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Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N resonance assignments of the N-terminal, 135-residue domain of KaiA, a clock protein from *Synechococcus elongatus*

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Biological context

An endogenous, self-sustaining circadian clock (also called an oscillator or pacemaker) modulates metabolic, physiological and behavioral activities of virtually all organisms with a period of ∼24 h. The fact that evolutionarily divergent organisms are commonly endowed with the ability to anticipate daily environmental oscillations suggests that a healthy circadian pacemaker is of fundamental importance to the survival of most life forms. In the past few years large strides have been made in elucidating the genetic and biochemical bases of the circadian clock in cyanobacteria, fungi, plants, insects, and mammals; however, the structural basis of any circadian clock remains unknown as no clock protein structures have been determined yet.

The basic timing oscillator of the cyanobacterium *Synechococcus elongatus* consists of three proteins KaiA, KaiB and KaiC whose expression and mutual interactions drive the circadian rhythm (Ishiura et al., 1998; Xu et al., 2000; Iwasaki et al., 1999). Point mutations in these proteins are known to alter these interactions and thereby the periodicity of the circadian clock. KaiA acts as the positive element of the oscillator by enhancing *kaiBC* expression and therefore maintains the robustness of the oscillation.

We have been able to identify a stable, independently folded domain for KaiA, which resists proteolytic cleavage by trypsin for up to 5 h. This domain,

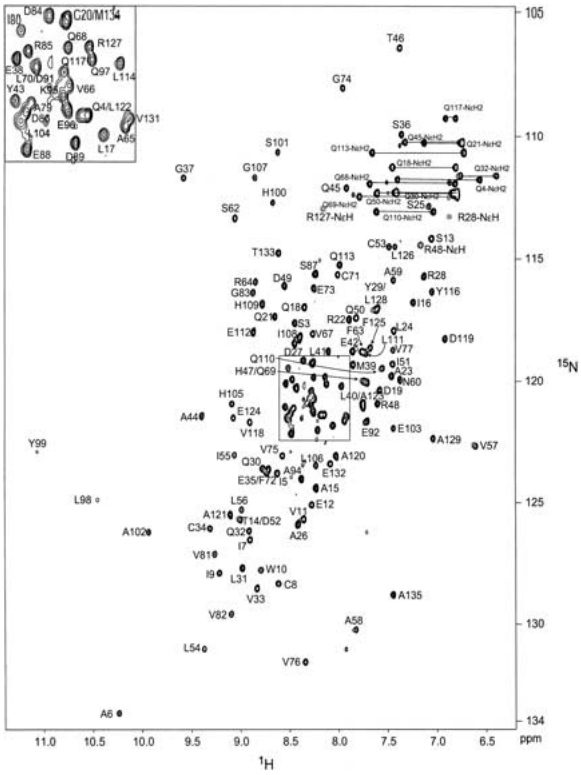

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum of 3 mM ^{15}N-labeled KaiA recorded on a 600 MHz Varian Inova spectrometer at 25 °C. The side chain NH_2 resonances of glutamine residues are connected by horizontal bars. The arginine $N_\epsilon H$ side chain resonances are also identified. Expansion of the region enclosed by the box is shown at the upper left corner of the spectrum.

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henceforth simply referred to as KaiA, consists of the first 135 N-terminal residues of KaiA (15.1 kDa).

Methods and Results

Protein expression and purification

The gene coding for residues 1–135 of *S. elongatus* KaiA was subcloned into the pET-32a+ 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 resuspended cell pellet was passed through a French press and the lysates were centrifuged at 20 000 g for 30 min. The resulting supernatant was run over a Ni-charged chelating column and then dialyzed against enterokinase cleavage buffer. Enterokinase was used for cleavage, and thioredoxin was separated from KaiA using a Ni-charged chelating column. The resulting KaiA was analyzed for purity using SDS-polyacrylamide gel electrophoresis, dialyzed against 50 mM NaCl, 20 mM sodium phosphate pH 7.0 and concentrated to 45 mg/ml. For double labeling typically 25 mg KaiA were obtained from 1 l of culture.

NMR spectroscopy

NMR samples contained 50 mM NaCl, 20 mM sodium phosphate at pH 7.0, 3 mM NaN_3, 0.1 mM DSS, 3 mM purified KaiA in a 95% H_2O / 5% D_2O solvent mixture. The spectra were recorded on Varian Inova 600 MHz spectrometer at 25 °C at the Biomolecular NMR Laboratory at Texas A&M University. ^1H, ^{13}C and ^{15}N chemical shifts were referenced to internal DSS (Markley et al. 1998).

Sequence specific backbone assignments of $^1H^\alpha$, $^1H^\beta$, $^1H^N$, $^{13}C^\alpha$, $^{13}C^\beta$ and ^{15}N were obtained from CBCA(CO)NH, CBCANH, HNHA (Wang and Bax, 1996) and HBHA(CO)NH (Grzesiek and Bax, 1993) 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 Sykes, 1994) analysis for $^{13}C^\alpha$ and $^{13}C^\beta$ suggests the presence of four short β-strands (residues 6–13, 79–83, 102–107 and 128–134) and four α-helices (residues 14–23, 37–46, 61–72 and 111–127) in KaiA. 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 the entire backbone ^{15}N, $^{13}C^\alpha$, $^{13}C^\beta$, $^1H^\alpha$,$^1H^\beta$ and $^1H^N$ resonances except Met1, the $^1H^N$ and ^{15}N of Leu2, and the backbone ^{15}N of the proline residues. A labeled ^{15}N-HSQC spectrum is shown in Figure 1. More than 98% of the aliphatic and amide side chain assignments have been completed. Due to the abundance of certain types of residues (12.6% Leu, 11.9% Ala, 9.6% Gln, 8.9% Glu, 8.9% Val) there is 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. The ^1H, ^{15}N and ^{13}C chemical shifts for KaiA have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 5031.

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