Experimental report

12/05/2019

Proposal: 8-03-924 **Council:** 4/2017

Title: Investigation on monomer exchange and synchronization on Kai circadian clock system

Research area: Biology

This proposal is a new proposal

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Samples: h-KaiC (AA,AE,DE,DA,WT)
75d-(KaiC_AA,AE,WT-KaiA) complex
75d-(KaiC_AA,AE,WT-KaiB) complex
75d-KaiC(AA,AE,DE,DA,WT)

Instrument	Requested days	Allocated days	From	To
D22	4	3	18/06/2018	21/06/2018

Abstract:

Biological clock, consisting of many biomolecular oscillators, has been attracted by researches from Biology to Mathematical physics because the clock mechanism is related with wide range of sciences. For example, there are three significant questions about biological clock: the oscillation mechanism, the synchronization mechanism between all oscillators and the temperature compensation mechanism. The circadian oscillator in cyanobacteria is the best system of choice for this research because its oscillator consists only of three proteins, KaiA, KaiB and KaiC, which display ATP-dependent complex-formation and dissociation with a 24-hour period. In the last few decades, the oscillation mechanism is being clarified. Therefore, we are focusing on the synchronization mechanism. Non-linear dynamics points out that there should be a weak interaction which works between oscillators at one phase point. We supposed that the weak interaction should be monomer exchange between the KaiC hexamers and succeeded to prove that the monomer exchange does exist. In this proposal, we will reveal the monomer exchange in detail and its relation with clock phase with deuteration-labelling SANS method.

Experimental Report

Proposal No. 8-03-924
Experiment Team: M. Sugiyama, H. Yagi, A. Martel, L. Porcar, G. Zaccai
Title: Investigation on monomer exchange and synchronization on Kai circadian clock system
Instrument: D22, Date of Experiment: from 18/06/2018 to 21/06/2018

[Introduction]

Kai clock system in cyanobacteria is one of the simplest circadian oscillator, which consists of only three kinds of proteins, KaiA, KaiB and KaiC, and ATP. This clock system displays ATP-dependent complex-formation and dissociation with a 24-hour period. Because of this simplicity, the Kai system is a research target to understand a biological clock with molecular scale in the last few decades. Under this line, we are focusing on a synchronization mechanism between oscillators, "How do they associate each other on the same timing?"

To understand the synchronization mechanism, it is inevitable to reveal dynamics of each protein in each phase. As shown in Fig.1, we succeeded to observe the clock oscillation with the Kai-system with small-angle x-ray scattering. Therefore, based on this result, we tried to observe dynamics of KaiA and /or KaiB with time-resolved inverse Contrast Matching Small-Angle Neutron Scattering (iCM-SANS) method [1-3].

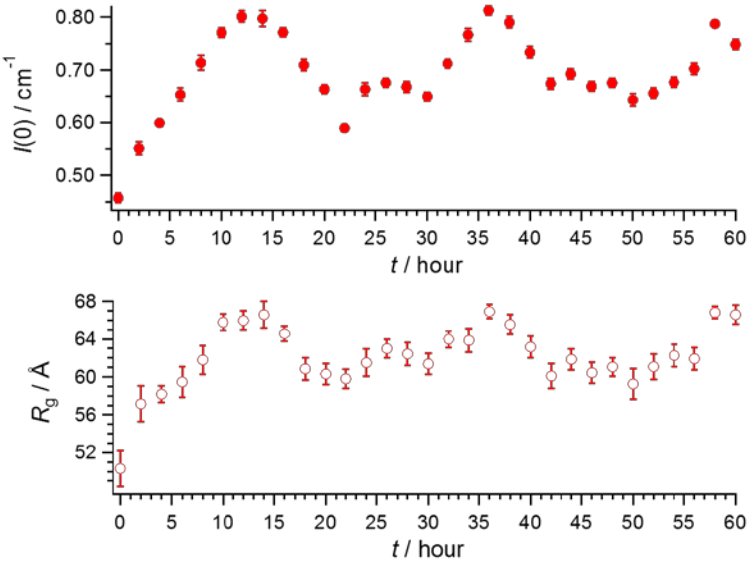

Fig.1. Clock oscillation of Kai-system. (upper) Zero angle scattering intensity, $I(0)$ and (bottom) radius of gyration, Rg.

[Experimental]

We prepared for three Kai clock system: #1. h-KaiA, h-KaiB and h-KaiC in D_2O (reference), #2. 75d-KaiA, h-KaiB and h-KaiC in D_2O, where KaiA is invisible and KaiB and KaiC are visible, #3. h-KaiA, 75d-KaiB and 75d-KaiC in D_2O, where KaiA is visible and KaiB and KaiC are invisible. Here, "h" means hydrogenated and "75d" does 75% deuterated proteins, which are visible and invisible in D_2O due to their scattering contrasts to D_2O, respectively.

As shown in Fig.2, the scattering contrasts of 75d-KaiA and 75d-KaiC were matched to that of D_2O: Since KaiB easily makes aggregates in solo solution, we did not observe matching of 75d-KaiB.

