Journal of Biomolecular NMR, **18:** 285–286, 2000.
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Letter to the Editor: Backbone resonance assignment and secondary structure of the 30 kDa Sud dimer from *Wolinella succinogenes*

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Received 3 August 2000; Accepted 18 September 2000

Key words: chemical shifts, homodimer, NMR assignment, polysulfide respiration, polysulfide sulfur transferase

Biological context

The proteobacterium *Wolinella succinogenes* is the first organism demonstrated to grow by oxidative phosphorylation with polysulfide (S_n^{2-}) as terminal electron acceptor (Klimmek et al., 1991). The periplasmic Sud protein which is induced in *W. succinogenes* growing by polysulfide respiration, has been previously proposed to serve as a polysulfide binding protein and to transfer polysulfide-sulfur to the active site of the polysulfide reductase (Klimmek et al., 1998). It consists of two identical subunits (15.3 kDa including a His tag of six histidine residues), each with a single cysteine residue, and does not contain prosthetic groups or heavy metal ions. Sud binds up to 10 polysulfide–sulfur atoms covalently to the single cysteine residue in each monomer (Klimmek et al., 1999). The transfer of sulfur from Sud to the reductase probably occurs in a complex of the two proteins (Klimmek et al., 1998). There exists no homologous protein structure. The resonance assignment of the Sud dimer is the first step of the structure determination and provides the basis for further studies of the protein complex.

Methods and results

Recombinant protein was obtained by over-expression in *Escherichia coli.* Details of the expression and purification have been described elsewhere (Klimmek et al., 1998). Protein samples labelled with ^{15}N, and $^{15}N/^{13}C$, were prepared by growing bacteria on isotope enriched minimal medium using ^{15}N ammonium chloride (Martek) and $^{13}C_3$ enriched glycerol (Martek) as main nitrogen and carbon source, respectively. For protein samples labelled with $^{2}H/^{15}N/^{13}C$, the bacteria were grown on Celtone®-dCN (Martek, deuteration degree: 97%). NMR samples of purified protein (0.6–1.2 mM dimer) were prepared in 50 mM sodium phosphate at pH 7.6, 1 mM polysulfide (S_n^{2-}), 13 mM sulfide, and 5% v/v $^{2}H_2O$. The protein was loaded with sulfur before dissolving in the described buffer. Sample tubes were flushed with nitrogen while filling and subsequently widely sealed in order to exclude oxygen from the sample volume. Under these conditions, it can be assumed that the protein remains sulfur-loaded during the NMR experiments.

NMR data were acquired at 300 K using Bruker DMX-600 and DRX-800 NMR spectrometers equipped with xyz-gradient ^{1}H, ^{15}N, ^{13}C triple resonance probeheads. The sensitivity and resolution of triple resonance experiments was improved by employing the TROSY technology (Pervushin et al., 1997; Salzmann et al., 1999). ^{1}H chemical shifts were referenced to internal DSS (2,2-dimethyl-2-silapentane-5-sulfonate sodium salt) at 0.00 ppm. ^{15}N and ^{13}C chemical shifts were calibrated indirectly using the appropriate gyromagnetic ratios (Wishart et al., 1995).

Backbone sequential resonance assignments were obtained with 3D HNCACB, HNCO, HN(CA)CO, and HNCAN (Löhr et al., 2000) experiments using $^{2}H/^{15}N/^{13}C$ protein samples. ^{15}N, ^{1}HN, ^{13}CO, $^{13}C^{\alpha}$, and $^{13}C^{\beta}$ chemical shifts of the protonated protein were confirmed using 2D [^{1}H,^{15}N]-HSQC, and 3D HNCA, HNCO, and H(C)CH-COSY experiments. $^{1}H^{\alpha}$ chemical shifts were obtained from a 3D HCACO

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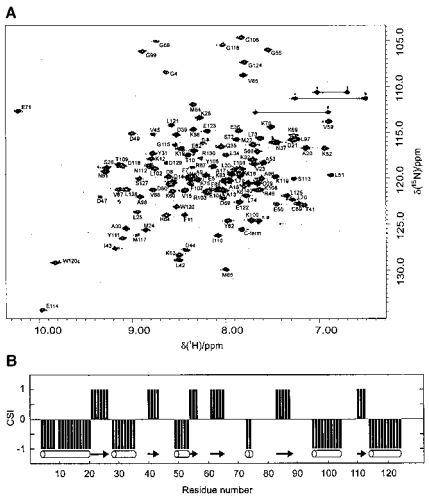

Figure 1. (A) 2D-[^{1}H,^{15}N]-HSQC spectrum of Sud at 0.5 mM concentration. Observed side chain amide moieties are connected by horizontal bars. (B) Plot of the consensus chemical shift indices derived from the $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, and ^{13}CO chemical shifts versus the residue numbers of Sud. Secondary structure elements are shown as arrows for β-strands and cylinders for α-helices.

experiment. Figure 1A shows a well-separated and assigned [^{1}H,^{15}N]-HSQC spectrum of the Sud dimer. The overall quality of the spectra was not always comparable to the shown [^{1}H,^{15}N]-HSQC. Frequently, a second set of signals with similar chemical shifts and line width appeared a few days after preparation of the sample which could be also combined in an almost complete sequential resonance assignment. As the intensities of the additional signals increased over time, this phenomenon might be attributed to slow aggregation at higher sample concentrations. Protein samples remained unchanged for a much longer time at lower sample concentrations ($\leqslant$ 0.5 mM dimer). In addition, spurious oxygen influences the buffer in which Sud is dissolved. Subsequently, the polysulfide sulfur chain bound to the single cysteine of Sud will be shortened which might lead to the observation of different species of protein molecules within the sample.

The CSI program by Wishart et al. (1997) was used to determine consensus chemical shift indices for all assigned residues (Figure 1B). The data revealed an α/β-protein with six α-helices and six β-strands which are likely to be parallel in this case. Here, it appeared that the $^{13}C^{\beta}$ chemical shifts were not very informative with regard to the secondary structure elements, especially for the identification of the β-strands. Therefore, the consensus CSI for Sud is based on the $^{13}C^{\alpha}$, ^{13}CO, and $^{1}H^{\alpha}$ chemical shifts. The obtained secondary structure is consistent with results obtained from a secondary structure prediction using the amino acid sequence of Sud (http://www.embl-heidelberg.de/predictprotein; Fisher et al., 1999).

Extent of assignments and data deposition

The resonance assignment is complete for all backbone nuclei ^{15}N and ^{1}HN (except for A1, D2, A92–A95), $^{13}C^{\alpha}$ (except for A1, A93–R94), ^{13}CO (except for A1, A92–R94), $^{13}C^{\beta}$ (except for A1, F88–A95), and $^{1}H^{\alpha}$ (except for A1, A92–R94, G116, S131). The correlation signals of the residues A1, D2, M3, G78, K90–A95, as well as S131 are absent in Figure 1A due to fast exchange of the corresponding amide protons with the solvent protons. Fast chemical exchange is also likely to be the reason for the absence of information within the segment F88-A95.

As the 3D H(C)CH-COSY spectrum suffered from a considerable overlap of signals, the reported $^{13}C^{\beta}$ chemical shifts consist of $^{13}C^{\beta}$ chemical shifts derived from the 3D-H(C)CH-COSY spectrum and $^{13}C^{\beta}$ chemical shifts derived from the HNCACB spectrum of the deuterated protein which were subsequently corrected according to the number of deuterons one and two bonds away from the $^{13}C^{\beta}$ nucleus (Gardener et al., 1997). The backbone resonance assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4776.

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