

INHIBITION OF A MITOTIC MOTOR PROTEIN – WHERE, HOW, AND CONFORMATIONAL CONSEQUENCES

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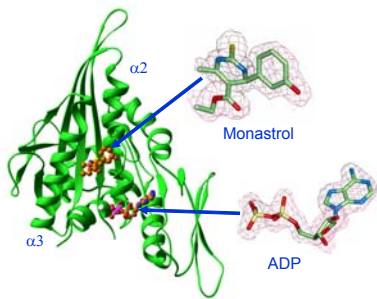
Introduction. Inhibition of the mitotic spindle is among the more successful strategies to control tumor growth in clinical oncology. Mitotic motor proteins, of which some are kinesins, are required for bipolar spindle formation during mitosis. Recently, a small molecule inhibitor of the mitotic kinesin Eg5 has been described.¹⁻⁴ Monastrol arrests mitosis by reversibly inhibiting KSP to perturb bipolar mitotic spindle formation. Prolonged mitotic arrest leads to apoptosis in tumor cells and to senescence or apoptosis in primary cells. Inhibition of KSP thus provides a novel and specific mechanism to target the mitotic spindle. In preclinical models, it results in formation of monoaster spindles leading to mitotic arrest. We report here the first inhibitor-bound structure of a mitotic motor protein. The 1.9 Å resolution structure of the motor domain of KSP, bound with the small-molecule monastrol and Mg^{2+} -ADP, reveals that monastrol confers inhibition by ‘induce-fitting’ onto the protein some 12 Å away from the catalytic center of the enzyme, resulting in the creation of a previously non-existing binding pocket. The structure sheds new insights into the biochemical and mechanical mechanisms of the mitotic motor domain. Inhibition of the KSP provides a novel mechanism to arrest mitotic spindle formation, a target of several approved and investigative anti-cancer agents. The structural information gleaned from this novel pocket offers a new angle for the design of anti-mitotic agents.

Method. KSP and monastrol complex crystals in the presence of ADP (Mg^{2+}) were obtained by the co-crystallization method. A complete data set was collected at APS, IMCA beam line to 1.9 Å resolution at -180°C, $R_{sym}=0.067$. The KSP ternary complex was crystallized in the $P2_12_12_1$ space group with cell dimensions $a = 69.3$ Å, $b = 79.5$ Å, and $c = 159.2$ Å. The KSP structure was determined with the molecular replacement method. The binary structure⁵ of KSP was used as the search model. The final model revealed two protein complexes (r.m.s.d. 0.45 Å) per asymmetric unit, along with 501 water molecules. There was one Mg^{2+} -ADP and one monastrol per protein molecule.

Results. The ternary structure of the motor domain of KSP at 1.9 Å resolution, bound with monastrol and Mg^{2+} -ADP, is shown in Figure 1 in a ribbon representation. Enlarged views of the bound conformation of monastrol and ADP are also given together with their respective electron density.

The ternary complex of KSP maintains the same overall structural features of the binary complex, typical of a kinesin motor. The active isomer of monastrol used in the study is the *S*-enantiomer. Monastrol binds to an induced-fit pocket 12 Å away from the nucleotide site of the protein. The inhibitor pocket is situated between helix $\alpha 3$ and the insertion loop (L5) of helix $\alpha 2$. Lining the newly formed pocket and surrounding the inhibitor are 20 residues. To generate the monastrol pocket, L5 of helix $\alpha 2$ relocates its

main-chain with a downward swing of $\sim 7 \text{ \AA}$. As a result, the side-chain of its W127 re-locates inward by $\sim 10 \text{ \AA}$, capping the entrance of the induced-fit cavity together with the side-chains of R119 (on helix $\alpha 2$) and Y211 (on helix $\alpha 3$). These two side-chains move outward towards the bulk solvent to yield space to accommodate the inhibitor. Helix $\alpha 2$ remains unaltered, and helix $\alpha 3$ shifts along its axis by 1 \AA . The conformational changes of KSP upon monastrol binding are not limited to the induced-fit site. The gap of the V-shaped helices of Switch-2, between R305 and G325, expands by about 6 \AA , resulting in a 20° wider opening. This rearrangement generates a space to allow the C-terminal neck-linker to lock onto Switch-2. In doing so, residues N358 to K362 of the neck-linker rotate by $\sim 120^\circ$ to dock into this enlarged crevice. In all, the concerted movement of Switch-2 and of the neck-linker region is the most significant conformational change of the KSP-ADP complex when monastrol binds.



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Figure 1

Conclusion. The ternary structure of the KSP motor domain reveals that monastrol transforms the insertion loop of helix $\alpha 2$ into a rigidified conformation and the neck-linker/Switch-2 cluster into the ‘locked’ conformation previously observed for some kinesin motors.

Reference

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