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# Measurement of the Isometric Force Exerted by a Single Kinesin Molecule

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The past year has brought remarkable advances in instrumentation, making it possible to measure the movements of a single motor protein and the associated forces with nanometer and piconewton precision on a millisecond time scale (Svoboda et al., 1993; Finer et al., 1994; Simmons et al., 1994). Discrete displacement steps were discovered for both kinesin (Svoboda et al., 1993) and myosin (Finer et al., 1994; Ishijima et al., 1994), and the isometric force transients exerted by myosin were measured (Finer et al., 1994). The studies of Svoboda et al. and Finer et al. were made possible by the use of a laser trap that attenuated the Brownian movement of the microscopic position-marking bead tethered to the motor or to its associated filament (for review, see Svoboda and Block, 1994). Without a laser trap, the displacement steps would be buried in the Brownian noise (Malik et al., 1994). A wide range of experiments can now be performed on a single molecule to elucidate the mechanism of the chemomechanical cycle. We have used the instrument developed by Finer and his colleagues (Finer et al., 1994; Simmons et al., 1994) in conjunction with a single-kinesin bead motility assay to confirm previous measurements of the discrete stepping of kinesin along a microtubule (Svoboda et al., 1993), and we have begun making new measurements of the isometric force transients produced by this motor.

In our assay, we used whole sea urchin sperm axonemes extracted in high-salt instead of microtubules from repolymerized tubulin to achieve improved adhesion to the surface of the microscope slide and to maintain the bead further away from the surface. A low concentration of squid brain kinesin was adsorbed onto 1-$\mu$m polycarboxylated latex beads that were precoated with casein; the kinesin concentration was adjusted until approximately half of the beads were translocation-competent, and then increased fivefold to yield a final average number of functional kinesins per bead of about 3–4. Under these conditions, the chances of two or more kinesins interacting simultaneously with the axoneme were less than 1%. In most cases, the ATP concentration was adjusted to 20 $\mu$M to allow sufficiently long pauses between steps, and the laser trap stiffness was set in the range of 0.02–0.06 pN/nm. The $x$ and $y$ displacements of the bead were monitored using a quadrant photodiode detector, and the data were sampled at 2 kHz. The assay system can be modeled as a bead held between a weak spring (the laser trap) and a strong one (an elastic tether between the bead and the motor head).

In exploring the behavior of kinesin, three types of preliminary experiments were conducted. In the first experimental protocol, herein referred to as a translocation experiment, the position of the trap was stationary and the motor pulled the bead away from the center of trap's parabolic potential well as it walked along the axoneme. In the second type of experiment, referred to as an isometric experiment, the position of the bead was "clamped" by feedback control of the optical trap. When the bead was slightly displaced by thermal or motor-induced forces, causing the image of the bead to shift away from the center of the photodiode detector, a rapid feedback signal to a pair of acousto-optic modulators repositioned the optical trap in such a way as to precisely counteract those forces. Because the trap behaves as a well defined spring (Simmons et al., 1994), the magnitude of its displacement needed to immobilize the bead reflects the forces exerted on the bead by the motor (with thermal fluctuations superimposed). Although we use the term "isometric" to describe these conditions, it should be noted that this refers only to the position of the bead; the motor head itself can translocate as far as its elastic tether will permit. In the third type of experiment, a rapid stretch or release of the elastic motor-to-bead linkage was achieved by abruptly repositioning the bead through a jump in the position of the trap along the microtubule. This type of experiment is analogous to rapid release and stretch experiments that have been performed on muscle fibers; however, in this case they were performed on single molecules.

## TRANSLOCATION EXPERIMENTS

Translocation experiments yielded five measurements of interest: step sizes, pause durations between steps, maximal forces, maximal distances traveled, and positional fluctuations about equilibrium position during pauses. To clearly discern individual steps, it was necessary to reduce the average duration of inter-step pauses by lowering the ATP concentration to about 20 $\mu$M.

