Biochemical Characterization of *C. elegans* Kinesin Bmk-1

ATP Driven Motor Protein Research Group
Toru Kurosaka[1)], Fofou Yonta Tostani [1)], Shunsuke Kumagai[1)]
Supervisor Shinsaku Maruta[1)2)]

1) Soka university Graduate School of Science and Engineering, Department of Biosciences, Maruta Laboratory (Laboratory of Bio-nanotechnology)
2) Soka university, Faculty of Science and Engineering, Department of Science and Engineering for Sustainable Innovation

Keywords: Bmk-1, Eg5, ATPase, motility assay, photochromic

1. Background and Aim of Study

Kinesin Eg5 is an ATP-driven motor protein belonging to the kinesin superfamily that plays important physiological roles in cell division, such as centrosome segregation and assembly of bipolar mitotic spindles during pre-finning division. STLC and monastrol are typical Eg5 inhibitors and well studied on their inhibitory mechanism and binding site. These inhibitors bind to the pocket composed of helix,α2,α3 and uniquely elongated loop, L5.

Previously we have developed novel photoresponsive Eg5 inhibitor to control Eg5 function photoreversibly. Caenorhabditis elegans (*C. elegans*) is well known model organism. *C. elegans* has a kinesin Bmk-1 with a structure of elongated L5 like Eg5. Recently overexpression of Bmk-1 was shown to extend the lifespan of *C. elegans*. However, biochemical properties of Bmk-1 have not been clarified yet.

In this study, we prepared motor domain of Bmk-1 using E.coli expression system and analyzed its biochemical characterization. Subsequently interaction of Bmk-1 with Eg5 model inhibitor was examined to apply photochromic inhibitors to Bmk-1. The purified Bmk-1 motor domain exhibited microtubules dependent ATPase activity. Also gliding velocity of microtubules along Bmk-1 adsorbed on a glass surface was measured by the established method of in vitro motility assay. The gliding velocity was much slower than that of Eg5. Interestingly the ATPase and motility activities of Bmk-1 were inhibited by the Eg5 specific inhibitor STLC. It is suggested that although Bmk-1 has different physiological role from Eg5, Bmk-1 may share common inhibitory mechanism with Eg5 for Eg5 inhibitors.

2. Materials and Methods

Preparation of kinesin Bmk-1.

After selecting the sequence containing the motor domain of Bmk-1, pET-21 a was used as the vector and transformed into E. coli DH5α for cloning, and plasmid DNA was extracted from the resulting colony. The obtained plasmid DNA was transformed into E. coli Rosetta 2 (DE3) for protein expression. The expression was confirmed in a small culture and purified in a large culture using a cobalt chelate column.

in vitro motility assay.

The *in vitro* motility assay was performed using a microtubule sliding motility assay, in which fluorescently labeled microtubules are observed moving along a kinesin anchored in a flow cell. For comparison, Eg5 and Bmk-1 were also performed under the same conditions, respectively. Observations were recorded by time-lapse photography of one image every 30 seconds. The recorded data was converted into a continuous image file in tiff. format every 30 seconds using NIH ImageJ, and the moving distance of each microtubule was measured using "Manual tracking". The microtubule sliding velocity was expressed in terms of the flat velocity calculated from the measured moving distance and the observation time, and a velocity distribution was created.

ATPase assay

The reaction solution was made with 120 mM NaCl, 30 mM Tris-HCl (pH 7.5), 2 mM MgCl2, against 0.5 μM kinesin. The reaction was stopped by adding TCA after 15 min, centrifuged at 15 K rpm for 5 min at 4°C, and 50 μl of the supernatant was added with Biomol The reaction was stopped by adding TCA after 15 min and centrifuged at 15 K rpm for 5 min at 4°C. 50 μl of the supernatant was incubated at 25°C for 30 min. The inhibitory effect of STLC was measured by absorbance at 630 nm in a microplate reader. 50 μM of STLC was added to the above reaction solution of kinesin.

3. RESULTS and DISCUSSION

3-1. Purification of Bmk-1.

