Photo-control of Mitotic Kinesin Eg5 using Photoresponsive Protein Aureochrome1

Kinesin Eg5 Research Group

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1. Background

In this study, I focused on mitotic kinesin Eg5 whose structure, biochemical properties and physiological functions have been clarified from the kinesin family of motor proteins to use for photo-controlled biomolecular machines. By forming dimers, conventional kinesin moves by a mechanism that walks on microtubules to transport vesicles containing neurotransmitters etc. On the other hand, kinesin Eg5 has an important physiological function responsible for cell division, which forms tetramers and forms spindles. The motor activity of these kinesins depends on the formation of these dimers and tetramers. Therefore, photo-reversible control of kinesin motor function can be expected by introducing a photoresponsive protein into a functional site related to multimerization.

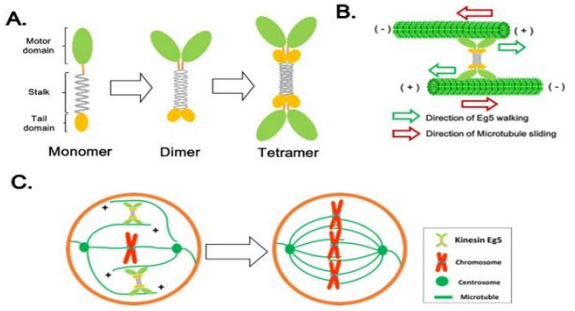

Fig.1 Structure and Physiological role of Mitotic kinesin Eg5. (A) Kinesin Eg5 formed a tetramer in physiological conditions. (B) Tetramer of kinesin Eg5 were crosslinks with anti-parallel microtubule. Microtubules are shifted opposite directions by Eg5 motor activity. (C) In M phase, kinesin Eg5 constitute stable bipolar spindle from microtubule.

[Aim of this study]

The aim of this study is to photoreversibly control bionanomachine, kinesin by artificially introducing photoresponsive protein as a regulatory nanodevice into the functional site of kinesin. In this study, the highly functional photoresponsive protein Aurochrome 1, is introduced into the motor protein kinesin Eg5, which is a biomolecular machine responsible for spindle formation, as a biomolecular machine unit for photodrive or light control, and the movement of kinesin is photocontrolled. Furthermore, for application to living organisms, these photo-regulated kinesin Eg5 are expressed in cells and their effects on photo-reversible cell function are investigated.

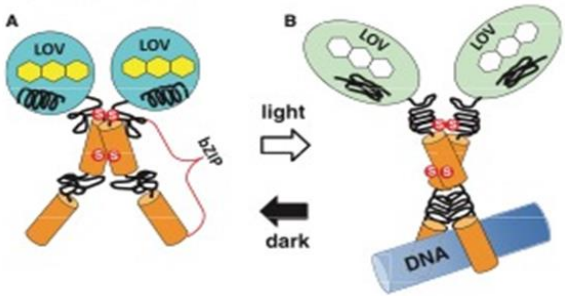

Fig.2 Structure of Eg5-Aurochrome 1 in blue light and dark

Aureochrome-1 (AUREO1) is a blue light (BL) receptor responsible for the BL-induced blanching of a stramenopile alga, Vaucheria frigida. BL induces the dimerization of monomeric AUREO1, which subsequently increases the affinity of this transcription factor for its target DNA.

Material and Method

First, prepare the plasmid DNA of Eg5 fused Aurochrome 1 with His-tag introduced at the C-terminal. Then, according to the method established by Maruta's lab., the expression is expressed using Escherichia coli, and isolation and purification are performed using a Co-chelate column. We also attempt to prepare several fusion proteins with different spacer lengths that connect Eg5 and Aurochrome 1 for efficient photoreversible tetramer formation. Maruta's lab has already obtained the plasmid DNA of Aurochrome 1 and succeeded in incorporating it into the E. coli expression system. Regarding kinesin Eg5, a method for preparing a variant of Eg5 has been established in Maruta's lab. It is at the stage where the preparation can be started concretely.

