Selective observation of clock protein during circadian oscillation of association-dissociation.

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The cyanobacterial circadian clock is composed of only three clock proteins KaiA, KaiB, and KaiC, and exhibits a phosphorylation oscillation of KaiC in 24-hour period. This phosphorylation oscillation is coupled with the association-dissociation between the three clock proteins. According to the previous study, the KaiA-KaiC (AC) complex (A_2C_6) is mainly formed during the phosphorylation process, and the KaiA-KaiB-KaiC (ABC) complex ($A_nB_6C_6$; $2 \leq n \leq 12$) is mainly formed during the dephosphorylation process, and that the oscillation of the degree of phosphorylation is caused by the change of complexes between AC and ABC complexs in a 24-hour cycle. The association-dissociation in 24-hour is observed as the oscillation of the forward scattering intensity for small-angle X-ray scattering (SAXS) (blue circles in Figure 1). However, the detail of kinetics for all proteins and their complexes in the solution during the cycle have not been completely elcidated. In this study, we especially focused on the kinetic behavior of KaiA during the association-dissociation oscillation.

We prepared the mixture of 100% deuterated (d)-KaiA, hydrogenated (h)-KaiB, and hydrogenated (h)-KaiC in 42 % D_2O solution to make d-KaiA visible and h-KaiB and h-KaiC invisible on a neutron scattering profile. Contrast matching small-angle neutron scattering (CM-SANS) measurement was conducted with SANS-U located at JRR-3 (Proposal#: 23556). A neutron beam at wavelength = 6.0 Å with 10 % of resolution was irradiated to the samples. The sample-to-detector-distance (SDD) was set to be 4000 which cover the q-range of 0.01 – 0.1 Å^{-1}. We acquired the scattering profile for 30 minutes every two hours.

Red circles in Figure 1 shows the time course of the forward scattering intensity ($I(0)$), which was obtained through Guinier analysis for the time resolved CM-SANS profile. Interestingly, the forward scattering intensities for SAXS and CM-SANS oscillated with opposite phases. Here, it is noted that the forward scattering intensities for SAXS and CM-SANS are proportional to the weight-averaged molecular mass of all components and KaiA, respectively. Considering the results of the CM-SANS, SAXS and analytical ultracentrifugation, we found that the number (n) of KaiA in the ABC complex ($A_nB_6C_6$) and the number of ABC complexes oscillate with opposite phases.

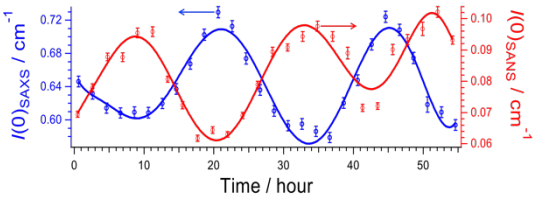

Fig. 1. Time course of forward scattering intensities of SAXS (blue circles) and CM-SANS (red circles).