemical shifts were referenced to external DSS, the ¹³C shifts were referenced indirectly to DSS using the frequency ratio ¹³C/¹H = 0.251449527 and ¹⁵N shifts were referenced indirectly to liquid ammonia using ¹⁵N/¹H = 0.101329118.

Extent of assignments and data deposition

TrbB₂₉ has 121 non-proline residues. Of these residues, 119 have been labeled on the HSQC (Fig. 1). All visible peaks on the HSQC were unambiguously assigned, correlating to over 98% of non-proline residues. Analysis of the triple resonance experiments provided over 98% of the ¹H and ¹⁵N assignments, the C_α and C_β resonances (Fig. 2), and the C' resonances of the protein. Importantly, over 97% of side-chain hydrogens expected from the H(CCO)NH-TOCSY are assigned, with the shifts being well-dispersed. C_α and C_β shifts have been assigned for the 11 proline residues that do not precede another proline (Pro-Pro). Furthermore, resonances have been assigned for 10 of the 11 proline C_γ atoms and for two of the 11 proline C_δ atoms. It must also be noted that the two residues for which we are lacking ¹H and ¹⁵N data, T50 and A51, compose the extreme N-terminus of the protein. In addition, 14 of the 15 NH₂ side-chains have been assigned in the HSQC, as well as the NH of the two tryptophan residues, W57 and W71. The sequence-specific chemical shift assignments for TrbB₂₉ have been deposited in the BioMagResBank (BMRB) under the accession number 19465. Further analysis of chemical shifts using TALOS+ and RAMA+ (Shen 2009) predicts secondary structure in agreement with a thioredoxin-like fold, as was expected from homology and functional studies (data not shown).

The HSQC profile of full-length TrbB was nearly indistinguishable from the TrbB₂₉ construct with the notable addition of roughly ten additional strong peaks located between 117-123 ppm in the nitrogen dimension and 7.8-8.5 ppm in the hydrogen dimension. No additional peaks were observed under varying temperature and buffer conditions, indicating that first 29 residues of TrbB are likely largely dynamic and unstructured. In addition, the lack of significant chemical shift perturbations of the remaining “core” residues demonstrates that truncation of TrbB's N-terminal “arm” does not significantly affect the integrity of the remaining structure to any significant degree (Figure 3).

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Figure 1. 2D ^1H - ^{15}N HSQC of reduced TrbB 29 at 600 MHz (^1H) with the assignments included. Backbone ^1H - ^{15}N correlations are labeled sequence-specifically and numerically according to the full-length unprocessed protein. Correlations connected by horizontal lines correspond to glutamine and asparagine side-chain NH_2 groups.

Figure 2.

Strips derived from ^{15}N planes of a 3D ^{15}N -edited CBCA(CO)NH (left strip of each pair) and a 3D ^{15}N -edited HNCACB spectrum (right strip of each pair) illustrating through-bond connectivities of the C_α and C_β carbon atoms from residues T158-M164.

Figure 3.
Chemical shift perturbation by residue for all assigned residues of reduced TrbB 29 compared to the full-length TrbB protein.