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In vitro motility of actin filaments powered by plant myosins XI

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ABSTRACT

The actomyosin network is thought to support fundamental processes of plant development and cell expansion such as polarized elongation of root hairs and the diffuse growth of epidermal and mesophyll cells. Inhibition of myosins via pharmacological treatments represents one of the key approaches for understanding of their roles in different cellular processes. However, the use of the standard plant myosin inhibitor, 2,3-butanedionemonoxime (BDM), is questioned as it requires a high concentration and may not be as specific as desired. By testing drugs that inhibit animal and yeast myosins V, the Staiger laboratory previously found pentabromopseudilin (PBP) as a potential inhibitor of plant myosins in vivo. In order to verify PBP as a plant myosin inhibitor in vitro, an actin filament gliding assay powered by chicken Myosin Va (MyoVa) was developed as a positive control using Total Internal Reflection Fluorescence Microscopy (TIRFM). Here, we partially purified a YFP-tagged Myosin XIK from *Arabidopsis thaliana,* and enriched it in the motility assay chamber by an antibody affinity-capture method. The enriched XIK-YFP showed actin binding activity and addition of ATP resulted in detachment of actin filaments (F-actin) from the protein, suggesting that the ATPase domain of the isolated myosin is partially functional. By testing the detachment frequency of myosin-bound F-actin, we demonstrated that PBP could effectively inhibit the ATP-dependent release of F-actin from the isolated XIK-YFP, suggesting that PBP is a potential plant myosin inhibitor.

KEYWORDS

In vitro motility assay, Myosin, pentabromopseudilin, actin, TIRF microscopy