Experimental report

10/03/2016

Proposal: 8-03-838 **Council:** 10/2014

Title: Structural study on clock protein:Relation between phosphorylation and its allosteric effect on the structure

Research area: Biology

This proposal is a new proposal

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Samples: KaiC-WT,AA,AE,DE,DT(75dC1-75dC2)
KaiC-WT,AA,AE,DE,DT(75dC1-hC2)
KaiC-WT,AA,AE,DE,DT(hC1-75dC2)
KaiC-WT,AA,AE,DE,DT(hC1-hC2)
KaiB

Instrument	Requested days	Allocated days	From	To
D22	4	2	24/07/2015	26/07/2015

Abstract:

Biological clock regulates many physiological activities and the wrong clock induces serious diseases. Therefore, to understand the biological clock system is remarkably important not only for a fundamental biology but also medical point of view. A circadian clock in cyanobacteria is the simplest and suitable one for research. This clock consists of only three proteins, KaiA, KaiB and KaiC, and display ATP dependent complex-formation and dissociation with a 24-hour period. Through intensive researches in the last few decades, it has been clarified that KaiC controls the clock phase by oscillating its phosphorylation state, and KaiA and KaiB modulate KaICs phosphorylation activity. However, the shortage of structural knowledge hinders fully understanding of this clock mechanism. In this proposal, using the phosphorylation-state-mimicking mutants and also conducting SANS by utilizing its features, contrast matching, 75%-deuteration and partial domatin-deuteration, we will reveal the relation between the phosphorylation state of KaiC and its allosteric effect on the structures of KaiC and its complex with KaiB.

Experimental Report

Proposal No. 8-03-838
Experiment Team: M. Sugiyama, H. Yagi, K. Kato A. Martel, L. Porcar, G. Zaccai
Title: Structural study on clock protein:
Relation between phosphorylation and its allosteric effect on the structure
Instrument : D22, Date of Experiment: from : 24/07/2015 To : 26/07/2015

[Introduction]

We have an intrinsic ~24-hour clock in our body. Thanks to this clock, our physiological function can work in an approximate 24 hour cycle. One of the simplest biological clocks is in cyanobacteria. It consists of three proteins, KaiA, KaiB and KaiC, which display ATP dependent complex-formation and dissociation with a 24-hour period. Amazingly, this clock is functional in vitro, in absence of daylight oscillation. This indicates that, contrarily to the conventional model of biological clocks, the clock mechanism does not involve a transcriptional/translational regulation process. Through intensive researches in the last few decades, it has been clarified that KaiC is the base unit and controls the clock phase by auto-phosphorylation and –dephosphorylation. KaiC is a homohexamer with six-fold symmetry. Its monomer unit consists of C1 and C2 domains, aligning parallel to the six-fold axis in the hexamer. The whole structure looks a pile of two doughnuts rings, C1 and C2 rings (Fig.1). The phosphorylation sites locate in C2 domain, 431Ser and 432Thr. KaiA and KaiB modulate KaiC activities: KaiA activates the auto-phosphorylation of KaiC and KaiB, inhibiting it, promotes KaiC dephosphorylation.

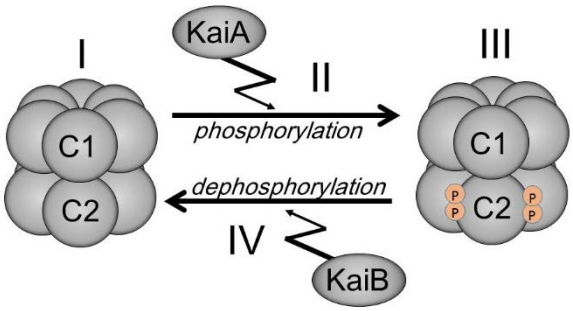

Fig.1. Oscillation process of circadian clock of *cyanobacteria* (orange dots represent phosphates groups).

Fig.1 shows the standard model of this clock system. The phosphorylation of the fully dephosphorylated KaiC (I) is activated by KaiA (1I) and induces conformational change of C1/C2 ring (III). KaiB sequentially connects with the C1/C2 ring under its phosphorylated conformation and promotes KaiC dephosphorylation. KaiB (and KaiA if it is connecting to KaiC) disconnect(s) from the dephoshorylated KaiC (IV) and the system returns to the initial state (I). In fact, as shown in Fig.2, zero-angle SAXS intensity (blue) reflecting the oligomerisation state and the phosphorylation state of KaiC (red) are strongly correlated in the time-evolution. This is a clear evidence that the oscillation is controlled by the phosphorylation of KaiC through interaction with KaiA and KaiB[1]. Although the biochemical aspects of this process are well understood, our knowledge remains limited from the structural point of view. For example, the connection side of KaiBs to KaiC, the number of connected KaiBs and its structures. The purpose of this work is to reveal the structure of KaiC-KaiB complex.

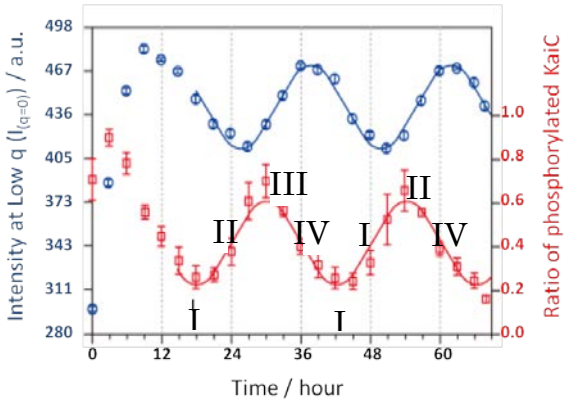

Fig.2.The scattering intensity extrapolated at q = 0 (in blue) and the proportion of phosphorylated KaiC [1] (in red) are strongly correlated along time. I-IV show the phosphorylation states.

