Experimental Report

Proposal: **8-03-801** **Council:** 10/2012

Title: SANS Study on Synchronization Kinetics on Clock Protein

This proposal is a new proposal

Researh Area: Biology

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Samples: Kai B (protein, 22.8kDa)
deuterated Kai C (protein 364kDa)
Kai A (protein, 130.6kDa)
Kai C (protein, 346kDa)

Instrument	Req. Days	All. Days	From	To
D22	3	2	25/07/2013	27/07/2013

Abstract:

A circadian clock in cyanobacteria is the simplest and interesting living oscillator. This oscillator consists of only three proteins, KaiA, KaiB and KaiC, and works in vitro with ATP. The oscillation process has been revealed by SAXS studies and so on. However, the synchronization mechanism in a large number of protein oscillators is still unknown. Recently, it is supposed that subunit exchange between KaiC proteins could be a possible candidate for the interaction making synchronization. Therefore, in this study, we will prove the existence of the subunit exchange between KaiC proteins and also reveal the relation between the subunit exchange and synchronization mechanism by time-resolve SANS experiment utilizing deuterated subunits: this technique has been established for measurement of subunit exchange by our group in the previous work.

Experimental Report

Proposal No. 8-03-801
Experiment Team: M. Sugiyama, H. Yagi, M. Hirai, K. Kato, and G. Zaccai.
Title : SANS Study on Synchronization Kinetics on Clock Protein
Instrument : D22, Date of Experiment: from : 25/07/2013 To : 27/07/2013

[Introduction]

A circadian clock is a time keeper in a living cell. The clock is characterized with three features: (#1) the clock oscillates self-sustainably with approximately 24 hours without any external cue, and (#2) the oscillatory period is almost independent upon the temperature, and (#3) the phase can be reset by periodical change of temperature, light, and so on. Recently, a remarkable circadian clock oscillator was discovered in cyanobacteria. Its oscillator consists of only three proteins, KaiA, KaiB and KaiC, and can be reconstructed in vitro with ATP as energy; KaiC is a main oscillator protein, which consists of six subunits having C1 and C2 domains with six-fold symmetry, and KaiA and KaiB are accelerator and inhibitor for phosphorylation of KaiC, respectively. SAXS studies revealed the detailed oscillation process, which is a 24 hrs-cycle of formation and dissociation of a complex by three proteins [1]; As shown in Fig.1, Firstly, the phosphorylation of KaiC is accelerated by KaiA connecting to KaiC and induces the structural deformation of C2 domain, next Kai B sequentially connects to the deformed C2 domain and starts to dephosphorylate KaiC, next KaiA and KaiB disconnect from the dephoshorylated KaiC and the system is going back to the initial state. In fact, as shown in Fig.2, zero-angle SAXS intensity reflecting the aggregation state and the phosphorylation state of KaiC are deeply related. This is a clear evidence that the oscilation is controlled by the phsophorlyration of KaiC through connecting/disconnecting KaiA and KaiB[1].

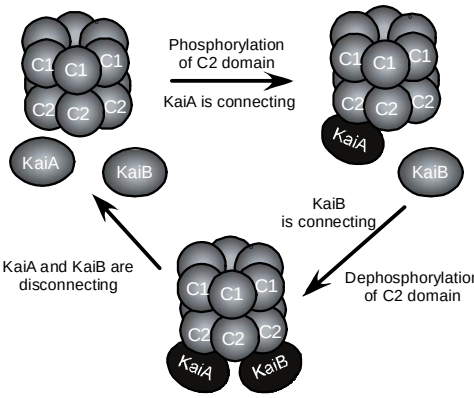

Fig.1. Oscillation process of circadian clock of *cyanobacteria*.

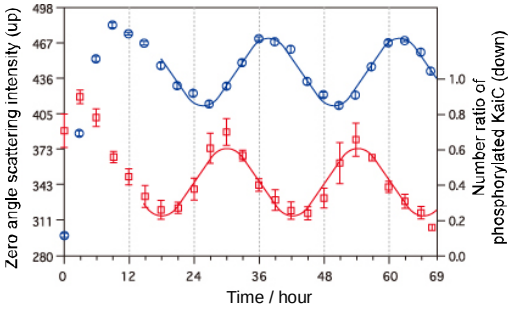

Fig.2. (up) Zero-angle scattering intensity and (down) number ratio of phosphrlyrated KaiC[1].

Now, we are interesting of the synchronization mechanism: for example, when two oscillator systems with different time phases are mixed, the mixed system generated a new oscillator system with new time phase. Recently, it is supposed that subunit exchange between KaiC proteins could exist as a possible candidate for the interaction of phase reduction, which makes synchronization between the Kai oscilators [2]. But no one has succeeded to observe it.