The Bmk-1 was first mass cultured and purified at 37°C for 4 h. It was expressed on Rosetta2 (DE3) and purified using a cobalt chelate column. SDS-PAGE results showed a band around the predicted molecular weight of 42,000 for Bmk-1, but it was thin and bands were seen at the top and bottom. This may be due to the degradation of Bmk-1 or the formation of dimers. The Bmk-1 was expressed on Rosetta2 (DE3) and purified using a cobalt chelate column (Fig. 1). 24 hours at 18°C resulted in a higher concentration of pure Bmk-1, while 4 hours at 37°C resulted in a higher concentration of impurity. The 4-hour condition at 37°C resulted in a higher concentration of pure Bmk-1 and a higher concentration of impurities.

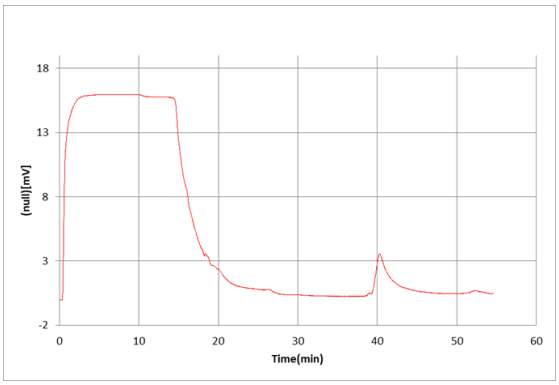
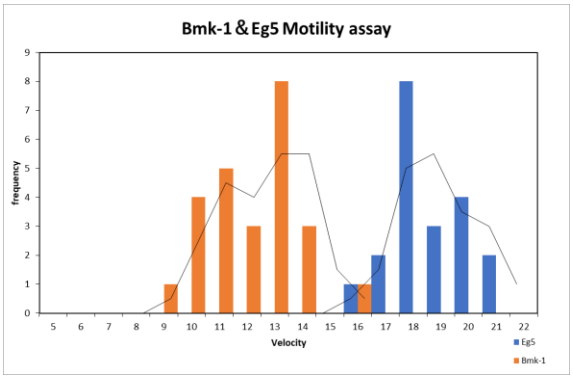


Fig. 1 Elution curve of Bmk-1.

3.-2. In vitro Motility assay for residence chemistry analysis

The in vitro motility assay was performed using the microtubule sliding motility assay, which observes the movement of fluorescently labeled microtubules along kinesin anchored in the flow cell. In vitro motility assay using purified Bmk-1 showed that Bmk-1 runs on microtubules, and compared with its homologue Eg5 (Fig.



2), Bmk-1 moves more slowly than Eg5.

Fig.2 in vitro Motility assay (Bmk-1 and Eg5)

3.-3. In vitro Motility assay for residence chemistry analysis

After polymerization of microtubules, microtubule-dependent ATPase activity assay was performed using purified Bmk-1. It was found that the activity was significantly lower in the absence of microtubules and significantly higher in the presence of microtubules. Next, ATPase activity was measured using STLC inhibitors in the presence of microtubules. Since the inhibitor STLC is a specific inhibitor of Eg5, it could be applied to Bmk-1, so we performed the experiment. The results showed that the activity was significantly lower when STLC was used.

4. CONCLUSION

Cloning of the C. elegans Bmk-1 gene and purification of Bmk-1 revealed that the best incubation temperature is at low temperatures, such as 18°C, and that the ion concentration is not the cause of the cloudiness at the dialysis stage. The results of microtubule-dependent ATPase activity assay also indicated that Bmk-1 is microtubule-dependent. In addition, STLC, a specific inhibitor of Eg5, showed an inhibitory effect on Bmk-1. in vitro Motility assay showed that Bmk-1 slides through microtubules at a slower rate than Eg5. Bmk-1 slides slower in microtubules than Eg5. In the future, we will test whether the photochromic inhibitor of Eg5 developed in Maruta's lab is effective, and attempt to photo-control Bmk-1 in vitro.

5. Future Plane

1 Verify the efficacy of photochromic inhibitors of Eg5 developed in Maruta lab.

2. Attempt to photo-regulate Bmk-1 in vitro.

6. References

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