Activation of Cardiac Sarcomere ATPase by CK-1122534, a Small Molecule Agent that Specifically Targets Cardiac Myosin

H Rodriguez, S Sylvester, X Qian, B Morgan, D Morgans Jr, F Malik, R Sakowicz

Cytokinetics, Inc. South San Francisco, CA

Abstract #174

Abstract

Current agents that improve cardiac contractility via second messenger activation and alteration of calcium homeostasis have significant safety drawbacks, likely related to their mechanism of action. A more direct approach to improving cardiac contractility is to activate the force generating enzyme cardiac myosin itself. Here we define the enzymology of such an agent, CK-1122534, a small molecule activator of the cardiac myosin ATPase.

CK-1122534 was characterized in a series of steady-state and transient kinetic biochemical assays. Skinned cardiac myofibrillar preparations and purified cardiac, smooth and skeletal muscle proteins were used.

In cardiac myofibrils, CK-1122534 increased the rate of ATP hydrolysis in the presence of calcium. CK-1122534 also increased the rate of ATP hydrolysis in a preparation consisting only of purified actin and cardiac myosin subfragment-1 (cS1), thus suggesting the target of CK-1122534 may be the motor domain of cardiac myosin. Furthermore, this activator had no detectable activity against myosins from smooth and skeletal muscle. Transient kinetic analysis of individual steps in the actomyosin enzymatic cycle was used to identify the key steps directly affected by the compound. It was found that the mechanism of action of the compound involves acceleration of the weak-to-strong transition of S1 binding to the thin filament without affecting the rate of release of myosin from the strongly bound state. No additional steps in the enzymatic cycle were significantly affected. The mechanism of action of CK-1122534 on the cardiac sarcomere should result in increased force-production without affecting relaxation. CK-1122534 provides a rare example of a small-molecule positive modulator of enzymatic function with a potential for improving cardiac contractility (1).

Introduction

There is much interest in small molecule agents that can modulate myosin function. Blebbistatin is an example of one such agent, it inhibits the ATPase activity of myosin IIs (4). Here we identify a small molecule agent CK-1122534 (figure 1), that specifically ***activates*** the ATPase activity of cardiac myosin. We have characterized the mechanism of action of this compound using steady state an transient kinetic methods.

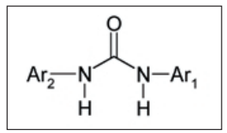


Figure 1: CK-1122534 is the member of an identified diaryl urea family.

Materials & Methods

Materials
CK-1122534 was synthesized at Cytokinetics Inc. All proteins used were produced by the protein production group at Cytokinetics Inc.

Steady state kinetics
All steady state kinetics 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 (PM10) buffering system. Assays were run at 25 °C unless otherwise specified. All measurements were carried out in a Molecular Devices SpectraMax plate reader.

Transient kinetics
Transient kinetic measurements were carried out on a Hi-Tech SF-61DX2 stopped-flow apparatus temperature controlled to 25 °C. On average, three transient traces were collected and averaged together. All data analysis was conducted using the KinetAsyst software.

ATP binding to cS1 was measured by monitoring the intrinsic tryptophan fluorescence of cS1. Nucleotide binding to acto-cS1 was monitored using the change in mantATP fluorescence. ADP release rates were determined by chasing mantADP from cS1 or acto-cS1 with a high concentration of ADP. ATP stimulated release of S1 from actin was monitored using pyrene labeled actin and measuring the change in pyrene fluorescence. Phosphate release was monitored using MDCC modified phosphate binding protein (PBP) (2).

Results

CK-1122534 activates cardiac myosin S1

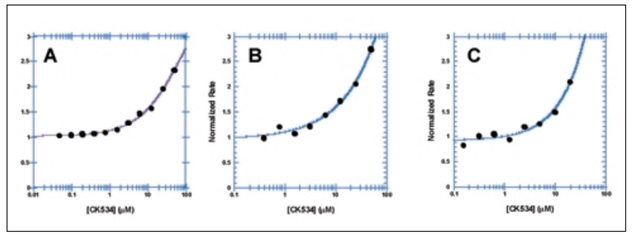


Figure 2: Dose dependent activation curves of CK-1122534 using: (A) purified cardiac actin and cardiac S1 (B) a calcium regulated system composed of cardiac S1 and a reconstituted thin filament and (C) skinned cardiac myofibrils assayed at 50% of maximal calcium activation. Kinetic measurements were obtained using a coupled enzyme system utilizing NADH. Rates are normalized to a DMSO control and corrected for the number of myosin heads.