In the oscillation experiment, the sample concentrations are 0.45mg/mL of KaiA, 0.45 mg/mL of KaiB and 1.8 mg/mL of KaiC and 3mM of ATP. The temperature was kept at 30C. Every 45 minute, we accumulated SANS intensities for 15 minutes in 65 hrs with D22 SANS camera.

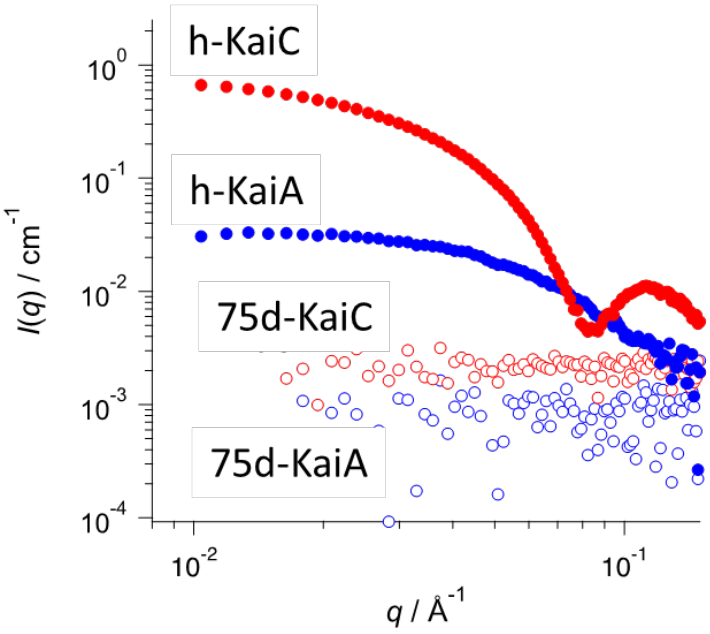

Fig.2. SANS profiles of h-KaiA (blue close circle), h-KaiC (red close circle), 75d-KaiA (blue open circle), 75d-KaiC (red open circle), respectively.

[Results and Discussion]

As shown in Fig.3, we clearly observed the clock oscillation of Kai system in D_2O. The phases between I(0) (and Rg) and phosphorylation of KaiC are different in $\pi/4$. This phase shift is agreement with the previous result [4]. Interestingly, the oscillation period became 31h hrs. The reason of this expanding is under consideration.

Now, we are analyzing the remaining two systems; 75d-KaiA, h-KaiB, h-KaiC and h-KaiA, 75d-KaiB, 75d-KaiC.

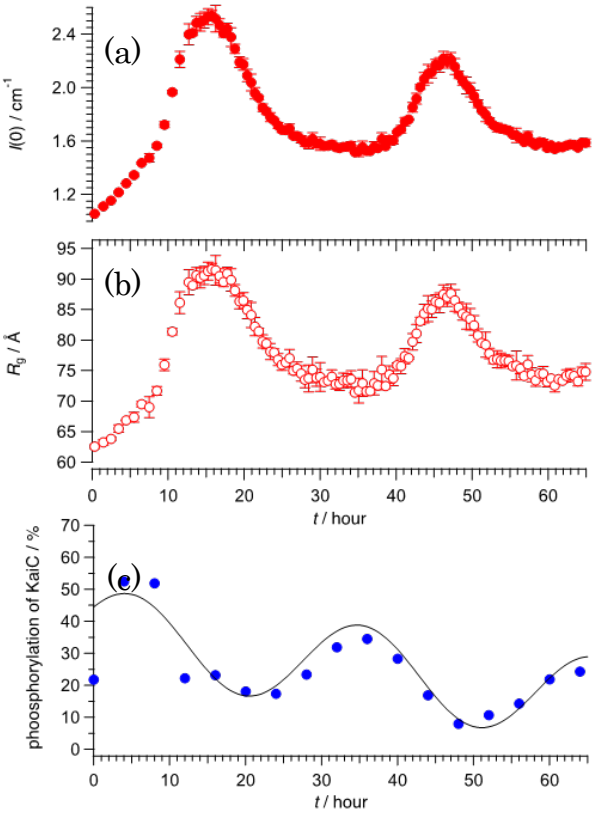

Fig.3. Clock oscillation of #1 system: (a) I(0), (b) Rg and (c) phosphorylation of

[References]

[1] M. Sugiyama, H. Yagi, T. Yamaguchi, K. Kumoi, M. Hirai, Y. Oba, N. Sato, L. Porcar, A. Martel, and K. Kato, *Journal of Applied Crystallography*, **47**, (2014) 430–435.

[2] M. Sugiyama, H. Yagi, K. Ishii, L. Porcar, A. Martel, K. Oyama, M. Noda, Y. Yunoki, R. Murakami, R. Inoue, N. Sato, Y. Oba, K. Terauchi, S. Uchiyama, and K. Kato, *Scientific Reports*, **6** (2016) 35567.

[3] R. Yogo, S. Yanaka, H. Yagi, A. Martel, L. Porcar, Y. Ueki, R. Inoue, N. Sato, M. Sugiyama, and K. Kato, *Biochemistry and Biophysics Reports*, **12** (2017) 1-4.

[4] S.Akiyama. *Cell. Mol. Life Sci*., **25** (2012).