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Letter to the Editor: Backbone NMR assignment and secondary structure of the 19 kDa hemophore HasA

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Biological context

HasA is secreted under iron-deficiency conditions by the Gram-negative bacteria *Serratia marcescens* considered as an opportunist pathogen. It is able to bind free heme as well as to take it up from hemoglobin and to transfer it to a specific outer membrane receptor HasR, which, in turn, delivers it to the bacteria. HasA thus allows the bacteria to grow in the absence of any other source of iron as host hemoglobin (Létoffé et al., 1994). HasA was the first hemophore (extracellular heme-binding protein acting as a heme carrier) to be identified. It is a monomer of 19 kDa (187 residues) which binds heme with a stoichiometry of 1 and with a very high affinity ($K_d < 10^{-8}$ M). It is secreted by a signal peptide-independent pathway which involves a C-terminal secretion signal and an ABC (ATP Binding Cassette) transporter. HasA does not present any sequence similarity with other known proteins, except with three proteins recently identified in *Pseudomonas aeruginosa* (HasAp), *Pseudomonas fluorescens* (HasApf) and *Yersinia pestis*. The structure determination of HasA, in both apo and hemo forms, will provide information for a better understanding of the heme uptake and release mechanisms of this new family of proteins. As the first stage of the study, we report here the ^{1}H, ^{15}N and ^{13}C sequence-specific resonance assignment and the secondary structure of the apoprotein.

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Methods and results

HasA was expressed in *E. coli* strain Pop3 (*araD139* Δ*lac-169 rpsL relA thi*) transformed with plasmid pSYC34(pAM238). Bacteria were grown to $A_{600} = 2.2$ in a 1 L bioreactor at 30 °C. Uniformly labelled samples were produced using M9 minimal medium containing 1 g/L $^{15}NH_4Cl$ and 2 g/L ^{13}C glycerol as the sole nitrogen and carbon sources, respectively, and complemented with 1 mg/L thiamine. Sample specifically ^{15}N labelled at Leu, Ile and Val was obtained in M9 minimal medium complemented with 0.2 g/L ^{15}N-Leu. The unexpected labelling of Ile and Val residues, checked by MALDI-TOF spectrometry, was due to the lack of specificity of the *E. coli* transaminase. Proteins were purified as described earlier (Izadi et al., 1997), after cleavage of the last nine C-terminal residues (Izadi-Pruneyre et al., 1999). NMR samples were about 2 mM in 10 mM sodium phosphate buffer, pH 5.6, in H_2O/D_2O (90/10 v/v).

All NMR spectra were recorded at 30 °C on a Varian Unity 500 spectrometer equipped with a triple resonance z-gradient probe. Resonances of Leu, Ile and Val residues were assigned from ^{15}N HSQC and ^{15}N-edited TOCSY-HSQC experiments using the specifically labelled sample. The sequence-specific backbone assignment was based on 3D HNCACB and 3D CBCA(CO)NH (Muhandiram and Kay, 1994). CO chemical shifts were obtained from the 3D HNCO data (Muhandiram and Kay, 1994). H_α were assigned using the ^{15}N-edited TOCSY-HSQC experiment (Zhang et al., 1994). Short- and medium-range NOE interactions were obtained from a ^{15}N-edited NOESY-HSQC

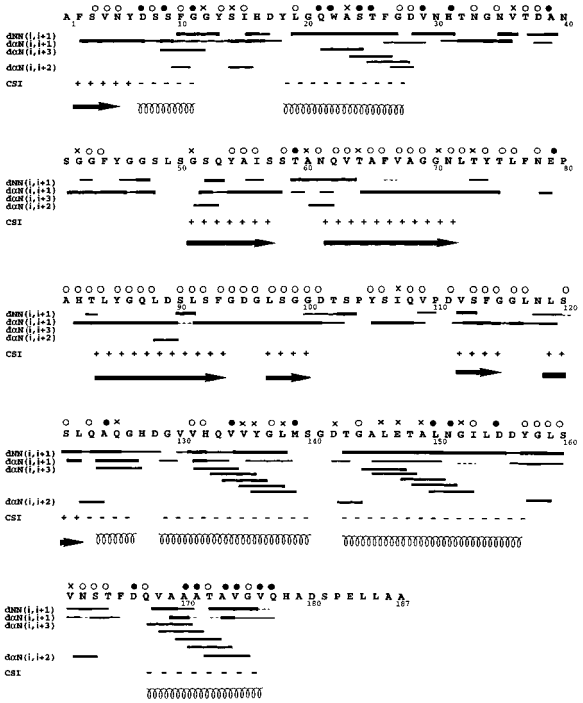

Figure 1. Primary sequence of HasA with a summary of observed short- and medium-range NOEs, CSI data and data on the $^{3}J_{NH-H\alpha}$ coupling constants. NOE intensities are categorised as either strong, medium or weak and shown accordingly by the thickness of the lines. The consensus CSI data obtained from H_α, $^{13}C_\alpha$ and ^{13}CO chemical shift data are represented by + and − signs which represent the consensus positive and negative deviations of the chemical shifts from random coil values observed in α-helices and β-strands, respectively. $^{3}J_{NH-H\alpha}$ are depicted as: open circles, $^{3}J_{NH-H\alpha}$ >8 Hz; filled circles, $^{3}J_{NH-H\alpha} < 6.5$ Hz; x, intermediate values. β-strands are represented by arrows and α-helices by loops.

experiment recorded with mixing times of 40 ms (Zhang et al., 1994). $^{3}J_{NH-H\alpha}$ coupling constants were evaluated from the HMQC-J spectrum (Kay and Bax, 1990; Wishart and Wang, 1998). All experimental data acquisition parameters are available as Supplementary Material from the authors. Chemical shifts were measured relative to external TSP for ^{1}H and calculated assuming $\gamma N/\gamma H = 0.101329118$ and $\gamma C/\gamma H = 0.251449530$. Data were transformed using the VNMR software and analysed with the program XEASY (Bartels et al., 1995) on a Silicon Graphics INDY workstation. All 3D data sets were zero-filled in the three dimensions and linear-predicted in the ^{15}N dimension.

The summary of the secondary structure elements identified in HasA is presented in Figure 1. Six α-helices and seven β-strands are observed. Characteristic NOEs, consensus of chemical shift index (CSI) data for H_α, C_α and CO (Wishart and Sykes, 1994) and data on the $^{3}J_{NH-H\alpha}$ coupling constants used to determine these elements are shown in the figure.

Extent of assignments and data deposition

Backbone amide assignments ($^{1}H^{N}$, ^{15}N) were obtained for all amino acids with the exception of Y18, S50, F77, and the proline residues P80, P104 and P110. A large percentage of the resonances was assigned for other nuclei: 97% for C_α, 92% for CO and 96% for H_α. The ^{1}H, ^{15}N and ^{13}C chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4309.

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