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Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C resonance assignment of the 23 kDa organomercurial lyase MerB in its free and mercury-bound forms

Paola Di Lello[a,b], Gregory C. Benison[a,c], James G. Omichinski[a,c,*] & Pascale Legault[a,*]
Departments of [a]Biochemistry and Molecular Biology and [c]Chemistry, University of Georgia, Athens, GA 30602, U.S.A.; [b]Dipartimento di Scienze Ambientali, Seconda Università di Napoli, Via Arena 22, 81100 Caserta, Italy

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Biological context

The toxicity of mercury is due to its great affinity for thiol groups in proteins (Clarkson, 1997). Organomercurial compounds, such as methylmercury (MeHg), are particularly toxic because they are hydrophobic and they efficiently permeate biological membranes (Shamoo, 1987). This enables them to bioaccumulate to high levels in the food chain. Bacteria isolated from mercury-contaminated sites have developed a detoxification system that allows them to survive in the presence of mercury-containing compounds. Their resistance is due to a series of proteins with specific roles in the detoxification pathway, which consists in transforming organomercurials and inorganic mercuric salts, into elemental mercury [Hg(0)] (Miller, 1999). Two enzymes, MerA and MerB, play key roles in this detoxification process. The organomercurial lyase MerB catalyzes the protonolysis of the carbon-mercury bond in organomercurials, resulting in the formation of ionic mercury [Hg(II)] and a reduced hydrocarbon (Begley et al., 1986). The ionic mercury is subsequently reduced to the less reactive elemental mercury by MerA, a specific mercuric reductase (Fox and Walsh, 1982). MerB does not show sequence homology with any known protein with an identified function (Pitts and Summers, 2002) and its three-dimensional structure is unknown. The elucidation of the solution structure of MerB can provide structural details and new insights in understanding the catalytic mechanism of this unique enzyme. Here we report the ^{1}H, ^{15}N and ^{13}C resonance assignment of free MerB and of the MerB/Hg/DTT complex.

*Present address and to whom correspondence should be addressed: Département de Biochimie, Université de Montréal, C.P. 6128 Succursale Centre-Ville, Montréal, Qc. Canada, H3C 3J7. E-mails: jg.omichinski@umontreal.ca; pascale.legault@umontreal.ca

Methods and experiments

The *merB* gene from *E. coli* (R831b) was cloned into a pET expression vector and overexpressed as the native protein in the host bacterial strain BL21(DE3) (Benison et al., in preparation). Uniformly (>98%) ^{15}N and ^{13}C/^{15}N-labeled MerB were obtained by growing the host cells in modified M9 minimal media. Uniformly (>98%) ^{13}C/^{15}N-labeled, partially (70%) ^{2}H-labeled MerB was obtained in the same way, except that the growth media contained 70% D_2O, 30% H_2O. The purification protocol for MerB will be provided elsewhere (Benison et al., in preparation). Samples for NMR studies contained 1.0–1.5 mM of MerB in NMR buffer (10 mM sodium phosphate buffer pH = 7.5, 10 mM NaCl, 7.5 mM DTT and 1 mM EDTA) in either 90% H_2O/10% D_2O or 99.9% D_2O. NMR samples of the MerB/Hg/DTT complex were prepared by titrating one equivalent of mersalyl [3-(hydroxymercuri)-2-methoxipropyl carbonyl phenoxyacetic acid], into a MerB sample containing L-DTT.

All the NMR spectra were collected at 300 K on Varian Unity^{Inova} 600 MHz and 800 MHz spectrometers. The backbone and side chains resonances of MerB were assigned using a combination of experiments, including 2D ^{1}H-^{15}N HSQC, 2D ^{1}H-^{13}C CT-HSQC, 3D HNCO, 3D HNCACB, 3D (HB)CBCA(CO)NNH, 3D (H)C(CO)NNH, 3D and 4D H(CCO)NNH, 3D HCCH-COSY, 3D HCCH-TOCSY, 3D HNHA, 3D ^{15}N-edited TOCSY-HSQC, 3D ^{15}N-edited NOESY-HSQC, 3D ^{13}C-edited HMQC-NOESY, 3D CT-HNCA (Yamazaki et al., 1994b), 3D CT-HN(CO)CA

