**ABSTRACT**

Blebbistatin is a specific inhibitor of myosin II and a valuable tool for dissecting the roles of myosin II motors. Blebbistatin has fluorescent properties that are modulated by the conformation of myosin. These properties were used to measure the binding affinity and binding kinetics of blebbistatin for myosin under differing enzyme conformations. Our studies indicated that the fluorescent properties of blebbistatin can be used as a convenient reporter of myosin conformations and may provide insight into the binding site of the inhibitor.

**INTRODUCTION**

Blebbistatin is a recently discovered small molecule inhibitor of Myosin II (1). A detailed study on the mechanism of this inhibitor has already been carried out on skeletal muscle myosin and suggests that the inhibitor interferes with the phosphate release step of the enzymatic cycle (2). We have found that blebbistatin inhibits the basal as well as the actin activated ATPase of bovine cardiac myosin subfragment-1 (S1). Additionally, we have also found that blebbistatin has fluorescent properties that are modulated by the conformation of myosin. The specificity of this inhibitor for myosin II in conjunction with the fluorescent properties that are reported here provide a valuable tool for probing the binding interactions of blebbistatin to other myosins and for monitoring conformational changes within these myosins.

**RESULTS**

**Blebbistatin has fluorescent properties that are modulated by the conformations of myosin**

The fluorescent properties of blebbistatin can be used to monitor blebbistatin binding.

**REFERENCES**


**MATERIALS & METHODS**

Materials

- Blebbistatin was obtained from Toronto Research. Bovine cardiac myosin subfragment-1 (S1) and actin were produced by the protein production group at Cytokinetics, Inc. (-)-S-blebbistatin was used for all studies.

Steady state kinetics

- All steady state kinetic measurements were made using an NaDH coupled assay system by monitoring the absorbance change at 340 nm in 10mM Pipes, 2mM MgCl2, 1mM DTT pH 6.8 containing nucleotide (1mM) was rapidly mixed with varying concentrations of cS1 in 10uM final. All spectra were corrected for background subtraction.

Fluorescence spectroscopy

- Fluorescence measurements were carried out on a Spex FluoroMax fluorimeter. The excitation wavelength was set to 434 nm with a 10 nm bandpass. All spectra were corrected for background subtraction.

Rapid kinetic measurements

- All rapid kinetic measurements were carried out on a FluorTec SF-450K stopped-flow apparatus temperature controlled to 25° C. The excitation wavelength was set to 434 nm and emission was collected through a 455 nm long pass filter. On average, three transient traces were collected and averaged together. All data analysis was conducted using the KintinMynt software.

**Figure 1:** The structure of blebbistatin. [Image]

**Figure 2:** Emission spectra of (-)-S-blebbistatin in the presence of bovine cardiac S1 under differing nucleotide states. Experiment was conducted at 10 uM blebbistatin, 5 uM bovine cardiac S1 and 1mM nucleotide. Spectra were background corrected. An enhancement in fluorescence as well as a blue shift are noticeable when blebbistatin binds to cS1 and these properties are further enhanced in ADP and ATP-like states of myosin.

**Figure 3:** Inhibition curves of (-)-S-blebbistatin against the (A) basal ATPase of bovine cardiac S1 (500 uM ATP) and (B) actin stimulated ATPase of bovine cardiac S1 (1000 uM ATP) at 24 uM. These data show that blebbistatin is capable of binding with cS1 and acto-cS1.

**Table 1:** Summary of the binding rates of blebbistatin to cS1 in different nucleotide states. The dissociation constants were computed from the ratio of the off and on rates.