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Monastrol stabilises an attached low-friction mode of Eg5

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Monastrol [1] is a small molecule that inhibits a mitotic kinesin called Eg5. In cells, monastrol dramatically arrests mitosis with a defect in the engagement and relative sliding of the two half-spindles [1,2]. We show here that monastrol stabilises a remarkable low-friction attached mode of Eg5 that binds stably to microtubules, yet can be readily slid along them. We will suggest that this mode is part of the normal Eg5 kinetic cycle ('on-pathway'), with significant consequences for the *in vivo* role of Eg5 and for the interpretation of experiments involving monastrol.

Monastrol inhibits Eg5 by an allosteric mechanism, binding to an Eg5–ADP complex and specifically inhibiting ADP release [3,4]. A recent crystal structure of the Eg5–ADP–monastrol ternary complex reveals that monastrol binds just beneath the active site, in a self-created pocket comprising elements of the α2 and α3 helices [5]. Monastrol does not bind to other kinesins. The crystal structure of the monastrol–ADP–Eg5 complex is very similar to that of a previously-determined structure of Kif1a [6] that was interpreted as a strong binding complex. Accordingly, it was suggested [5] that monastrol stabilises a strong binding state of Eg5. In this case, 'strong binding state' refers to a stable, stereospecifically attached state that can bear force, as opposed to a weak binding state that detaches rapidly and cannot bear force. Consistent with the idea that monastrol stabilises a strong binding state, monastrol inhibits Eg5-driven motility in surface sliding assays, but does not cause the microtubules to dissociate from the Eg5 surface [1]. On the other hand, kinetic data indicate that monastrol stabilises an ADP state of Eg5, and Eg5–ADP is reported to be a weak binding state with a low affinity for microtubules [7].

To find out whether the ternary Eg5–monastrol–ADP complex is binding weakly or strongly to microtubules, we ran 'tug-o'-war' motility assays in which microtubules move on mixed surfaces of kinesin (rat brain kinesin 1, kif5b) and Eg5 (*Xenopus* Eg 5.2). Monastrol inhibits only the Eg5 component on these mixed surfaces, allowing us to ask whether the rat kinesin can overcome the frictional resistance due to monastrol-inhibited Eg5. Figure 1 shows that on surfaces of Eg5 alone, microtubules slid at 0.07 μm s^{-1}, while on rat kinesin alone, microtubules slid at 0.56 μm s^{-1}. On mixed surfaces of Eg5 and kif5b, microtubules moved at 0.24 μm s^{-1}, indicating that the more slowly cycling Eg5 motor works as a brake on the rat kinesin-driven motility. When we added monastrol, microtubules on the Eg5 surface arrested, while the velocity of those on the mixed surface approximately doubled to 0.46 μm s^{-1}. We thus find that monastrol decreases the drag force exerted by the microtubule-bound Eg5 heads, implying that the ternary Eg5–ADP–monastrol complex indeed tends to remain attached to microtubules, but that the attachment exerts negligible molecular friction.

Our data thus indicate that monastrol stabilises an Eg5–ADP state or states that bind microtubules but can be slid along them with little frictional resistance. Is this monastrol state on-pathway for the Eg5 ATPase? We reasoned that if monastrol is stabilising and enriching a weakly binding Eg5–ADP state that is on-pathway, then it should be possible to produce monastrol-like inhibition of Eg5 just by adding ADP. For rat kinesin, the affinity of the motor for ADP is similar to that for ATP, so that adding equimolar ADP and ATP reduces velocity by about 50% [8]. For Eg5-driven sliding, the inhibitory effects of added ADP are dramatically enhanced by adding monastrol (Figure 1B). This synergy is consistent with an allosteric mechanism in which monastrol increases the affinity of Eg5 for ADP, thereby stabilising a pre-existing, on-pathway, Eg5–ADP conformation. We supported this interpretation using proteolytic fingerprinting to demonstrate a monastrol-dependent increase in the ADP binding affinity of Eg5 (Supplemental Data).

Supposing that the low-friction Eg5–ADP "monastrol state" is indeed on-pathway for the normal kinetic cycle of Eg5, how would it fit into the kinetic scheme, and what might be its function? Figure 1C shows a possible scheme, in which the "monastrol state" of Eg5 is a composite of on-pathway attached and detached trapped ADP states in rapid equilibrium. Wild-type Eg5 self-assembles into antiparallel tetramers [9]. The proposed on-pathway low-friction state of Eg5 would have interesting consequences for the function of tetramers. Individual two-headed Eg5 molecules are non-processive [10], but an array of connected tetramers would be processive, because attachment would be maintained by low-friction Eg5–ADP heads. In this model, a single power stroke by an individual head within an array can move the whole array along the microtubule. Potentially analogous long-lived low friction states have been reported for the kif1a kinesin [11] and for dynein–ADP–vanadate [12]. By allowing a single power stroke to slide an oligomeric team of connected heads, processivity of the team can be maintained even where individual strokes are appreciably smaller than the 8nm axial distance between successive binding sites on the microtubule.