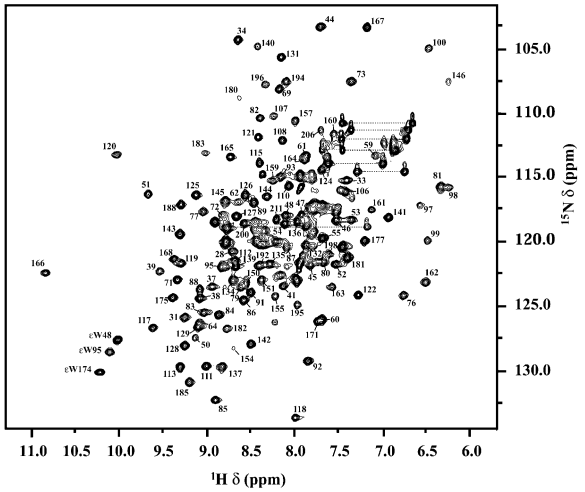

Figure 1. Two-dimensional ^{1}H-^{15}N HSQC spectrum of uniformly ^{15}N-labeled MerB in NMR buffer at 300 K and pH = 7.5. For clarity, the assignment of several residues has been omitted.

(Yamazaki et al., 1994a), 3D H(CC-CO)NH-TOCSY (Lin and Wagner, 1999), 2D (Hβ)Cβ(CγCδ)Hδ and 2D (Hβ)Cβ(CγCδCε)Hε. Chemical shifts of all proton, carbon and nitrogen nuclei were referenced externally to that of DSS at 0 ppm. NMR data were processed using the NMRPipe/NMRDraw package and analyzed with PIPP and NMRView.

Extent of assigments and data deposition

The 2D ^{1}H-^{15}N HSQC spectrum of free MerB is characterized by very good chemical shift dispersion both in the ^{1}H and ^{15}N chemical shift dimensions (Figure 1). The first 25 amino acids at the amino terminus of MerB were not observed in the 2D ^{1}H-^{15}N HSQC spectrum (Figure 1), probably due to the rapid amide proton exchange at pH 7.5 and/or line broadening. For the ^{1}H, ^{15}N and ^{13}C backbone nuclei of the remaining 187 residues (26–212), nearly complete assignment was achieved: 93% of the $^{13}C^{\alpha}$, 88% of the $^{1}H^{\alpha}$, 92% of the $^{1}H^{N}$, 87% of the ^{15}N, and 87% of the $^{13}C'$ resonances were assigned. For residues 26-212 of free MerB, the extent of the assignment for the aliphatic and aromatic side chains (^{1}H-bound ^{13}C, ^{1}H-bound ^{15}N and ^{1}H nuclei, except for ^{1}H-^{15}N and ^{15}N of Lys and Arg) was 68%: 66% of the side chains were completely assigned; 27% were partially assigned, and 7% were unassigned.

As was the case for free MerB, the 2D ^{1}H-^{15}N HSQC spectrum of the MerB/Hg/DTT complex was characterized by very good dispersion (data not shown). Only a few amide signals were observed in this spectrum for the first 25 amino acids. For the remainder of the protein (residues 26–212), a nearly complete assignment was achieved for the backbone ^{1}H, ^{13}C and ^{15}N resonances of the MerB/Hg/DTT complex: 98% of the $^{13}C^{\alpha}$, 95% of the $^{1}H^{\alpha}$, 97% of the $^{1}H^{N}$, 92% of the ^{15}N, and 89% of the $^{13}C'$ were assigned. For residues 26-212 of the MerB/Hg/DTT complex, the extent of the assignment for the aliphatic and aromatic side chains was 68%: 68% of the side chains were completely assigned; 29% were partially assigned, and 3% were unassigned.

It is important to note that some assignments are missing for free MerB and the MerB/Hg/DTT complex due to the absence of signals in various spectra, likely as a result of conformational heterogeneity. ^{15}N relaxation studies of free MerB and of the MerB/Hg/DTT complex have indicated higher backbone flexibity for regions defined by residues 146–161, residues 178–184 and for residues 205–212 (not shown), and these regions are poorly defined in our initial structure calculations.

The ^{1}H, ^{13}C and ^{15}N chemical shift assignments for free MerB (accession number #6047) and for the MerB/Hg/DTT complex (accession number #6055) have been deposited in the BioMagnResBank (www.bmrb.wisc.edu).

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