In our assay, the behavior of the motor can only be inferred from observations of the behavior of the bead and, hence, it was important to characterize the properties of the motor-to-bead linkage. The adsorption of kinesin to the bead is

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nonspecific, and its geometry is poorly defined. It's not clear whether the tail, the stalk, or both are binding to the surface of the bead. However, it was reported that the tether is stretchable and behaves like a nonlinear spring (Svoboda et al., 1993). We performed a tentative calculation of the spring constant based on the equipartition theorem after measuring the mean-squared displacement of the bead from its equilibrium position during pauses between translocation steps. This assumes that the fluctuations in the signal were entirely thermal and therefore amounted to $k_BT/2$ of energy on average. In almost every case, the spring constant was found to lie in the range of 0.2–0.4 pN/nm, meaning that the elastic element of the molecule was about 10 times stiffer than the laser trap. In most cases, the rms noise did not change appreciably as a function of the optical force exerted on the bead. However, we also found many cases in which it decreased to about half of its initial value as the force rose to about 5 pN.

In most cases, stepwise displacement of the kinesin-coated bead was evident. By direct observation of the positional data a wide range of bead-step sizes was apparent. With the laser trap stiffness set at 0.02 pN/nm most of these bead-steps appeared to have a magnitude of about 7–8 nm, but a number of 4–5 and 11–13 nm steps could also be seen. These findings are generally consistent with the conclusions of Svoboda et al. (1993) that kinesin moves from one tubulin dimer to the next. However, the observation of occasional smaller or larger steps raises several other possibilities: kinesin may have a weak affinity for the $\alpha$-tubulin subunit, the motor may have episodes when either one or both of its heads are bound to the microtubule, or the two heads may occasionally interact with adjacent protofilaments while translocating on the seam of a microtubule.

The number of consecutive steps taken before detachment varied inversely with trap stiffness. Because the trap behaves as a linear spring, there appeared to be an approximate threshold load above which the processivity of the motor was lost. In our experiments, this threshold resided around 5–6 pN. It is not clear what property of the assay system is responsible for the existence of a threshold force. One possibility is that greater forces can pull the bead and its motor sufficiently far away from a tubulin binding site during the motor's brief dissociation period to preclude reattachment. This possibility is suggested further by the finding that the maximal forces produced by kinesin were higher when examined under isometric conditions, as described below.

## ISOMETRIC FORCES

In the isometric experiments, the bead was held in place with a tolerance of ~1 nm, while the motor was allowed to develop force. Typical force transients developed slowly and lasted for up to several seconds before the motor released from the axoneme. The maximal forces measured under isometric conditions (5–10 pN) were consistently greater than the maximum force against which a bead could translocate in the translocation experiments. However, the force transient data records showed that the force developed in a discontinuous fashion and that force maxima were rarely reached all at once. Hence, the maximal forces probably do not represent the force associated with the hydrolysis of a single ATP molecule. On the other hand, some abrupt force steps of 1–3 pN were observed, which we believe reflect the mechanics of the molecule during one ATPase cycle. Decreases in isometric force were also observed, which may be due to slippage of the motor. This result is distinct from that obtained from myosin with the same instrument (Finer et al., 1994). In the case of myosin, the force developed rapidly to a maximal value and then vanished, presumably reflecting the transition to a strongly bound, force-producing state followed by the dissociation of myosin from the actin filament. By contrast, in the case of kinesin, the motor usually remained firmly attached to the microtubule after a force increment. This finding is consistent with kinesin's processive movement.

## RAPID RELEASE AND STRETCH MEASUREMENT

In the isometric experiments described above, the bead remains stationary while the motor head is thought to translocate along the microtubule, thereby stretching the linkage. To gain further insight into this phenomenon, we have begun experiments in which the feedback position of the bead is rapidly shifted by a fixed increment along the microtubule during a pause in the motor's activity. Depending upon the direction, this produced a rapid stretch or release of tension in the kinesin-to-bead linkage. So far only 8 nm shifts have been tried. The concomitant change in tension, from an initial force in the range of 2–5 pN, was found to be on the order of 2–3 pN with either a stretch or a release. The measured force change indicates a spring constant of about 0.3 pN/nm for the linkage, which is consistent with measurements from the translocation experiments described above. These results also suggest that many of the force steps taken by the motor correspond to an 8-nm stretch of the linkage and, hence, may be associated with the movement of the motor to a new tubulin binding site. The total stretch of the linkage in the isometric experiments may be greater than 20 nm in some cases, but this may represent the combined effects of stretching the polypeptide and a gradual detachment of part of the kinesin stalk/tail from the bead surface. In contrast to muscle fibers, a rapid release in tension was not followed by a rapid recovery of force. However, our experiments were performed at low ATP concentration, and under these conditions, the motor was very likely paused in a nucleotide-free (rigor-like) state when the rapid releases were carried out.

