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Letter to the Editor: Backbone (^{1}H, ^{15}N, ^{13}C) resonance assignments of a 21 kDa construct of *S. aureus* peptide deformylase

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Biological context

Mankind has come to a critical point with regard to bacterial infections. The emergence of new strains of antibiotic-resistant bacteria makes the current battery of antibiotics unsuccessful in the treatment of infections caused by these bacteria. Thus new classes of antibacterial agents must be found to relieve the pressure brought on by the resistant bacteria e.g., antibiotic-resistant *Staphylococcus aureus* related infections. *S. aureus* is the cause of many life threatening hospital infections including skin lesions, deep-seated skin infections and pneumonia.

Three areas of cellular functions have been targeted by antibiotics, and these include cell-wall biosynthesis, DNA replication and protein synthesis. In prokaryotes, protein synthesis begins with a formylated methionine, N-formylmethionine (fMet) (Kozak, 1983). After translation initiation, the formyl group is removed by peptide deformylase (Takeda et al., 1968). The deformylation process is necessary to produce the final mature protein and essential for bacterial survival (Mazel et al., 1994). However, fMet is not used in translation initiation in cytoplasmic protein synthesis in eukaryotic cells. For this reason potent inhibitors of *S. aureus* peptide deformylase (pdf) could selectively block growth of bacterial cells with the benefit of low toxicity in humans. This makes pdf an attractive target for structure-based drug design of potent inhibitors once the NMR resonance assignments are made and the secondary and tertiary structure is determined. Peptide deformylase is a metallopeptidase which uses iron as a catalytic metal for amide hydrolysis (Rajagopalan et al., 1997). Studies on other replacement metals (zinc, nickel or cobalt) give stable variants that have partial or full catalytic activity. Peptide deformylase from known strains of bacteria contains the HEXXH motif of the zinc metalloproteases superfamily. The X-ray and NMR structures of *E. coli* pdf have been reported and the results show that there are no significant differences between the structures with each replacement metal (Dardel et al., 1998; Hao et al., 1999). The *S. aureus* pdf construct used in this study has little sequence homology (<33%) to the *E. coli* enzyme with the exception of the catalytic site residues, G58–G60, E109–S113, H154-H159 (HEXXH), which are completely conserved. The length of our pdf construct was 189 amino acids including a hexa-His C-terminal tag. Here we report the backbone ^{1}H, ^{15}N and $^{13}C_{\alpha}$, $^{13}C_{\beta}$ and $^{13}C'$ assignments of the zinc form of *S. aureus* peptide deformylase.

Methods and experiments

S. aureus pdf was expressed in *E. coli* K12 using M9 glucose medium containing thiamin, $ZnCl_2$ and ampicillin. For labeled (^{15}N or ^{15}N/^{13}C) protein M9 was prepared using $^{13}C_6$-glucose and $^{15}NH_4Cl$ as the sole carbon and nitrogen sources. The affinity tagged protein was purified with a Ni-NTA resin, ultrafiltration, anion exchange and column chromatography. No modification of the purification was made for labeled protein.

All NMR spectra were recorded on a Bruker DRX-600 at a temperature of 37 °C. NMR samples (un-

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labeled, ^{15}N labeled and ^{13}C,^{15}N labeled) contained 0.3–1.0 mM protein, 20 mM K-phosphate buffer, 0.02% NaN_3 in 93% H_2O and 7% D_2O at a pH of 7.4. Typical NMR acquisition parameters (C is complex points) for 2D-HQSC were ^{15}N, ^{1}H ($400^{C} \times 512^{C}$) and 3D experiments (HNCA, HNCOCA, HNCO) were ^{13}C, ^{15}N, ^{1}H ($40^{C} \times 46^{C} \times 512^{C}$) and (HNCACB, CBCACONH, HBHACONH) were ($66^{C} \times 44^{C} \times 512^{C}$). ^{1}H chemical shifts were referenced to DSS at 0 ppm and ^{13}C and ^{15}N chemical shifts were referenced using chemical shift referencing ratios, where $\gamma_C/\gamma_H = 0.251449530$ and $\gamma_N/\gamma_H = 0.101329118$ (Wishart et al., 1995). All of the NMR data was processed with NMRPipe and visualized/analyzed with NMRDraw (Delaglio et al., 1995). Some semi-automatic analysis was done using Felix Assign97 from Molecular Simulations, Inc. (MSI).

Sequential assignments for ^{1}HN, ^{15}N, $^{13}C_\alpha$ and $^{13}C_\beta$ were derived from HNCA, HNCOCA, HNCACB and CBCACONH experiments; H_α and H_β assignments were derived from HBHACONH and $^{13}C'$ from the HNCO experiment. The HNCA, HNCOCA and HNCO experiments used in this study were recent versions of gradient-enhanced pulse sequences (Kay et al., 1992). During the analysis (HNCA, HNCOCA) of the sequential connectivity of many ^{13}C resonances it became clear that there were multiple correlations that were difficult to assign because of chemical shift redundancy. In many cases the frequency of the $^{13}C_\beta$ chemical shift (HNCACB, CBCACONH) confirmed the correct assignment. When this was not the case, each correlation was explored with respect to the possible $^{13}C_\alpha$ and $^{13}C_\beta$ (i$-$2) and (i$+$1) residues to correctly locate a particular residue with respect to its sequence in the protein. After locating these particular residues the assignment process for pdf proceeded well until about 50% of the backbone resonances were identified, with another 30% requiring much more effort (analyzing adjacent i$-$1, i$-$2, i$-$3, i$+$2 and i$+$3 residues) because of resonance overlap or very weak correlations. The final $\sim$17% were assigned with the help of Felix Assign97 from MSI.

Extent of assignments and data deposition

The quality of the data is shown in Figure 1, which depicts a strip plot of the HNCACB and CBCACONH for residues 129–136 of *S. aureus* pdf. The residues of the C-terminal hexa-histidine tag were not observed in the data, thus the assignments pertain to residues 1–183 of pdf. Total assignments determined were 96% of ^{1}HN, 96% of ^{15}N, 98% of $^{13}C_\alpha$, 98% of $^{13}C_\beta$, 93% of H_α, 93% of H_β, and 95% of $^{13}C'$. Data from the 3D ^{15}N-NOESY-HSQC were used to verify and substantiate the assignments made from the triple-resonance J-coupling based experiments.

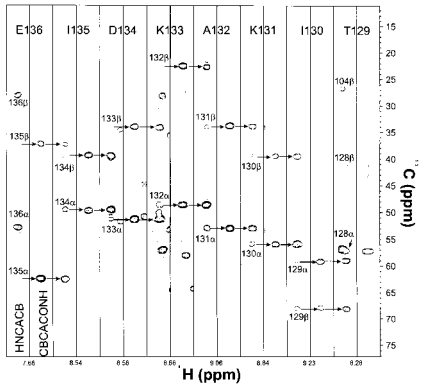

Figure 1. A strip plot depicting the quality of the triple resonance data showing the connectivity between the HNCACB and CBCACONH experiments for residues 129–136.

The ^{1}H, ^{15}N and ^{13}C chemical shift assignments for *S. aureus* peptide deformylase have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4834.

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