Single Kinesin Molecules Stressed with Optical Tweezers

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ABSTRACT Using the optical tweezers to pull on microtubules, we have stretched and twisted single kinesin molecules adsorbed to glass surfaces. Preliminary measurements suggest that the mechanical system is very compliant, with an apparent stretch of 120 nm with <2 pN of force. Although measurements of the series compliance of the bead-microtubule structure are still in progress, the kinesin attachment site does not slip with stretch. However, under torsional stress, kinesin appears to slip. With torques <2 pN-μm ~1 Hz in 2 mM AMP-PNP, there is no apparent limit to the number of revolutions that the microtubule can rotate around the kinesin attachment site (n = 44). Preliminary data from other nucleotide conditions are similar. Although there are rare instances of torsional elasticity where the attachment site unwinds, the restoring forces are not constant with angular position, also indicating slippage. Mechanisms of mechanochemical transduction must account for linear force generation in the presence of angular "slippage."

Mechanochemical enzymes such as kinesin move through cyclical interactions with a filamentous polymer in a stereospecific fashion. Kinesin moves toward the plus end of microtubules, so that the relative orientation of the microtubule polymer is critical. Using the optical tweezers to perturb kinesin-microtubule interactions, we sought evidence for the mechanical consequences of stereospecific force generation.

Our strategy is shown in Fig. 1. Limiting concentrations of kinesin (<0.5 ng/ml) are adsorbed to glass coverslips in the presence of casein so that interactions of individual kinesin molecules were studied. Mechanical forces were applied with optical tweezers to particles attached to the microtubules. Two types of mechanical perturbations are possible with this geometry: stretch and twist.

Fig. 2 shows a preliminary estimate of the stretch in the system. As the stalling power of the optical tweezers decreased, the bead slowly displaced until it escaped the maximum force of the optical tweezers at 150 nm (Kuo and Sheetz, 1993). Beyond 250 nm, optical forces on the bead become nominal and the bead-microtubule shortens. Linear extrapolation indicates a "stretch" of 120 nm with <2 pN, suggesting a large series compliance.

Twisting forces produced unexpected results (Table 1). In the rigor-inducing nucleotide analog, AMP-PNP, there was no limit to the number of revolutions (30 max) that kinesin could be twisted. Torques were <2 pN-μm at rates ~1 Hz. After torsional stress (>5 revolutions), microtubules usually did not unwind; unwinding >0.5 revolution within 15 s of release was occasionally observed. However, all torsional stresses caused kinesin to slip. Other rigor-like nucleotide conditions (AMP-PCP or apyrase) were similar.

Since the motor domains of the kinesin molecule form from a dimer of two identical polypeptides, the two domains are arranged with rotational symmetry. These domains must rotate 180° each cycle if they alternately bind to microtubules. The axis of rotation could be within each motor domain or at the center of the whole kinesin molecule. Twisting is an intrinsic part of mechanochemical transduction by an alternating, stereospecific binding model. If twisting were part of the power stroke, then there must be some torsional rigidity for efficient transmission of force.

For these reasons, it is surprising that there is no limit to twisting the kinesin molecule. However, the relaxation of torsional stress may be outside the force-generating domains.

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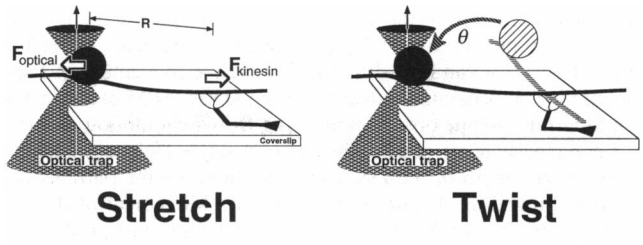

FIGURE 1 Experimental strategy.

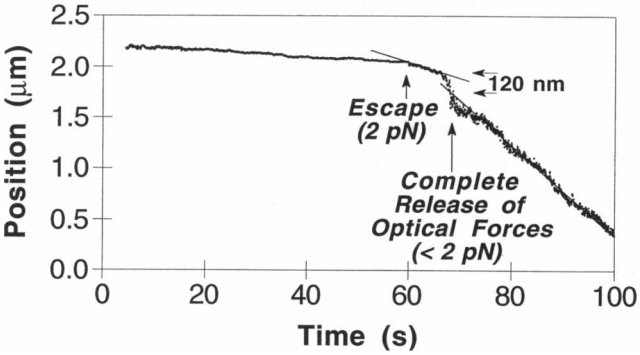

FIGURE 2 Series compliance.

TABLE 1 Responses after twisting single kinesin attachment sites in 2 mM AMP-PNP (n = 44)

Behavior	% Attachments
No unwinding	93%
Unwinding	7%
Limit to twisting	0%

All twists >5 revolutions (30 max). Unwinding is >0.5 rev within 15 s after release.

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REFERENCES

Kuo, S. C., and M. P. Sheetz. 1993. Force of single kinesin molecules measured with optical tweezers. *Science.* 260:232–234.