In vivo, Eg5 tetramers are believed to crosslink pairs of antiparallel spindle microtubules. Kapoor and Mitchison [2] reported that under monastrol treatment Eg5 in the spindle remains fixed while the microtubule lattice treadmills (fluxes) beneath the motor, and suggested that the Eg5

molecules might be anchored to a separate spindle matrix. Our data suggest an alternative possibility, that the bulk population of Eg5 might be attached in a low friction state to antiparallel spindle microtubules, allowing treadmilling to continue without shifting the position of attached Eg5 molecules. Monastrol treatment of pre-assembled spindles collapses the spindles. Our data suggest that this is likely to be due to an active disengagement of antiparallel spindle microtubules, perhaps produced by minus-end-directed spindle motors that in ordinary circumstances oppose and balance the plus-end-directed sliding action of Eg5.

Supplemental data

Supplemental data containing experimental procedures are available at http://www.current-biology.com/supplemental

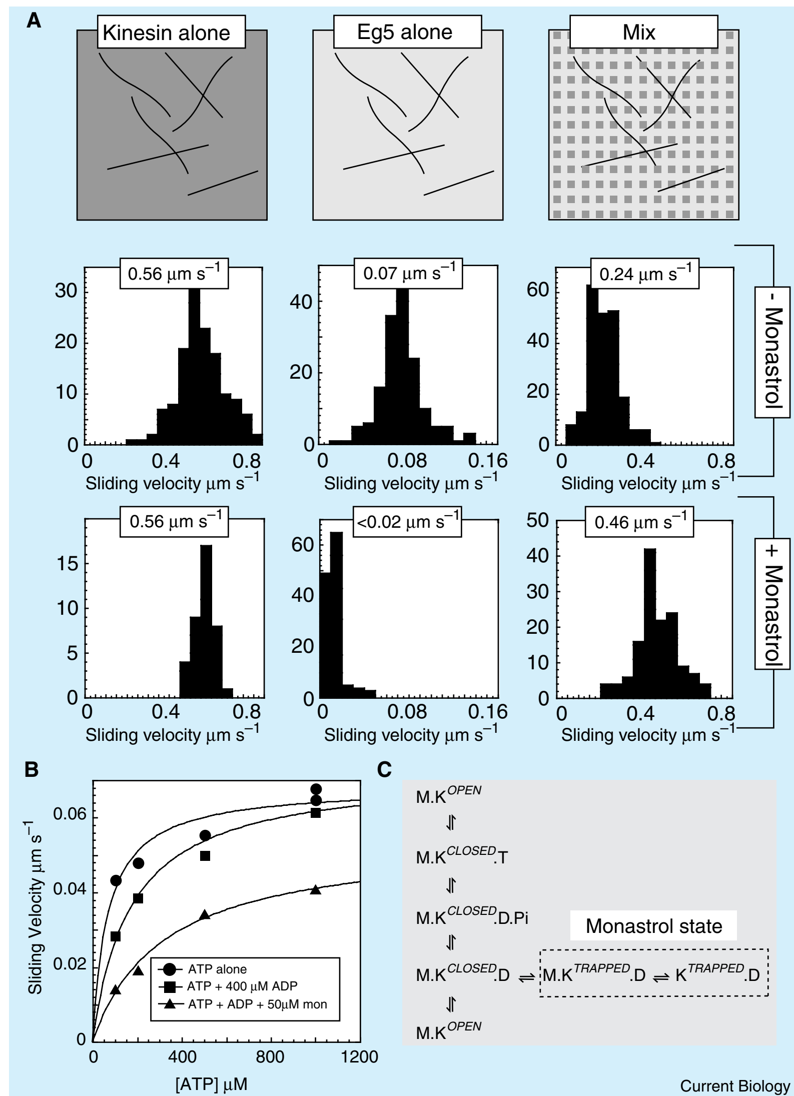

Figure 1. Effects of monastrol in tug-o'-war sliding assays.
Microtubule sliding assays were run in ~20μl flow cells and motility visualised using video-enhanced DIC microscopy. Monastrol inhibition was produced by flowing in motility buffer (MB) containing 400μM monastrol. The inhibition was titratable (Supplemental Data) and reversible on wash-out.
(A) Effects of monastrol on kinesin alone, Eg5 alone , or a 1:1 mix, as indicated.
(B) ATP concentration-dependence of motility in MB or MB plus 400 μM ADP or MB plus 400 μM ADP plus 50μM monastrol. The fits are hyperbolic binding curves, with maxima 0.07, 0.07 and 0.05 μm s-1, respectively, and apparent affinities for ATP 70 μM, 170 μM and 320 μM, respectively.
(C) Kinetic scheme (M = microtubules, K = kinesin). Microtubule binding and release occurs via a composite state composed of free and bound $K^{TRAPPED}$.ADP heads in rapid equilibrium. Monastrol is proposed to enrich this weakly-binding composite state.

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