[Experimental]

To observe the subunit exchange between KaiC proteins in the clock oscillation, we have to make a specific experimental system: Even though there are there proteins, KaiA, KaiB, and KaiC, in the oscillation system, it is possible to observe KaiC proteins and also their subunit exchange if it exists. For this purpose, we employed a combination technique with protein deuteration and solvent contrast variation methods, which was previously applied to observe the subunit exchange in the proteasome α7 rings[3].

We prepared for non-deuterated KaiA, KaiB, KaiC proteins and deuterated KaiC protein. It is well known that, in 40% D_2O solution, non-deuterated protein is matched out and only deuterated protein can be observed by SANS. Therefore, when we mixed with non-deuterated KaiA (h-KaiA), KaiB (h-KaiB), KaiC (h-KaiC) proteins and deuterated KaiC (d-KaiC) protein in 40% D_2O solution, if the subunit exchange exits between KaiC proteins, we observe the decrease of SANS intensity.

The difficulty of the above-mentioned experiment could be the high background because of incoherent scattering from hydrogen in the solvent where there is 60% H_2O. Therefore, we also tried to prepare for the protein which is matched out in 100% D_2O solution: the deuteration ratio of the protein is 75%. We also confirmed this protein, 75% deuterated

protein, was matched out in 100% D_2O solution.

[Results and Discussion]

Unfortunately, we have a problem about configuration of the disk chopper. Therefore, we discussed the total scattering intensities instead of the SANS profiles.

Fig 3 shows the time evolution of total intensities of two experimental systems: System A consists of h-KaiA, h-KaiB, h-KaiC and d-KaiC in 40%D_2O (Fig.4(a)) and system B consists of d-KaiC in 40%D_2O (Fig.4(b)). The oscillation period of system A is found to be 24.5 hr and that of system B is 27 hr. We supposed that this difference resulted in the difference of kinetics of the subunit exchange. Now the detailed analysis is in progress.

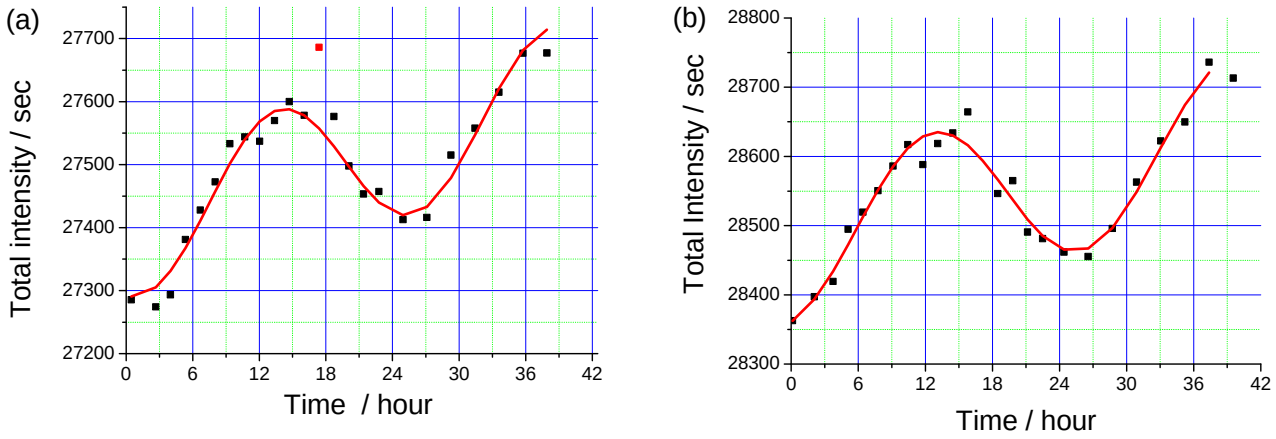

Fig 3. Time evolution of the total SANS intensity. (a) System A consists of h-KaiA, h-KaiB, h-KaiC and d-KaiC in 40%D_2O and (b) system B consists of d-KaiC in 40%D_2O.

The matching out of 75% deuteration protein in 100% D2O solution has been successfully confirmed and the result was publised [4].

[References]

[1] Structural and dynamic aspects of protein clocks: *S.Akiyama. Cellular and Molecular Life Sciences*, **25** (2012).

[2] Origin of the resilience of the cyanobacterial circadian clock, *H.Ito, etal., Nat. Struct. Mol. Biol.*, **14**, 1084 (2008),

[3] Kinetic Asymmetry of Subunit Exchange of Homooligomeric Protein as Revealed by Deuteration-Assisted Small-Angle Neutron Scattering: *M. Sugiyama, K.Kato, G.Zaccai, M.Hirai, etal., Biophysical J.* **101** 2037 (2011).

[4] Conformational characterization of a protein complex involving intrinsically disordered protein by small-angle neutron scattering using the inverse contrast matching method: a case study of interaction between α-synuclein and PbaB tetramer as a model chaperone: *M.Sugiyama, H.Yagi, M.Hirai, L.Porcar, A.Martel, K.Kato, etal., J. Appl. Cryst.*, **47**, 430-435 (2014).