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Letter to the Editor: ^{1}H, ^{13}C, ^{15}N backbone and sidechain resonance assignments of apo-NosL, a novel copper(I) binding protein from the nitrous oxide reductase gene cluster of *Achromobacter cycloclastes*

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Biological context

Many metalloenzymes utilize metal ions such as copper to perform the unique chemistry required to activate inorganic molecules, and often require metallochaperones for proper assembly of the enzymatic metal centers (Rosenzweig and O'Halloran, 2000). One such protein is the terminal enzyme of the denitrification pathway, nitrous oxide reductase (N_2OR), which converts nitrous oxide (N_2O) to dinitrogen (N_2). Transcription of the operon *nos*DFYL, located downstream of the N_2OR structural gene *nos*Z, is obligatory for the production of enzymatically active N_2OR and contains the genes necessary for correct assembly of the catalytic Cu_Z copper cluster (Zumft et al., 1990; Wunsch et al., 2003). These *nos* proteins also show significant homology to bacterial ATP binding cassette (ABC) transporter systems that are usually associated with the transport of small molecules and proteins across bacterial inner membranes with concomitant ATP hydrolysis (Zumft et al., 1990). The *nosL* gene is conserved across five denitrifying organisms suggesting that it plays a crucial function for nitrous oxide reductase biogenesis and assembly (McGuirl et al., 1998, 2001). Sequence homology analyses indicate that NosL is a periplasmic lipoprotein anchored to the outer bacterial membrane most likely via a thioether bond between a lipid and the N-terminal cysteine of the mature protein (Zumft et al., 1990; Dreusch et al., 1996). The *nosL* gene from *Achromobacter cycloclastes* was identified and subsequently cloned in an *E. coli* expression system for recombinant protein expression (McGuirl et al., 1998). Recombinant NosL is expressed as a periplasmic protein with its N-terminal cysteine replaced with Met-Asp to avoid potential problems in purification and characterization that may arise from the lipid anchor moiety. The 18.5 kDa highly soluble recombinant NosL has been found to bind copper(I) in a specific and 1:1 stoichiometric fashion, even in the relatively oxidizing environment of the periplasm, suggesting a highly specific copper-binding function for NosL (McGuirl et al., 2001).

In an effort to determine the 3D structure of NosL and to better characterize its function with respect to copper trafficking and copper cluster assembly within N_2OR and the *nos* protein system, multidimensional solution NMR studies have been undertaken on both the apo- and copper(I) holo- forms of NosL from *Achromobacter cycloclastes.* Herein, we report the (^{15}N, ^{13}C, ^{1}H) resonance assignments of backbone and sidechain atoms of recombinant apo-NosL.

Methods and experiments

Nucleotides 7479-8000 of NosL from *A. cycloclastes* were cloned as described previously (McGuirl et al., 1998) into the pET-20b+ vector (Novagen Inc., WI). ^{15}N and ^{13}C labeled NosL was produced by growing bacterial cell cultures in MOPS media enriched with [^{15}N]NH_4Cl and D-Glucose-$^{13}C_6$ (Cambridge Isotope Labs, MA) as the sole nitrogen and carbon sources, respectively. Following purification using anion exchange chromatography, final NMR conditions were set to 1.0 mM NosL, 100 mM sodium phosphate, 1 mM d_{10}-DTT, 1 mM EDTA, pH 6.5.

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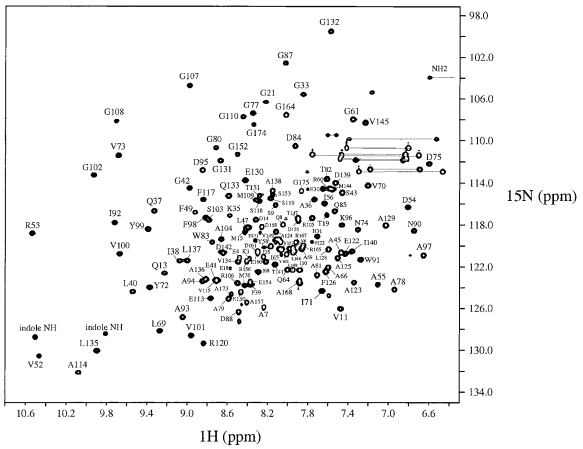

