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Letter to the Editor: Sequence-specific resonance assignments of the N-terminal, 105-residue KaiC-interacting domain of SasA, a protein necessary for a robust circadian rhythm in *Synechococcus elongatus*

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Biological context

Metabolic, physiological and behavioral activities of virtually all organisms are modulated with a period of approximately 24 h, which is due to an endogenous, self-sustaining circadian clock (Dunlap, 1999). The fact that circadian clocks are found in evolutionarily divergent organisms suggests that the ability to anticipate daily environmental oscillations is of fundamental importance to the survival of most life forms. However, the structural basis of any circadian clock remains unknown. Both the period and amplitude are important components of a healthy circadian oscillation. Disruption of the gene *sasA* from the cyanobacterium *Synechococcus elongatus* shortens the period and reduces the amplitude of the circadian rhythm (Iwasaki et al., 2000). Interactions between SasA and KaiC, one of the three clock proteins (Ishiura et al., 1998; Xu et al., 2000), form a feedback loop and amplify the central KaiA, KaiB, KaiC timing loop to normal levels. SasA, a histidine kinase, interacts with KaiC through its N-terminal domain, which shares 26% sequence identity and 60% similarity with KaiB. It is this N-terminal domain of SasA, which we will refer to as N-SasA, that we are investigating using NMR. Here we report sequential chemical shift assignments and the tentative secondary structure of the N-terminal 105 residues.

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Methods and results

Protein expression and purification: The gene coding for residues 1–105 of SasA of *S. elongatus* was subcloned into the pGEX-2T vector, and *Escherichia coli* BL21(DE3) was transformed with the resulting plasmid. Bacteria were grown at 37 °C in minimal medium containing $^{15}NH_4Cl$ as the only nitrogen source, and with either $^{13}C_6$-glucose or unlabeled glucose. Cells were induced by making the cell culture 1 mM in IPTG and harvested by centrifugation after 4 h. The cells from 1 l of culture were resuspended in 100 ml of PBS buffer (200 mM NaCl, 20 mM phosphate, 1% Triton X-100, pH 7.4) and passed through a French Press. The lysates were centrifuged at 20,000 *g* for 30 min. The resulting supernatant was run over a Glutathione Sepharose column and eluted with glutathione (5 mM glutathione, 200 mM NaCl, 20 mM Tris, pH 8.3). Thrombin was used for cleavage, and GST was separated from N-SasA with a Glutathione Sepharose column. The resulting N-SasA was analyzed for purity using SDS-polyacrylamide gel electrophoresis, dialyzed against 100 mM NaCl, 20 mM sodium phosphate pH 7.0 and concentrated to 0.6 mM. Approximately 12 mg of uniformly ^{13}C, ^{15}N doubly enriched N-SasA were obtained from 1 l of culture.

NMR spectroscopy: Spectra were recorded on a Varian Inova 600 MHz spectrometer at 25 °C at the Biomolecular NMR Laboratory at Texas A&M University. ^{1}H, ^{13}C and ^{15}N chemical shifts were referenced to internal DSS (Wishart and Case, 2001).

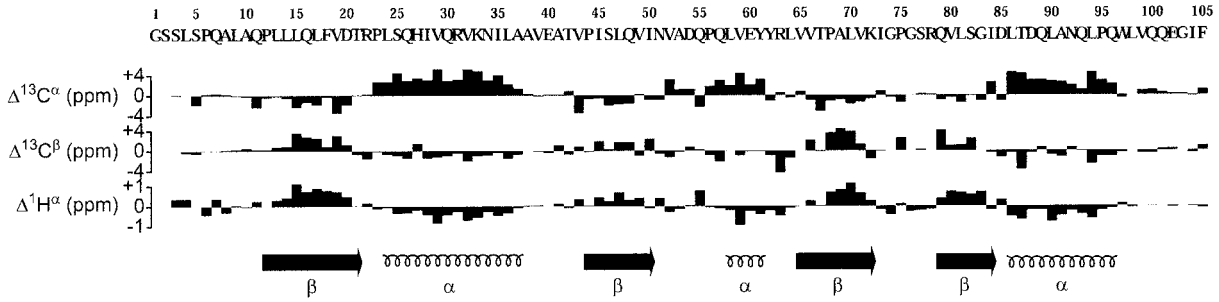

Figure 1. Secondary chemical shifts of $^{13}C^{\alpha}$, $^{13}C^{\beta}$, and $^{1}H^{\alpha}$ relative to random coil values as a function of residue number. Also shown are the stretches of secondary structure elements identified using these secondary shifts. Solid arrows represent stretches of β-strands and curly lines represent stretches of α-helices. NOEs were also used to determine the locations of secondary structure elements shown here.

