Journal of Biomolecular NMR, **23:** 71–72, 2002.
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Letter to the Editor: Assignment of the ^{1}H, ^{13}C, and ^{15}N signals of *Synechocystis* sp. PCC 6803 methemoglobin

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Received 4 February 2002; Accepted 5 March 2002

Key words: hemoglobin, low-spin heme, NMR resonance assignments, *Synechocystis*

Biological context

The unicellular cyanobacterium *Synechocystis* sp. PCC 6803 is extensively used in mechanistic and regulatory investigations of oxygen-producing photosynthesis. The sequence of the *Synechocystis* genome became available in 1996 (Kaneko et al., 1996) and among the three thousand potential protein-coding genes, a single copy of a hemoglobin gene was identified. The corresponding primary structure contains only 124 amino acids, some thirty fewer than in vertebrate β globins. *Synechocystis* hemoglobin is related to other prokaryotic (e.g., strains of *Mycobacterium*, *Legionella*, and *Campylobacter*) globins and unicellular eukaryotic (e.g., *Paramecium, Chlamydomonas*, and *Tetrahymena*) globins. These proteins, referred to as truncated hemoglobins (trHbs), share a low level of sequence identity and are thought to play distinct roles within their source organisms. Among putative functions are O_2 sensing, O_2 scavenging, NO detoxification, and participation in terminal oxidase complexes (Wittenberg et al., 2002).

In native vertebrate globins, the octahedral ferrous ion is coordinated by the four nitrogens of the porphyrin ring and the 'proximal' histidine. The sixth site is available for ligand binding. In contrast, several trHbs exhibit a propensity for heme ligation with two amino acids, one of which has to be displaced upon O_2 binding. This displacement step may serve to modulate ligand affinity and cause conformational changes of functional utility. Hexacoordinate trHbs are therefore expected to reveal unique ways in which the globin fold supports and controls heme reactivity. In order to guide the *in vivo* study of *Synechocystis* hemoglobin and determine the extent of structural rearrangement due to ligand binding, work is in progress to solve the structure of the protein in the hexacoordinate state and the CO-bound state.

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Methods and experiments

The gene for *Synechocystis* hemoglobin (slr2097) was cloned, and its product over-expressed in *E. coli*. The purification protocol, which includes urea unfolding of the protein packed in inclusion bodies, refolding during gel filtration chromatography, addition of ferric heme, and anion exchange chromatography, has been published elsewhere (Scott and Lecomte 2000; Lecomte et al., 2001).

NMR samples contained 0.6 to 2 mM protein on a heme basis in 95% H_2O/5% D_2O, buffered at pH 7.5 with 20 mM phosphate. Data sets were also collected in D_2O with the same buffer. The NMR spectra of this ferric form (methemoglobin) are typical of a low-spin ($S = \frac{1}{2}$) complex. The overall appearance is similar to that of the oxidized state of the heme-containing domain of cytochrome b_5. The protein ligands to the heme were identified as His46 and His70 in the wild-type protein by applying NMR methods suitable to low-spin heme proteins (Lecomte et al., 2001). In this letter, we report the ^{1}H, ^{13}C and ^{15}N assignments for the remainder of the protein.

NMR spectra were obtained at 25 °C on a Bruker DRX-600 spectrometer (14.1 T, operating at a ^{1}H frequency of 600.13 MHz). The ^{1}H spectra were referenced to the water resonance at 4.76 ppm; ^{13}C signals were referenced to internal dioxane at 67.8 ppm and ^{15}N signals were referenced indirectly to the proton

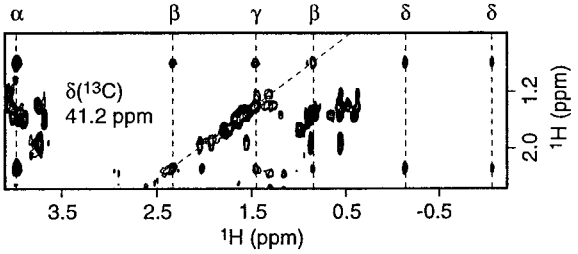

Figure 1. A plane of the HCCH-TOCSY data collected on ferric *Synechocystis* sp PCC 6803 hemoglobin. The ^{13}C chemical shift is that of Leu73 Cβ. Leu73 is within 10 Å of the iron ion and is nevertheless entirely detected.

frequency (Live et al., 1984). Data sets used for assignments included: homonuclear experiments (2QF-COSY, 2Q, TOCSY, and NOESY) and heteronuclear experiments (^{1}H-^{15}N HMQC, ^{1}H-^{13}C HMQC, HCCH-TOCSY, HCCH-COSY, HCC(CO)NH, ^{1}H-^{13}C HSQC-NOESY, HNCA, HNCO, HN(CA)CO, CBCA(CO)NH, CBCANH, ^{1}H-^{15}N TOCSY-HSQC and ^{1}H-^{15}N NOESY-HSQC). Solvent suppression was achieved with a WATERGATE scheme (Piotto et al., 1992) where necessary. Experimental description (Cavanagh et al., 1996) and parameters (Lecomte et al., 2001) are available elsewhere. Data sets were processed using FELIX 97.0 (Molecular Simulations Inc., San Diego, CA) or NMRPipe (Delaglio et al., 1995).

Extent of assignments and data deposition

In *E. coli*, Met1 is efficiently cleaved and Ser2 is the N-terminal amino acid. All of the backbone peptide ^{1}H and ^{15}N resonances were assigned, except for the ^{15}N of Thr3. All of the ^{1}Hα and ^{13}Cα were obtained, and most (97%) of the backbone ^{13}CO. Side chain assignments of non-exchangeable ^{1}H and ^{13}C were better than 80% complete; the missing information pertained primarily to Arg, Lys, degenerate methylene groups, and quaternary carbons. Several aromatic ring ^{13}C signals were assigned with an HMQC experiment where resolution in the ^{1}H and ^{13}C dimensions allowed.

Line broadening in the N-terminal 3 residues indicated conformational exchange on an intermediate time scale. The protein contains four histidines not coordinated to the iron (His33, His77, His83, and His117). The ^{1}H-^{13}C HMQC experiment, performed on a uniformly ^{15}N-^{13}C-labeled sample, distinguished unambiguously the Cδ2H and Cε1H signals of these four residues. At basic pH, these side chains were found predominately in the Nε2H tautomeric state. Overall the perturbations introduced by the paramagnetic center were moderate and did not interfere with the assignment process. Figure 1 illustrates the quality of the ^{13}C data for a residue located in dipolar contact with the paramagnetic heme group. Some of the largest chemical shift deviations from statistical values could be attributed to H-bonding (amide H of Asn80 shifted downfield by H-bonding to His83 Nδ1) or ring current effects ($C\beta H_3$ of Ala69 shifted upfield by Tyr65). The assignments are sufficient to determine the structure of the protein and to analyze the magnetic properties of the iron ion in the bis-histidine ferric state. These studies are in progress. Partial identification of heme resonances has been published (Lecomte et al., 2001). Protein ^{1}H, ^{13}C and ^{15}N chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number BMRB-5269.

Acknowledgements

This work was supported in part by National Institutes of Health Grant GM-54217 and in part by National Science Foundation Grant MCB-91182. An ^{15}N-separated NOESY spectrum was acquired at 800 MHz on a Varian INOVA spectrometer at the Yale NMR facility and used for results confirmation. The authors thank Dr Melanie Cocco for collecting the data. The authors also thank Ms Nancy Scott and Ms Christie Vu for protein preparation.

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