Experimental report

15/02/2020

Proposal: 8-03-938 **Council:** 4/2018

Title: Structural transition of clock protein complex induced by change of phosphorylation state

Research area: Biology

This proposal is a new proposal

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Samples: 75dKaiC+75dKaiB+hKaiA complex
75dKaiC_AA+hKaiA complex + 75dKaiC_DD+75dKaiB complex
75dKaiC_AA+hKaiA complex + 75dKaiC_DT+75dKaiB complex

Instrument	Requested days	Allocated days	From	To
D22	3	3	19/10/2018	22/10/2018

Abstract:

Every life on the earth has a circadian clock, which is a biological oscillator with 24-hrs period. 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, in vitro, display ATP dependent complex-formation and dissociation with a 24-hour period. It has been clarified that the particular complexes are formed depending upon the phosphorylation state of KaiC. Therefore, determining the correlation between the structure of complex and KaiC phosphorylation level is crucial to understand the mechanism of clock oscillation. Recently, in the higher phosphorylation state of KaiC, KaiCB complex absorbs twelve KaiAs and forms KaiCBA complex: it is supposed to induce dephosphorylation of KaiC. However, the complex was obtained under the extrem condition. Therefore, there are questions that "Does KaiCB complex really absorb KaiA under the native condition?" and "If it does, is the complex (KaiCB+12KaiA) produced?". The aim of this proposal is to reveal the formation process of the KaiCBA complexes by using size-exculsion chromatgraphy and iCM-SANS (SEC-iCM-SANS) in combination.

Experimental Report

Proposal No. 8-03-938
Experiment Team: M. Sugiyama, H. Yagi, A. Martel, L. Porcar, G. Zaccai
Title: Structural transition of clock protein complex induced by change of phosphorylation state
Instrument: D22, Date of Experiment: from 19/10/2018 to 22/10/2018

[Introduction]

Every life on the earth has a circadian clock, which is a biological oscillator with 24-hrs period. One of the simplest oscillator is *cyanobacteria*'s one: the oscillator consists of only three proteins, KaiA, KaiB and KaiC, and they repeat association–disassociation cycle. Surprisingly, this system can be reconstructed *in vitro* and its cycle is observed even without daylight oscillation (Fig.1(a), [1]). Therefore, it has been intensively investigated within last decades.

There are two oscillations with deferent scale in Kai-clock system: One is an oscillation inside a molecular and the other is between proteins. The former is as follows. The base unit of the oscillator is KaiC, which is a homohexamer with six-fold symmetry (Fig.1(b)). Its monomer unit consists of C1 and C2 domains, aligning parallel to the six-fold axis in the hexamer. The C2 domain contains two phosphorylation sites: Ser431 (S) and Thr432 (T). The oscillation inside a molecular is phosphorylation-dephosphorylation cycle in these sites: S/T→S/*p*T→*p*S/*p*T→*p*S/T→S/T, *p* indicates that the residue is phosphorylated (Fig.1(b)), as shown with a red line and circles in Fig.1 (a). The later one, the oscillation between proteins, is the formation and splitting of complexes of KaiCA, KaiCB and KaiCBA. (a blue line in Fig.1(a) and "*p*"s are in Fig.1(b)).

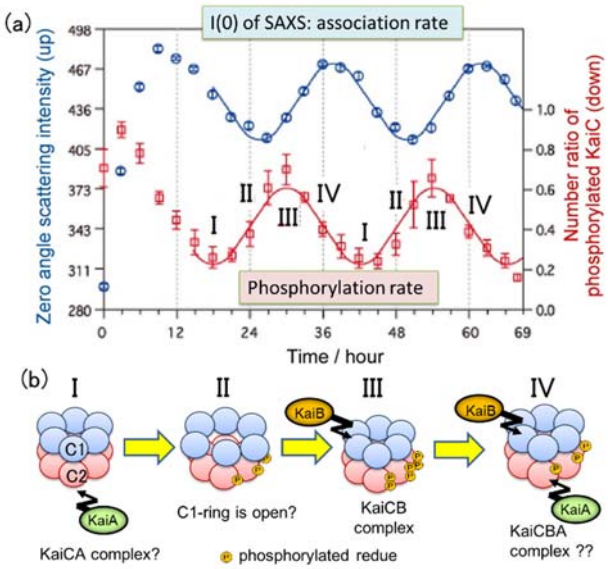

Fig.1. (a) Oscillation process of circadian clock of cyanobacteria. Red (lower) curve shows fraction of phosphorylated KaiC and blue (upper) curve does zero-angle SAXS intensity [1]. (b) Supposed structural change on the process of phosphorylation of KaiC and formation of complexes of KaiA, KaiCB, KaiCBA. I-IV indicate the clock phases.

These two levels oscillation is considered to be tightly correlated. Along this line, it is proposed that KaiA and KaiB play respectively the roles of accelerator and inhibitor of KaiC phosphorylation as depicted in the standard oscillation model (Fig.1(b)). The relation in the time phases is considered as follows:

[Phase I→II]: KaiA transiently interacts with C2 domain and promotes phosphorylation which induces a conformational change of C1 domain, unlocking the C1 ring.