CK-1122534 is specific for cardiac myosin

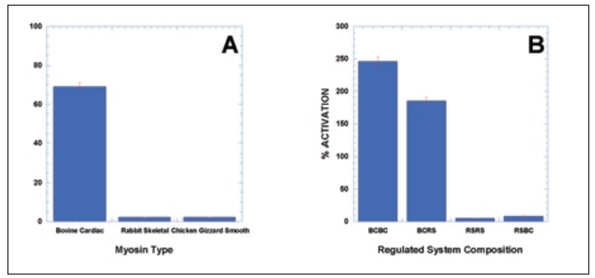
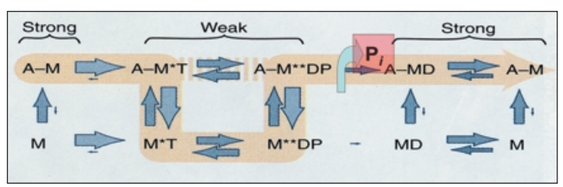


Figure 3: (A) Shows the effect of a single high dose of CK-1122534 (40 μM) on actin-stimulated ATPase of different myosin II types. (B) the effect of CK-1122534 (40 μM) on hybrid cardiac/skeletal Ca-responsive actomyosin systems. This experiment was performed at the 50% of maximal calcium activation. The first two letters denote the myosin source and the last two denote the thin filament source (BC=bovine cardiac, RS=rabbit skeletal). It is evident that only cardiac myosin is activated by CK-1122534 irrespective of the thin filament used.



A=actin T=ATP D=ADP M=myosin DP=ADP*Pi

Figure 4: The generalized kinetic scheme for bovine cardiac myosin (3).

CK-1122534 accelerates the actin dependent Pi release

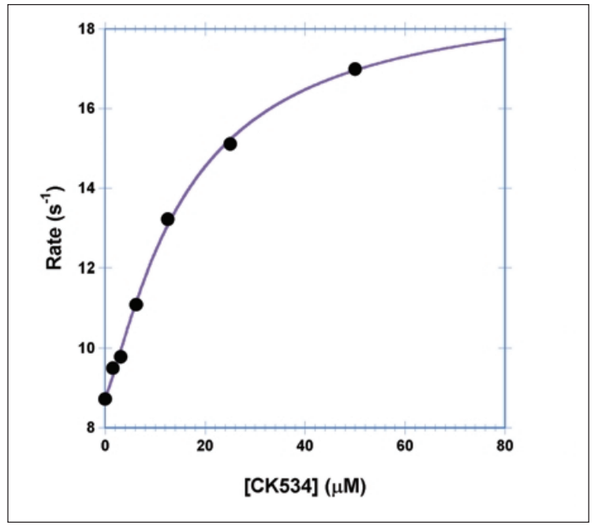


Figure 5: The rate of phosphate release from cardiac actomyosin was measured as a function of compound concentration. The kinetic scheme in figure 4 has the step in question highlighted. The experiment was conducted under a single turnover condition using fluorescence detection. MDCC modified PBP was used as a fluorescent probe.

	DMSO Control	CK-1122534
ATP binding to cS1	$0.92x10^6$ $M^{-1}s^{-1}$	$1.2x10^6$ $M^{-1}s^{-1}$
ATP binding to acto-cS1	$2.3x10^6$ $M^{-1}s^{-1}$	$2.2x10^6$ $M^{-1}s^{-1}$
Pyrene actin release	$2.8x10^6$ $M^{-1}s^{-1}$	$3.2x10^6$ $M^{-1}s^{-1}$
ADP release from cS1	0.4 s^{-1}	0.4 s^{-1}
ADP release from acto-cS1	118 s^{-1}	119 s^{-1}

Table 1: Summary of the rate constants determined for individual kinetics steps in the myosin kinetic cycle. Experiments involving CK534 were conducted at a final compound concentration of 50 μM. Equivalent volume of DMSO was added to no-compound controls

References

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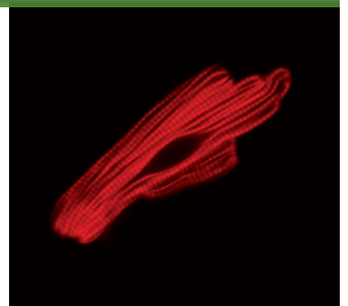
4. Kovacs, M *et al*. Mechanism of blebbistatin inhibition of myosin II. *J Biol Chem* (2004).



Summary/Conclusion

We have shown that the small molecule compound CK-1122534 can activate the ATPase of bovine cardiac myosin in various reconstituted systems and in skinned bovine cardiac myofibrils. Additionally, this activation is specific for cardiac myosin and is not the result of an interaction with the regulatory apparatus of the sarcomere.

Transient kinetic analysis of the individual kinetic steps of cardiac myosin suggests that the rate of phosphate release from the actin associated step is increased in the presence of the compound in a dose responsive manner. No additional steps in kinetic cycle were affected by the presence of CK-1122534.

Additional studies indicate that CK-1122534 increases the contractility of adult cardiac myocytes without affecting the calcium transient consistent with a sarcomere based mechanism (1).

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