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Letter to the Editor: Backbone NMR assignments of the metal-free UreE from *Bacillus pasteurii*

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Biological context

Urease is a nickel-containing enzyme that catalyzes the hydrolysis of urea. Increased pH arising from this reaction is critical to the virulence of several human and animal pathogens (Mobley and Hausinger, 1989). UreE, a urease accessory protein, transports nickel ions into the urease active site (Colpas and Hausinger, 2000). The crystal structure of the UreE from *Bacillus pasteurii* (*Bp*UreE) has been recently solved as a zinc-bound form, where the C-terminal residues containing the conserved histidines were not visible (Remaut et al., 2001). This crystal structure showed two forms of tetrameric states sharing a zinc ion in the dimer-dimer interface. However, it has been revealed that the tetrameric state of *Bp*UreE could be formed only with a highly concentrated sample in the presence of metal ions, and the physiologically relevant unit of *Bp*UreE in solution is a dimer (Ciurli et al., 2002). The *Bp*UreE dimer has a molecular mass of 34.8 kDa, and consists of two identical subunits, each 147 amino acids long. Recently, it has been revealed that the nickel binding increases the stability of *Bp*UreE, probably by altering the conformation or the dynamic property of the protein (Lee et al., 2002). However, the detailed picture of the nickel-binding effect remains obscure at the structural level, since the structure of both the metal-free and the nickel-bound *Bp*UreE is not available. In this report, we describe the complete backbone NMR assignments of the metal-free *Bp*UreE dimer. The results would provide fundamental information for further research to reveal the detailed molecular mechanism of the nickel-UreE interaction and the metal center assembly in the urease system.

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Methods and results

Triply labeled [90% ^{2}H, u-^{15}N/^{13}C]-*Bp*UreE was prepared from the overproducing *Eschelichia coli* strain DH5α containing the plasmid pKBurE (Lee et al., 2002). The cells were grown at 37 °C in M9 media supplemented with ^{13}C-glucose (2 g l^{-1}) and $^{15}NH_4Cl$ (1 g l^{-1}) in 90% D_2O/10% H_2O. The purification of BpUreE was performed as reported previously (Lee et al., 2002).

All of the NMR spectra were obtained with protein dissolved in 20 mM sodium phosphate buffer (pH 6.5) containing 0.5 M NaCl, 1 mM EDTA, and 1 mM NaN_3, on a Bruker DRX 600 and 800 spectrometers at 308 K. The following TROSY-based triple resonance spectra were recorded on the [90% ^{2}H, u-^{15}N/^{13}C]-*Bp*UreE: HNCA, HN(CO)CA, HN(CA)CB, HN(COCA)CB, HNCO, HN(CA)CO. 2D [^{1}H-^{15}N]HSQC and 3D ^{15}N-edited NOESY-HSQC spectra of the [90% ^{2}H, u-^{15}N]-*Bp*UreE were also obtained. All NMR spectra were processed and analyzed using the NMRPipe/NMRDraw software (Delaglio et al., 1995) and NMRView program (Johnson and Blevins, 1994).

We observed that the number of peaks in all of the spectra was in good agreement with that expected from the number of residues in one subunit of *Bp*UreE. Thus, our data demonstrate that the two subunits of

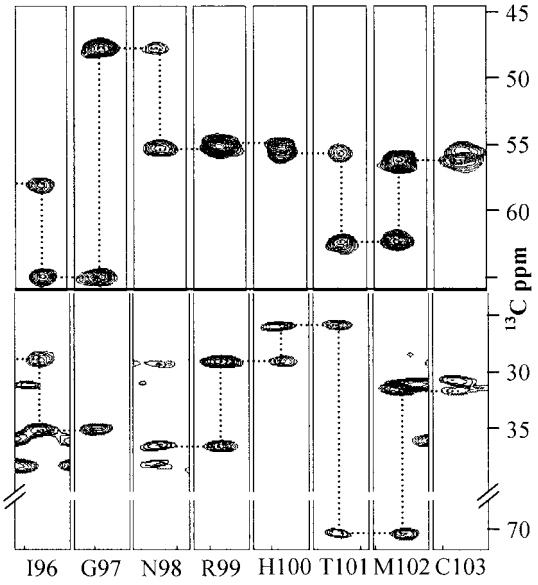

Figure 1. Sequential linking according to ^{13}C correlations. Selected strips from 3D TROSY-HNCA and TROSY-HN(CO)CA (upper panel), and those from 3D HN(CA)CB and HN(COCA)CB (lower panel) spectra were superimposed respectively. Dotted lines show the connectivities for the region I96 to C103.

the metal-free *Bp*UreE dimer in solution are symmetric. Sequential assignment was achieved by verifying and linking of peak clusters, as described by Won et al. (2000). The individual peak clusters could be sequentially linked according to the inter- and intra-residue correlations of ^{13}C ($^{13}C^{\alpha}$, $^{13}C^{\beta}$, ^{13}CO) chemical shifts, and sequential $^{1}H^{N}$ NOE connectivities in helical regions.

Generally, the high-affinity metal-binding to a protein such as the nickel-binding to *Bp*UreE (Lee et al., 2002) is expected to result in a severe broadening of the resonances from the binding residues. In the present work, as shown in Figure 1, the resonances from the His100 residue, which is conserved as a nickel-binding site (Remaut et al., 2001), was unambiguously assigned, indicating that our data were obtained for the protein in the metal-free state.

Extent of assignments and data deposition

Deuteration of *Bp*UreE combined with TROSY methodology was essential to overcome the high molecular weight (34.8 kDa) of the protein. Consequently, the backbone amide assignments ($^{1}H^{N}$, ^{15}N) were obtained for 142 of the 144 possible amide resonances (147 residues minus two prolines and the N-terminal residue), and complete assignments were achieved for ^{13}C nuclei ($^{13}C^{\alpha}$, $^{13}C^{\beta}$, and ^{13}CO). All of the assigned chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5484.

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