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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments for the Gallium protoporphyrin IX-$HasA_{sm}$ hemophore complex

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Biological context

HasA hemophores represent a new class of bacterial extracellular proteins which now contains four members, identified in *Serratia marcescens*, *Pseudomonas. aeruginosa*, *Pseudomonas fluorescens* and *Yersinia pestis*. The role of these proteins is to take up free or hemoprotein bound heme and to shuttle it back to a specific outer membrane receptor HasR. The *Serratia marcescens* $HasA_{sm}$ hemophore is a monomer of 19 kDa (187 residues) which binds b heme with a stoechiometry of one and with a very high affinity ($Ka > 10^8\ M^{-1}$, Izadi et al., 1997). Crystal structure of its holo-form reveals an original α/β fold and an unusual pattern of b heme ligation with a His/Tyr residues pair as iron ligands. Heme is maintained by two long loops and is very exposed to solvent (Arnoux et al., 1999). Because flexibility of the binding site might play an important role in the heme uptake and release mechanisms, the present study was undertaken to study the dynamics of both free and complexed $HasA_{sm}$. Heme iron within holo-$HasA_{sm}$ is in a low spin ferric state and its very low redox potential value precludes its reduction in aerobic conditions. Therefore, the complexed form of $HasA_{sm}$ was made up with a diamagnetic metallo-porphyrin, the Gallium protoporphyrin IX (GaPPIX), to prevent from resonance shifts and from relaxation effects induced by paramagnetism. Backbone resonance assignments for apo-$HasA_{sm}$ has been already given (Izadi-Pruneyre et al., 1999a). Complete ^{1}H, ^{15}N and ^{13}C backbone and $^{13}C_{\beta}$ resonance assignments for the GaPPIX loaded hemophore are reported here.

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Methods and experiments

$HasA_{sm}$ was expressed in *E. coli* strain Pop3 (*araD139* Δ*lac-169 rpsL relA thi*) transformed with plasmid pSYC34 (pAM238) (Létoffé et al., 1994). ^{15}N-^{13}C uniformly labelled apo-$HasA_{sm}$ was 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. Protein was purified as described earlier after cleavage of the last nine C-terminal residues (Izadi-Pruneyre et al., 1999b). GaPPIX-$HasA_{sm}$ complex was made up by adding saturating amounts of GaPPIX to apo-$HasA_{sm}$ as previously described (Wolff et al, submitted for publication). NMR sample was 2 mM in 20 mM sodium phosphate buffer pH 5.6 in H20/D20 (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. Chemical shifts were referenced directly/indirectly from the proton frequency of the DSS resonance at 0.00 ppm. Data were processed using NMRpipe software (Delaglio et al., 1995) and analysed with the program XEASY (Bartels et al., 1995) on silicon graphics INDY workstation. The following NMR experiments were performed: 2D ^{15}N TROSY-HSQC and 3D TROSY-HNCACB, TROSY-CBCA(CO)NH, TROSY-HNCO, HNHA and HBCBCA(CO)HA. The pulse sequences of experiments were taken as implemented from the Varian

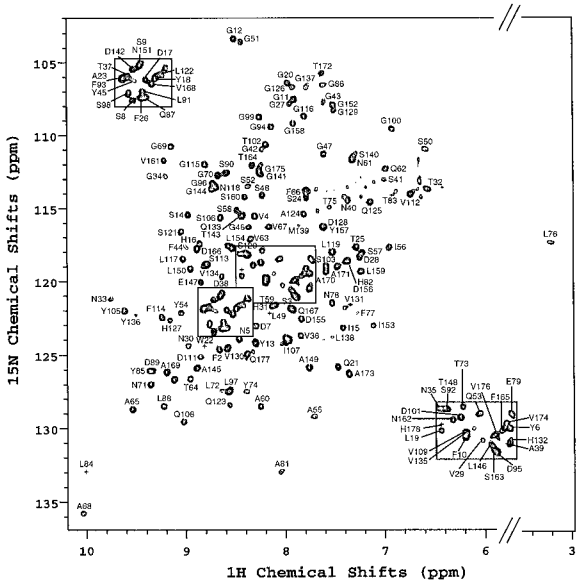

Figure 1. ^{1}H-^{15}N TROSY-HSQC spectrum of GaPPIX ^{15}N-^{13}C uniformly labeled $HasA_{sm}$ complex (^{1}H resonance frequency 500 MHz, 30 °C, pH 5.6).

proteinpack (http://www.varianinc.com), except for the HBCBCA(CO)HA.

Sequence specific assignments were greatly facilitated by the previously reported assignments of the apo-protein backbone resonances: 70% of the $^{1}H_N$, ^{15}N and ^{13}CO resonances could be assigned by the direct comparison of the HNCO spectra of both apo and complexed forms of $HasA_{sm}$. Other experiments allowed to confirm and complete the GaPPIX-$HasA_{sm}$ backbone resonance assignments.

Extent of assignments and data deposition

All ^{1}H, ^{15}N and ^{13}C backbone and $^{13}C_\beta$ resonances of $HasA_{sm}$-GaPPIX complex have been assigned, with the exception of the backbone amide resonances of Ala1, the H_α resonance of Gly95 and all resonances of His178. Due to the protoporphyrin ring current effects, unusual chemical shift values are observed such as the 3.34 ppm for the Leu76 amide proton, as seen in the ^{1}H-^{15}N HSQC spectrum (Figure 1). Minor peaks were observed for most of the binding pocket residues, likely corresponding to a second orientation of the metallo-protoporphyrin IX, as already observed in most myoglobines, hemoglobines and other metalloproteins and in agreement with the crystal structure of the heme-$HasA_{sm}$ complex (Arnoux et al., 2000). The assignments corresponding to the major form for residues in the binding pocket have been deposited in BioMagRes Bank (http://www.bmrb.wisc.edu) under accession number 5081.

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References

Arnoux, P., Haser, R., Izadi, N., Lecroisey, A., Delepierre, M., Wandersman, C. and Czjzek, M. (1999) *Nat. Struct. Biol.*, **6**, 516–520.

Arnoux, P., Haser, R., Izadi-Pruneyre, N., Lecroisey, A. and Czjzek, M. (2000) *Proteins*, **41**, 202–210.

Bartels, C., Xia, T., Billeter, M., Güntert, P. and Wüthrich, K. (1995) *J. Biomol. NMR,* **6**, 1–10.

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Izadi, N., Henry, Y., Haladjian, J., Goldberg, M.E., Wandersman, C., Delepierre, M. and Lecroisey, A. (1997) *Biochemistry,* **36**, 7050–7057.

Izadi-Pruneyre, N., Wolff, N., Castagné, C., Czisch, M., Wandersman, C., Delepierre, M. and Lecroisey, A. (1999a) *J. Biomol. NMR*, **14**, 193–194.

Izadi-Pruneyre, N., Wolff, N., Redeker, V., Wandersman, C., Delepierre, M. and Lecroisey, A. (1999b) *Eur. J. Biochem.*, **261**, 562–568.

Kay, L.E. (1993) *J. Am. Chem. Soc.*, **115**, 2055–2057.

Létoffé, S., Ghigo, J.M. and Wandersman, C. (1994) *Proc. Natl. Acad. Sci. USA.*, **91**, 9876–9880.