These results are still preliminary and specific numbers for the displacement- and force-step sizes still await confirmation from additional experiments. Nevertheless, the qualitative features that we observed already provide interesting insights into the behavior of the motor.

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## DISCUSSION

*Session Chairperson:* Margaret A. Titus
*Scribe:* Laura Romberg

DEAN ASTUMIAN: It seems that there are fairly long periods of time where no stepping is taking place, but you still have a pretty significant fluctuation. Has anyone done an analysis where you parcel those second period of time into about 10 ms and analyze the variance in there as a function of time?

CHRIS COPPIN: No.

ASTUMIAN: Because basically what you'd hope to get out of that is the answer to the question of whether you're having ATP binding and then being hydrolyzed and released without making a productive step. With the idea being that when ATP is on, the variance might be different than when ATP is off.

COPPIN: You're talking about a fluctuation like this?

ASTUMIAN: Yes, I'm talking about things like right there, around 1.5 s, for example. It looks to me like there are periods of time which are very constrained, and then less constrained in their motion.

COPPIN: We do see things like this that look like a little dip. We don't see systematic changes or rapid changes in the RMS noise in the trace.

ASTUMIAN: And so you've actually looked and done some nice. . . .

COPPIN: Qualitatively.

ASTUMIAN: OK. Because it would probably take some fairly heavy statistical analysis of the data, but you would be expecting a bimodal distribution.

COPPIN: Yes. Thank you. That's a good idea.

VINCENZO LOMBARDI: When you make your stepping length, and you record stepping force, did you try to see if there is a linear relationship between the size of the length step and the size of the force step?

COPPIN: Not yet.

STEFAN HIGHSMITH: Can the dwell time, the time between steps, be accommodated to the biochemical cycle rates?

COPPIN: We haven't actually measured the ATPase activity under these identical conditions, but that's something we certainly plan to do, and will try to correlate the two.

DAVID WHITE: But the answer is yes in principle it can be, because you could do those transients, and Justin (Molloy) will show you those on myosin this afternoon.

STEVE BLOCK: Chris, this harks back to a conversation we had earlier, but I thought it might be useful to share it with other people. The forces that you see when you go into the isometric clamp situation are somewhat higher—7, 8, 9 pN—than the stall forces that have been seen in steady state measurements by Joe Howard's group and my own group, of about, say, 6 pN. I was wondering if you wanted to comment on why you see this discrepancy and what you think might be going on.

COPPIN: As I see it, there are at least two possibilities. One is simply that for some unknown reason, the head can produce more force when there's an active resistance. I don't have any model to explain that. One easier possibility is that if there is such a thing as a powerstroke, that powerstroke could take place faster than the feedback. So that here's my motor head and here's the trap trying to counteract the force (he demonstrates). It's moving step, step, step, and then it reaches the normal stall force. If it can make one step that's really fast, faster than the feedback, it's going to make one more step, and raise the force maybe from 5 to 5.2 or 5.3 pN or so. And then the feedback pulls the bead back to where it was, and you can get an extra 2.5 pN or so of force out of that. That's one possibility, and we'll have to control for that.

JOE HOWARD: Chris, what do you measure in the orthogonal direction? Do you see steps in the orthogonal direction? Or is it possible that the different forces are because it's moved over to a different protofilament? And so there's a different spacing between the motor and its binding site on the filament.

COPPIN: If we manage to pick an axoneme that's lined up with one of the axis of the quadrant photodiode detector, then there are no steps at all in the orthogonal direction. Sometimes, there a very low-frequency drift, but there are no steps that correlate with what we see in the longitudinal direction.

TOSHIO YANAGIDA: Recently, we have measured the force from a single kinesin molecule with a microneedle, a different method, and we found that the peak force is about 9 pN or so, so that our results are consistent with his results.