Results and Discussion

Biochemical analysis of Kinesin Eg5 fused Aurochrome 1 and experiment on the photo-control of monomer-multimer conversion.

1. Absorption spectra and fluorescent spectra measurement In order to confirm whether the photoreversible isomerization of the chromophore in the prepared fusion protein Kinesin Eg5-Aurochrome 1 is retained, the change in the absorption spectrum with light irradiation is measured. In the case of Eg5-Aurochrome 1, the absorption spectrum after light irradiation at each wavelength of 450 nm blue light and in the dark is measured and compared with the absorption spectrum of Aurochrome 1 to see if normal photoisomerization is induced

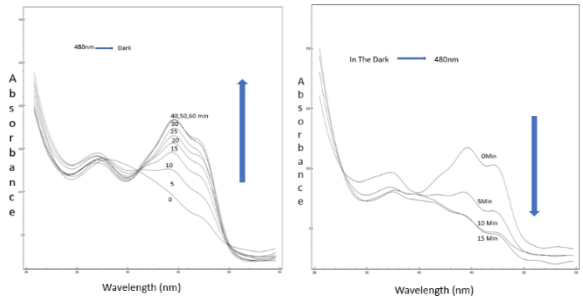

Fig.3 EG5-Aureochrome 1 Absorption Spectrum Measurement.

2. Experiment to show photoreversible control of monomer-multimer conversion

Analysis by size exclusion column chromatography: Eg5-Aurochrome 1 is analyzed using size exclusion column chromatography (SEC) to photoreversibly transfer monomer-tetramers. The change in elution time of Eg5 fused Aurochrome 1 irradiated with 450 nm blue light and in the dark, in SEC-HPLC is analyzed.

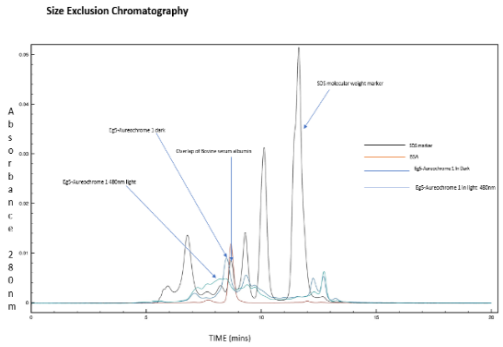

Fig.4 Monitoring Eg5-Aurochrome 1 multimer by size exclusion chromatography

3. ATPaseactivity measurement

In order to investigate whether the motor domain of the recombinant Kinesin Eg5 fused Aurochrome 1 expressed in E. coli retains normal ATPase activity, ATPase activity and microtubule-dependent ATPase activity are measured in the absence of microtubules and are Compare with conventional kinesin.

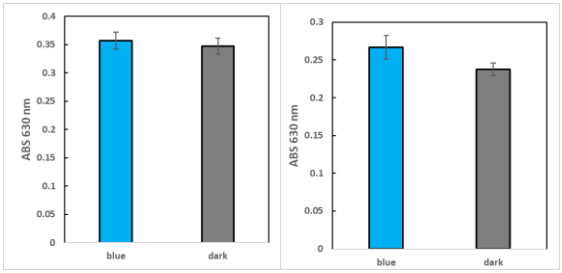

Fig.5 Photo control of ATPase activity in the a) presence of microtubules b) absent of microtubules

Conclusion: We have successfully demonstrated that we can incorporate Eg5-Aurochrome 1, and this enables the reversible control of ATPase activity though not significant and show Multimer formation by blue light radiation and in the dark.

Future Plan

-Making a mutant of eg5-Aureochrome by truncating the amino acid residues present in the basic region of Aurochrome 1 so I can have significance result at the level of ATPase assay.

Refrence:

(1) Istsuki, K; Hiroto, N; and Osamu, H; (2020) Biochemistry 59,2592-2601