Figure 1. Two-dimensional ^{1}H-^{15}N-HSQC spectrum of uniformly ^{15}N-labeled apo-NosL. Several resonances (∼6) are unlabelled in the spectrum, and correspond to amide resonances which could not be assigned sequentially in the three-dimensional NMR experiments. Resonances connected by a thin line correspond to signals from side chain NH_2 of glutamines and asparagines.

All NMR spectra were acquired at 30 °C on a four-channel Bruker DRX-600 spectrometer, with a triple ^{15}N, ^{13}C, ^{1}H inverse resonance probe equipped with triple axis gradients. All data were processed and analyzed using NMRPipe, PIPP, and Xwinnmr version 3.1 (Bruker Inc.) software packages. Sequential ^{1}H/^{13}C backbone and side-chain chemical shift assignments were extracted from standard heteronuclear NMR experiments (HNCA, HNCACB, CBCA(CO)NH, C(CO)NH, HBHA(CO)NH, HC(CO)NH, HNCO, and ^{15}N-edited ^{1}H-^{1}H TOCSY). ^{1}H, ^{15}N, and ^{13}C chemical shift dimensions were indirectly referenced to DSS.

Extent of assignments and data deposition

Without counting NosL's fourteen proline residues, 11 out of a total of 161 amide resonances remain unassigned, corresponding to a sequential assignment of ∼93%. Of the eleven unassigned NH resonances, two correspond to Met 1 and Asp 2, while the others include the missing ^{1}H/^{15}N amides of Thr 16, Leu 20, Tyr 23–Leu 29, and Ser 50. Cα/Cβ and CO chemical shifts were determined for 166 and 141 residues of the protein's 175 amino acids, respectively. ∼94% of the Hα/Hβ resonances were assigned and complete assignment of sidechain ^{13}C and ^{1}H resonances were made for the vast majority of the residues that were identified. Backbone and sidechain (^{1}H, ^{15}N, ^{13}C) resonance assignments have been deposited in the BioMagResBank in Madison (WI) under the accession number 5595.

The two-dimensional ^{1}H-^{15}N-HSQC correlation spectrum of apo-NosL is shown in Figure 1. One of the striking features of this spectrum is the very large chemical shift dispersion of the amide protons ($\Delta^{1}H_{N}$ of ∼4.0 ppm), suggesting that apo-NosL is rich in β sheet content. Preliminary CSI analysis also points to the presence of several α-helical regions. Some minor additional NMR signals detected in the CBCACONH and HNCACB spectra of apo-NosL could not be fully assigned, and may be indicative of slow conformational exchange processes. Interestingly, the residues that remain unassigned encompass and flank the Cu(I) binding residue, Cys24, (McGuirl et al., 2001), suggesting that this region may be relatively disordered in the apo-form of the protein.

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References

Dreusch, A., Riester, J., Kroneck, P.M.H. and Zumft, W.G. (1996) *Eur. J. Biochem.*, **237**, 447–453.

McGuirl M.A., Bollinger, J.A., Cosper, N., Scott R.A. and Dooley, D.M. (2001) *J. Biol. Inorg. Chem.*, **6**, 189–195.

McGuirl, M.A., Nelson, L.K., Bollinger, J.A., Chan, Y. and Dooley, D.M. (1998) *J. Inorg. Biochem.*, **70**, 155–169.

Rosenzweig, A.C. and O'Halloran, T.V. (2000) *Curr. Opin. Chem. Biol.*, **4**, 140–147.

Wunsch, P., Herb, M., Wieland, H., Schiek, U.M. and Zumft, W.G. (2003) *J. Bacteriol.*, **185**, 887–896.

Zumft, W.G., Viebrock-Sambale, A. and Braun, C. (1990) *Eur. J. Biochem.*, **192**, 591–599.