[Experimental]

The difficult point is to observe the structure at one time phase because the system is in constant motion. In other words, we have to stop the clock at the target phosphorylation state. Therefore, we prepared the phosphorylation-state-mimic (PhaseIV) mutants of KaiC, $KaiC_{DT}$. There is another difficulty to reveal the structure of KaiC-KaiB complex: it is to measure only KaiB in the complex. For this purpose, we conducted small-angle neutron scattering with contrast variation technique (CV-SANS). Because the scattering length density (SLD) of protein is matched to that of 40% D_2O (=60%H_2O), by observing the oligomer consisting of

deuterated KaiB and native KaiC in 40% D_2O solution, only deuterated KaiB can be observed, This is usual CV-SANS method. However, with this method, it is difficult to obtain the scattering profile with statistical precision, especially in the higher q-range, because of the intensive incoherent scattering coming from 60%H_2O in the solvent. To overcome this difficulty, we prepared 75% deuterated protein, of which SLD is matched to the SLD of 100% D_2O. Therefore, we measured of the complex with 75% deuterated $KaiC_{DT}$ and native KaiB in 100% D_2O. As a result, we measured the structure of KaiB in the complex.

[Results and Discussion]

Figure 3 shows that the prepared 75% deuterated $KaiC_{DT}$ was matched out in 100% D2O as expected. Figure 4 shows the scattering profiles of three complexes: native-$KaiC_{DT}$ + native-KaiB, native-$KaiC_{DT}$ + 75%-deuterated -KaiB, and 75%-deuterated-$KaiC_{DT}$ + native-KaiB. As shown in the figure, the calculated SANS profile of hexameric ring of KaiB.well-reproduced the scattering profile of the complex of 75%-deuterated-$KaiC_{DT}$ + native-KaiB. This result strongly supported that KaiBs make the hexameric ring on KaiC at Phase IV.

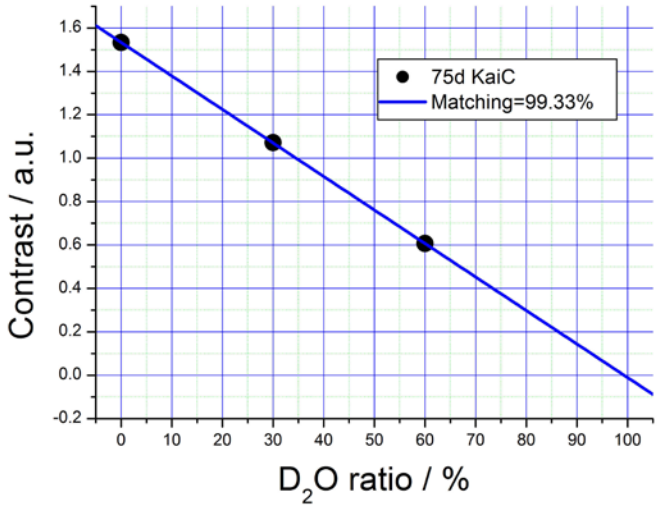

Fig.3. Scattering contrast of 75% deuterated $KaiC_{DT}$. Closed circles indicated the square roots of zero angle scattering intensities in 0 %, 30% 60% D_2O solvents. The matching point is calculated to be 99.73% D_2O solvent.

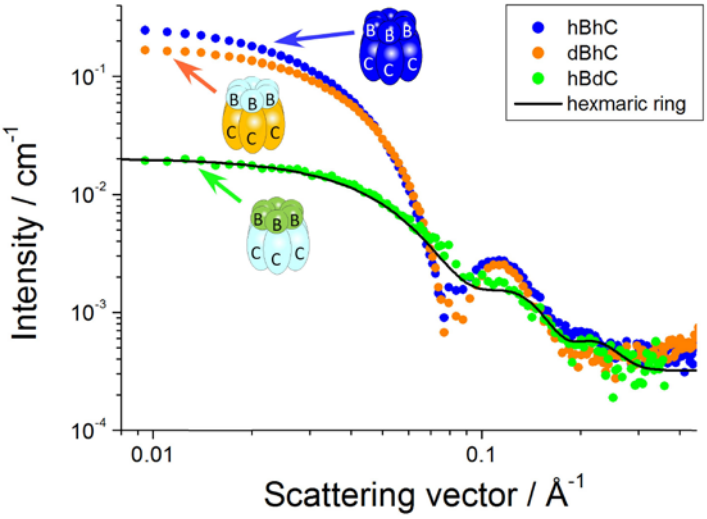

Fig.4. Scattering profiles of three complexes: native-KaiCDT + native-KaiB (blue), native-KaiCDT + 75%-deuterated -KaiB,(orage) and 75%-deuterated-KaiCDT + native-KaiB (green). The solid line is the calculated SANS profile of hexameric ring of KaiB.

[References]

[1] Structural and dynamic aspects of protein clocks: S.Akiyama. Cell. Mol. Life Sci., 25 (2012).

[2] Insight into cyanobacterial circadian timing from structural details of the KaiB–KaiC interaction: J.Snijder, et al., PNAS, 111(4) (2014) 1379.

[3] Conformational characterization of a protein complex involving intrinsically disordered protein by small-angle neutron scattering using the inverse contrast matching method: M.Sugiyama et al., J. Appl. Cryst., 47 (2014) 430.

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