Introduction

Eg5, the human kinesin-5 motor protein, is required for formation of the mitotic spindle which is essential for the completion of mitosis. The full length monomer contains ~42 kD N-terminal motor domain that binds both ATP and microtubules. Functioning as a homotetramer, the motor domain of Eg5 binds to microtubule plus ends, enabling it to walk along microtubules and move toward the plus ends of the mitotic spindle. This action is required for a proper spindle assembly and mitosis progression (Ref. 2). Inhibitors of Eg5 lead to mitotic arrest and cell apoptosis. Thus, these proteins are attractive drug targets for antimitotic therapy. Current antimitotic chemotherapeutic drugs target the spindle MTs interfering with spindle dynamics. While effective, they have various side effects, including neurotoxicity.

Cells were harvested by centrifugation and lysed using an Emuliflex. Cell debris was removed by centrifugation, and the supernatant (SN) was loaded onto a 50 mL column. The histidine fusion protein was eluted from the Ni column in wash buffer with 30% buffer B (30%). Fusion protein was digested with tobacco etch virus (TEV) protease before purification using a second ion exchange chromatography run.

The Klp61F-L5 chimera protein crystals we obtained (FigE) need to be improved in order to yield a high-resolution structure of the Klp61F-L5 chimera motor domain–drug complex. Despite using the same crystallization conditions and protocol, there is a noticeable difference from the Klp61F-L5 crystals obtained in previous successful crystallization experiments (FigE). We believe this could be due to a difference in both protein and conditions, but that we are using a different expression construct than that which was used in the prior effective crystallization trials.

The Klp61F-L5 chimera protein crystals from effective crystallization trials are much better for data collection than the crystals obtained from our present experiments. The data collection was performed at room temperature (22°C). However, our understanding of protein functions is limited by our inability to observe key interactions at the atomic level. Atomic level contacts that mediate allosteric inhibition of Eg5 have not been identified. These questions is difficult as there are no reported crystal structures of the Klp61F motor domain for comparison studies. The atomic level contacts mediating allosteric inhibition of Eg5 have not been identified.

We suspect effector binding elicits parallel structural changes in Klp61F–L5 as those that occur in human Eg5. However, answering these questions is difficult as there are no reported crystal structures of the Klp61F motor domain for comparison studies. The atomic level contacts mediating allosteric inhibition of Eg5 have not been identified. Therefore, we sought to determine the crystal structure of the Klp61F-L5 chimera motor domain alone and in complex with ATP. Analysis of these crystal structures may provide insight into the nature of the Eg5 allosteric mechanisms and the mechanism of allosteric inhibition by drugs that bind the L5 pocket.

The mechanism of allosteric inhibition in human Eg5 is conserved in Klp61F, and may also be conserved in other kinesins. Allosteric inhibition may be reconstituted in all kinesins by targeting the corresponding L5 pocket or modeling the corresponding L5 loop after that of Eg5. Allosteric inhibition is a key consideration in drug design.

Methods

Expression constructs were transformed with a modified J47T vector (J47T) into E.coli host strain, and the cells were grown at 27°C for 48 hours. The cells were collected, and the supernatant was loaded onto a 50 mL column.

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Results

The Klp61F-L5 chimera protein crystals we obtained (FigE) need to be improved in order to yield a high-resolution structure of the Klp61F-L5 chimera motor domain–drug complex.

Allosteric inhibition may be reconstituted in all kinesins by targeting the corresponding L5 pocket or modeling the corresponding L5 loop after that of Eg5.

A high resolution structure of the Klp61F-L5 chimera motor domain–drug complex will reveal detailed information on the atomic contacts that mediate allosteric inhibition.

Our results indicate that residue changes in the chimera L5 loop, although conservative, destabilize the chimera protein and alter proteolysis.

Alterations in the protein purification process are needed to improve upon crystallization trials, which have the potential to inform our understanding of protein functions.

Better understanding of kinesin protein function may lead to the development of molecular-targeted therapies for various human cancers.