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Letter to the Editor: Backbone ^{1}H, ^{13}C, and ^{15}N resonance assignments for a 29 kD monomeric variant of *Pseudomonas aeruginosa* dimethylarginine dimethylaminohydrolase

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Biological context

The enzyme dimethylarginine dimethylaminohydrolase (DDAH) is responsible for the metabolism of asymmetric methylarginine residues (AMAs) to citrulline and mono- or di-methylamines. Free methylarginines occur as a result of post-translational modification of proteins, with release as metabolites in the cell cytosol, plasma and tissues as a result of protein turnover. In mammals, the asymmetric methylarginines, N^{η},N^{η}-dimethylarginine (ADMA) and N^{η}-methylarginine (L-NMMA), but not symmetric N^{η},N'^{η}-dimethylarginine (SDMA), are endogenous inhibitors of all nitric oxide synthase (NOS) isoforms (Leiper and Vallance, 1999). By controlling local levels of AMAs, DDAH activity is implicated in the regulation of nitric oxide (NO) production, which gives rise to interest in the therapeutic potential of the modulation of DDAH activity (MacAllister et al., 1996). High levels of NO are often reported in septic shock, neurodegenerative diseases and certain inflammatory conditions. In some instances the blockade of overproduction of NO, generally associated to the expression of inducible NOS (iNOS), is of pharmacological interest (Vallance and Leiper, 2002). NO is a versatile signalling molecule involved in many physiological processes, and in view of the systemic physiological effects of NO in mammals, a major pharmaceutical objective is to obtain isoform-specific NOS inhibitors.

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A potential alternative approach to inhibit excess NO production is to target the activity of DDAH. Two isoforms of mammalian DDAH have been identified: DDAH I, predominantly expressed in neuronal tissue, and DDAH II, expressed in vascular tissue (Tram et al., 2000). Although the inhibition of one DDAH isoenzyme will not inhibit a specific NOS isoform, it might limit NO synthesis in a tissue-specific manner. As part of ongoing efforts to derive structural information for DDAH, the crystal structure of the more tractable bacterial *Pseudomonas aeruginosa* DDAH (*Pa*DDAH) homologue has recently been solved (Murray-Rust et al., 2001). The structure revealed that *Pa*DDAH exists as a 58 kD homodimer with each protomer adopting a β/α-propeller fold. In addition to the crystallographic studies, NMR methodology has been applied to further investigate the enzyme's active site dynamics and to better understand the ligand and inhibitor binding profiles. We report here, the backbone NMR resonance assignments of the exclusively monomeric (29 kD) double mutant (R40E-R98H) of *Pa*DDAH, recently characterised by our group (Plevin et al., 2004). These data for the bacterial enzyme provide a basis for future NMR-based investigations of DDAH ligand and inhibitor design.

Material and experiments

Sample preparation

Uniformly [^{2}H, ^{13}C, ^{15}N]-labelled R40E-R98H *Pa*DDAH was over-expressed in BL21(DE3) *E. coli*

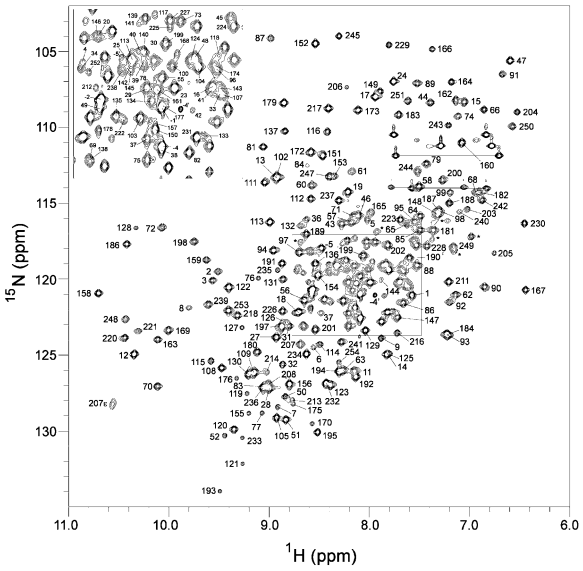

Figure 1. Assigned 2D ^{13}C-decoupled ^{1}H-^{15}N HSQC spectrum of [^{2}H,^{15}N,^{13}C]-labelled R40E-R98H *Pa*DDAH recorded on a 500 MHz Varian UNITYplus spectrometer at 298 K. Cross-peaks assigned by an asterix (*) are aliased arginine and lysine side-chain amide resonances.

competent cells grown in M9 minimal media, containing 1.0 g/L $(^{15}NH_4)_2SO_4$, and 2.0 g/L $^{13}C_6$-glucose (M.J. Plevin et al., 2004, submitted). Purification of *Pa*DDAH was achieved using nickel ion affinity chromatography, followed by cleavage of the polyhistidine tag with the recombinant tobacco etch virus (rTEV) protease and a final size exclusion chromatography step. R40E-R98H *Pa*DDAH NMR sample was prepared at 1.5 mM concentration in 20 mM phosphate buffer (pH 7.0), 100 mM NaCl, and 9:1 H_2O/D_2O.

NMR spectroscopy

NMR spectra were acquired at 298 K on Varian UNITYplus spectrometer (operating at a nominal ^{1}H frequency of 500 MHz) equipped with a triple resonance (^{1}H, ^{13}C, ^{15}N) probe including Z-axis pulse field gradients. Sequence-specific resonance assignments were obtained by combining the data from the following 3D deuterium decoupled, gradient sensitivity enhanced triple resonance experiments: HNCO, HNCA, HN(CO)CA, HN(CA)CB and HN(COCA)CB (Yamazaki et al., 1994). All spectra were processed using NMRpipe/NMRDraw (Delaglio et al., 1995) and analyzed using ANSIG v3.3 (Kraulis et al., 1994). Chemical shifts were indirectly referenced to DSS.

Extent of assignments and data deposition

Analysis of the triple resonance experiments allowed identification and the sequence-specific assignments for 239 out of the 241 R40E-R98H *Pa*DDAH (254 less 13 prolines) backbone ^{15}N and amide proton resonances. Definitive assignments have not been obtained for Ser-21 and His-22. Figure 1 shows an assigned 2D ^{13}C-decoupled ^{1}H-^{15}N HSQC spectrum of [^{2}H, ^{13}C, ^{15}N]-labelled R40E-R98H *Pa*DDAH at pH 7.0, recorded at a ^{1}H frequency of 500 MHz. From the assigned amide resonances, we were able to obtain 99, 99, and 93% of Cα, Cβ and CO chemical shifts, respectively. All of the backbone resonances for the active site catalytic triad, composed of residues Glu-114, His-162 and Cys-249, have been unambiguously assigned. Further NMR investigations will therefore provide a better understanding of *Pa*DDAH molecular dynamics upon ligand binding. The backbone ^{1}H, ^{13}C, and ^{15}N chemical shifts of R40E-R98H *Pa*DDAH have been deposited at BioMagResBank under accession number 6074.

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