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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C assignments of the carboxy-terminal domain of the transmembrane electron transfer protein DsbD

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Biological context

The electron transfer protein DsbD, also known as DipZ and found in the cytoplasmic membrane of Gram-negative bacteria, is unique in that it employs an intra-molecular disulfide cascade to transfer electrons from cytoplasmic thioredoxin to the periplasmic disulfide isomerases, DsbC and DsbG, and to the cytochrome *c* maturation pathway via CcmG (DsbE) and/or CcmH (Rietsch and Beckwith, 1998, and references therein). DsbD consists of three distinct domains: two periplasmic domains at the N- and C-termini which are separated by a transmembrane domain of eight helices (Page et al., 1997; Gordon et al., 2000), referred to elsewhere as the α, γ and β domains, respectively (Katzen and Beckwith, 2000). Each domain contains a conserved pair of cysteines essential for electron transfer, believed to occur via the sequential reduction of disulfide bonds. Initially cytoplasmic thioredoxin reduces the disulfide bond of the transmembrane domain, the resulting thiols in turn reduce the disulfide bond of the C-terminal domain, which then reduces the disulfide bond of the N-terminal domain (Katzen and Beckwith, 2000). Essential for elucidation of the mechanism by which DsbD transports electrons across the cytoplasmic membrane are the structures of the individual domains and the interactions between them. The crystal structure of the N-terminal domain has recently been published (Goulding et al., 2002), but there is no corresponding structure for the C-terminal domain.

The C-terminal domain of DsbD contains a CXXC-motif at its active site, characteristic of the thioredoxin superfamily of thiol-disulfide reductases, and is often predicted to adopt the same structure as thioredoxin, despite the lack of any appreciable sequence similarity outside the CXXC-motif or experimental evidence. Overexpressed versions of the C-terminal domain of DsbD from *E. coli* retain the ability to reduce the N-terminal domain *in vitro* (Collet et al., 2002; K.M.W. Bushell and S.J. Ferguson, unpublished results), in agreement with *in vivo* results (Katzen and Beckwith, 2000); this implies the structural integrity of this isolated domain is maintained. The crystal structure of CcmG (DsbE) (Edeling et al., 2002) exemplifies how differences in structure and protein sequence around the CXXC-motif are crucial to the function and specificity of the active site. Therefore, given the small degree of overall sequence homology between the C-terminal domain of DsbD and thioredoxin, it is important to determine the structure of the C-terminal domain of DsbD. Here we report preliminary NMR studies of the C-terminal domain of DsbD from *E. coli*; we present substantially complete ^{1}H, ^{13}C and ^{15}N assignments and describe the major elements of secondary structure.

Methods and experiments

Two versions of the C-terminal domain of DsbD from *E. coli* were used, both were purified from periplasmic extracts of *E. coli* using a C-terminal His-tag as described previously (Gordon et al., 2000). The first was EGX134 (Gordon et al., 2000), a 160 amino acid protein in which the N-terminus consists of nine amino acids from the expression vector preceeding V409 of the native sequence and the C-terminus has thirteen amino acids following P546 . The second was DZC1, a 137 amino acid protein obtained by subcloning the DNA sequence of interest into

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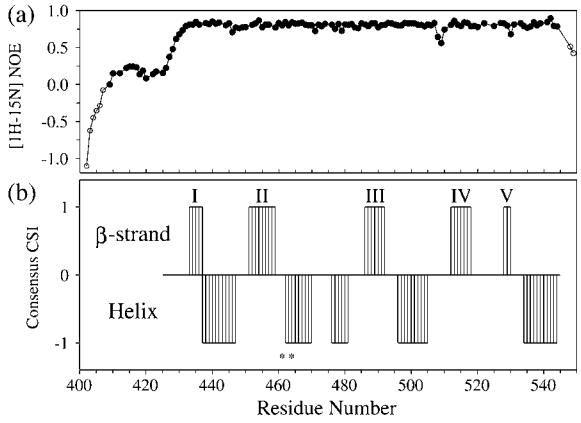

Figure 1. (a) [^{1}H-^{15}N] heteronuclear NOE ratios for EGX134. Filled circles represent residues derived from the native sequence. (b) Consensus chemical shift indeces for DZC1; β-strand and helical regions are indicated. The active site Cys residues are shown (*).

pET22b(+) and purifying the protein as before, which has two amino acids from the expression vector preceeding residue T425 of the native sequence, giving the new N-terminal sequence MDTQTH. ^{15}N-labelled EGX134 and ^{15}N/^{13}C-labelled DZC1 were produced using ^{15}N-NH_4Cl and ^{13}C-glucose enriched M9 minimal medium supplemented with 1mM thiamine. NMR samples contained 1.5 mM oxidised protein in 95% H_2O/5% D_2O at pH 6.5 and 25 °C.

Sequential assignments were carried out using ^{15}N-labelled EGX134 and 3D ^{15}N-edited TOCSY-HSQC and NOESY-HSQC experiments; analysis of these spectra resulted in complete assignment of the $^{1}H^{N}$ and ^{15}N resonances. A ^{1}H-^{15}N heteronuclear NOE experiment was important for identifying the structured regions of the EGX134 construct. The ^{1}H-^{15}N NOEs, shown in Figure 1a, indicate that the first 29 residues of the EGX134 construct, 20 of which are from native DsbD, are very mobile. The 29 N-terminal residues have chemical shifts close to random coil values and do not give rise to any long range NOEs. On this basis, DZC1, a new construct in which the first 25 residues of EGX134 are removed, was designed and used for the ^{15}N-^{13}C-labelled sample. This sample has chemical shifts, for residues 425 to 546, that are identical to those measured for EGX134. The sequential assignments obtained from the ^{15}N-labelled sample were confirmed with a 3D HNCA experiment and complete ^{13}C and ^{1}H assignments were obtained using 3D HCCH-TOCSY, HNCO and CBCA(CO)NH experiments.

Figure 1b shows the consensus chemical shift indeces (CSI) determined from the Hα, Cα, Cβ and C′ shifts of DZC1 (Wishart and Sykes, 1994). The protein contains alternating β-strand and helical secondary structure. Long-range HN-HN and HN-Hα NOEs indicate that the β-strands are arranged in a single β-sheet in the order I-III-II-IV-V. Strands I, III and II have a parallel orientation while strands II, IV and V are anti-parallel. The active-site CXXC-motif is located between β-strand II and the second helix. These results show that the C-terminal domain of DsbD has a pattern of secondary structure nearly identical to that of thioredoxin (Holmgren et al., 1975), consistent with previous proposals. A full 3D structure determination is in progress.

Extent of assignments and data deposition

Backbone assignments are essentially complete for residues 425–546 in the C-terminal domain of DsbD. A total of 98% of the ^{1}HN, ^{15}N, Hα, Cα, Hβ, Cβ and 93% of the C′ resonances were assigned. Chemical shifts have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under the accession number BMRB-5497.

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