Kinetics analysis of protein complex in Kai-circadian clock system

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The cyanobacterial circadian clock consists of three proteins, KaiA, KaiB, and KaiC, and has been extensively studied as a model of a transcription–translation-independent circadian clock. The circadian rhythm of this system is observed as an approximately 24 h oscillation in the phosphorylation degree of KaiC (Fig. 1(a)). KaiC phosphorylation is promoted by its association with KaiA, whereas its association with KaiB promotes dephosphorylation. Thus, the phosphorylation oscillation of KaiC is generated through the association and dissociation of the three Kai proteins. Elucidating the dynamics of the complexes formed by the Kai proteins—that is, which complexes are present, their relative abundances, and how these populations change over time—is therefore essential for understanding the operating mechanism of the circadian clock.

The dynamics of these complexes can be monitored through oscillations in the forward scattering intensity, ($I(0)$), measured by time-resolved small-angle X-ray scattering (SAXS) (Fig. 1(b)). Because ($I(0)$) is proportional to the number average of the squared molecular mass, its oscillation indicates that the complexes formed in solution change with a period of approximately 24 h. However, analytical ultracentrifugation (AUC) have suggested that various species with different stoichiometries may coexist in solution, including A_2, B_4, C_6, A_2C_6, B_6C_6, and $A_nB_6C_6$ ($2 \leq n \leq 12$). Because the $I(0)_{SAXS}$ represents an ensemble average over all components in solution, it has been difficult to resolve the dynamics of the individual complexes from SAXS data alone.

In this study, we therefore used time-resolved contrast-matching small-angle neutron scattering (CM-SANS) to selectively monitor the dynamics of a specific protein component. To focus on the dynamics of KaiA, which contributes to KaiC phosphorylation, time-resolved CM-SANS were performed in 42% D_2O, where hydrogenated (h) KaiB and KaiC were contrast-matched and rendered nearly invisible to neutrons, whereas deuterated (d) KaiA remained visible. This approach was intended to selectively observe the dynamics of KaiA-containing complexes.

We obtained scattering data in which the contribution from $A_nB_6C_6$ complexes with large KaiA occupancies, (n), was enhanced (Fig. 1(c)). Interestingly, the $I(0)_{CM\text{-}SANS}$ oscillated in antiphase with the $I(0)_{SAXS}$ (Figs. 1(b) and 1(c)). Combined analysis with separately obtained AUC revealed that, during the phosphorylation phase—the pink region in Fig. 1, where the $I(0)_{SAXS}$ was minimal and the $I(0)_{CM\text{-}SANS}$ was maximal—the population of $A_nB_6C_6$ involved in dephosphorylation reached its minimum, while their KaiA occupancy, (n), reached its maximum. In contrast, during the dephosphorylation phase—the light-blue region in Fig. 1, where the $I(0)_{SAXS}$ was maximal and the $I(0)_{CM\text{-}SANS}$ was minimal—the population of $A_nB_6C_6$ reached its maximum, while the KaiA occupancy (n) reached its minimum.

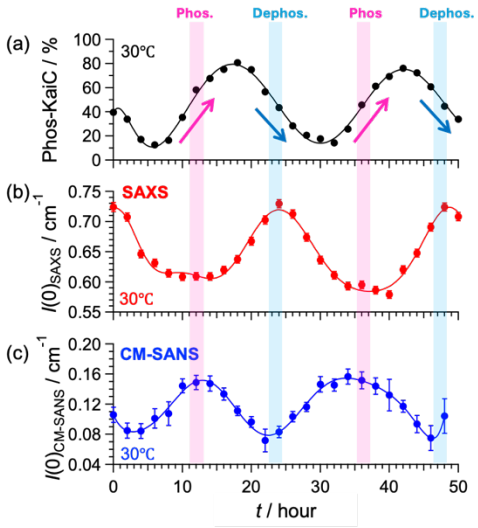

Fig. 1. Circadian oscillation in a mixture of d-KaiA, h-KaiB, and h-KaiC in 42 %D_2O. Time courses of (a) the KaiC phosphorylation degree, (b) $I(0)_{SAXS}$, and (c) $I(0)_{CM\text{-}SANS}$.