Sequence specific backbone assignments of $^{1}H^{\alpha}$, $^{1}H^{\beta}$, $^{1}H^{N}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and ^{15}N were obtained from CBCA(CO)NH, CBCANH, HNHA (Wang and Bax, 1996) and HBHA(CO)NH experiments. Aliphatic assignments for the side chains were obtained from C(CO)NH, H(CCO)NH (Grzesiek et al., 1993) and H(C)CH-COSY experiments while side chain amide assignments were taken from CBCA(CO)NH, CBCANH and HSQC experiments. Chemical shift index (Wishart and Case, 2001) analysis for $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and $^{1}H^{\alpha}$ and NOEs indicated the presence of four β-strands (tentatively residues 12-21, 44-51, 65-72, and 79-84) and three α-helices (tentatively residues 24-37, 58-61, and 86-96) in N-SasA (Figure 1). Assignments of the aromatic protons were obtained from 3D and 4D NOESY spectra. Data processing and analysis were carried out on a PC running Linux using the software packages NMRPipe (Delaglio et al., 1995), PIPP and STAPP (Garrett et al., 1991).

Extent of assignments and data deposition

By combining the information from the heteronuclear experiments, we were able to assign 99% of the backbone ^{15}N, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, $^{1}H^{\alpha}$, $^{1}H^{\beta}$ and $^{1}H^{N}$ resonances. Due to the abundance of certain types of residues (16.2% Leu, 13.3% Gln, 12.4% Val), there was a significant degree of overlap in the H(C)CH-COSY spectrum; however, these side chains were well resolved in the C(CO)NH and H(CCO)NH experiments. Assignments of the chemical shifts of aliphatic protons was 95.1% complete and 81.5% of aromatic protons have been assigned. Of the possible 55 side chain NH and NH_2 proton chemical shifts, 10 have been assigned. None of the chemical shifts of the first two residues were assigned. The secondary structure pattern is βαβαββα (Figure 1) and preliminary structures of N-SasA revealed that it has a thioredoxin-like fold (Cave et al., 2001). The high sequence similarity of N-SasA to KaiB strongly suggests that KaiB will be found to adopt a similar backbone fold. The ^{1}H, ^{15}N and ^{13}C chemical shifts for N-SasA have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 5141.

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References

Cave, J.W., Cho, H.S., Batchelder, A.M., Yokota, H., Kim, R. and Wemmer, D.E. (2001) *Protein Sci.*, **10**, 384–396.

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

Dunlap, J.C. (1999) *Cell*, **96**, 271–290.

Garrett, D.S., Powers, R., Gronenborn, A.M. and Clore, G.M. (1991) *J. Magn. Reson.*, **95**, 214–220.

Grzesiek, S., Anglister, J. and Bax, A. (1993) *J. Magn. Reson. Ser. B*, **101**, 114–119.

Ishiura, M., Kutsuna, S., Aoki, S., Iwasaki, H., Andersson, C.R., Tanabe, A., Golden, S.S., Johnson, C.H. and Kondo, T. (1998) *Science*, **281**, 1519–1523.

Iwasaki, H., Williams, S.B., Kitayama, Y., Ishiura, M., Golden, S.S. and Kondo, T. (2000) *Cell*, **101**, 223–233.

Wang, A.C. and Bax, A. (1996) *J. Am. Chem. Soc.*, **118**, 2483–2494.

Wishart, D.S. and Case, D.A. (2001) *Methods Enzymol.*, **338**, 3–34.

Xu, Y., Mori, T. and Johnson, C.H. (2000) *EMBO J.*, **19**, 3349–3357.