[Phase II→III]: KaiB binds to the unlocked C1 ring, forming KaiCB complex.

[Phase III→IV]: Free KaiAs bind to the KaiBC complex, forming KaiCBA complex and the dephosphorylation of KaiC starts.

[Phase IV→I]: The KaiC dephosphorylation leads to the release of KaiA and KaiB from the KaiCBA complex, and the cycle can start again.

This assumption is derived from the observation (Fig.1(a), [1]): The zero-angle SAXS intensity (blue), reflecting the oligomerisation level, is strongly correlated with the phosphorylation state of KaiC (red). Therefore, ***determining the correlation between the structures of complexes and KaiC phosphorylation level is crucial to confirm this scheme of regulation.***

As the first result, we successfully solved the structure of KaiCB complex (phase III)[2] with inverse Contrast Matching SANS (iCM-SANS) method (No. 8-03-875, annual report 2016, p64-55, [3]) and the structure in solution is in agreement with crystallographic analysis [4]. The next research target is the clarification of structure of KaiCBA complex which could be generated in phase IV.

Recently, a combined analysis of cryo-EM data with native mass spectrometry reported a structure of KaiCBA where 12 KaiA monomers were connected to the outer edge of KaiB

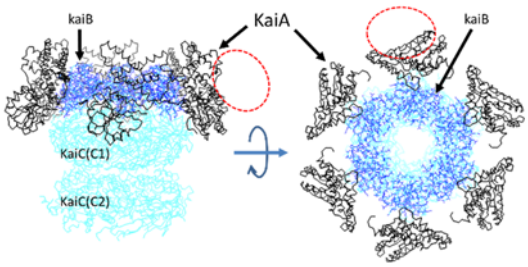

Fig.2. Structure of KaiCBA [5]. Black, blue and cyan indicate KaiA, KaiB and KaiC, respectively. Red circles show the supposed position of missing KaiA.

in KaiCB complex [5] (Fig.2), suggesting that 1) KaiA can bind to a KaiCB complex, and 2) the availability of free KaiA could be the restricting factor controling the phase III to phase IV transition. However, from the strucural point of view, the KaiCBA complex, shown in the report [5], only C-domains of KaiAs which are connecting to KaiB are visible but N-domains of KaiAs (red circles in Fig.2) are still unknown. Therefore, we have tried to solve the whole structure of KaiCBA complex in solution.

[Experimental]

KaiCBA complex is easy to make the aggregation. Therefore, it is inevitable to conduct to SEC-SANS, which is only available with D22 in the world. In addition, we should focus on the configuration of 12 KaiA in the complex because the structure of KaiBC complex has been known. Therefore, we combined iCM-SANS and SEC-SANS to observe the configuration of 12 KaiA in the complex.

The sample was KaiCBA complex consisting of hydrogenated KaiA, 75%-deuterated KaiB and 75%-deuterated KaiC in 100%D_2O buffer. In this condition, we can observe only the configuration of 12 KaiA in the complex with iCM-SANS method. To remove the aggregates, we injected the sample solution to SEC-SANS system: the flow rate around the complex was 0.07mL/min and the column was superpose6 increase 10/300GL.

[Results and Discussion]

Figure 3(a) shows the elution chart of SEC-SANS, meaning that KaiCBA was eluted out. Making average the SANS data between C and D in the elution, the SANS profile of KaiCBA was obtained. As shown in Fig.3(b), the obtained SANS profile of KaiCBA showed no upturn in the lower *Q*-range and its gyration radius was found to be 78.1±1.0 Å, which was the smallest value in all previous data.

Now, we are analyzing the configuration of 12 KaiA coupling with computational simulation.

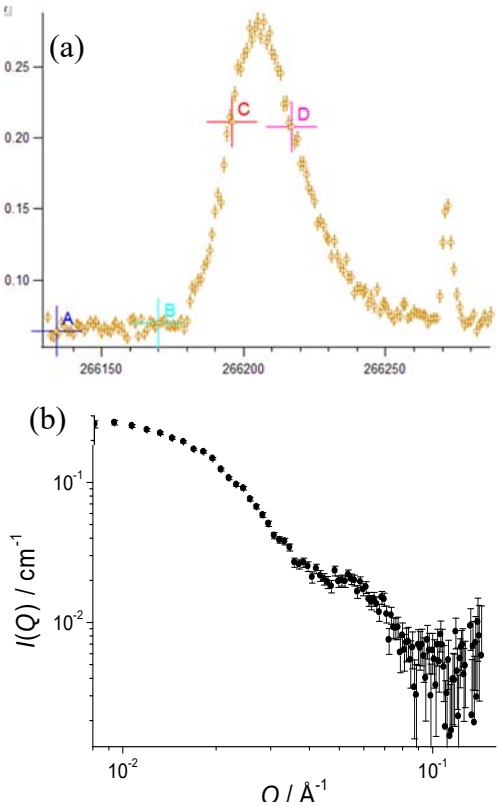

Fig.3. (a) Elution chart. SANS data between C and D were averaged. (b) Averaged SANS data.

[References]

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[4] R. Tseng et al., *Science*, **355** (2017) 1174–1180.
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