Stepping and Stretching

HOW KINESIN USES INTERNAL STRAIN TO WALK PROCESSIVELY*

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The ability of kinesin to travel long distances on its microtubule track without dissociating has led to a variety of models to explain how this remarkable degree of processivity is maintained. All of these require that the two motor domains remain enzymatically “out of phase,” a behavior that would ensure that, at any given time, one motor is strongly attached to the microtubule. The maintenance of this coordination over many mechanochemical cycles has never been explained, because key steps in the cycle could not be directly observed. We have addressed this issue by applying several novel spectroscopic approaches to monitor motor dissociation, phosphate release, and nucleotide binding during processive movement by a dimeric kinesin construct. Our data argue that the major effect of the internal strain generated when both motor domains of kinesin bind the microtubule is to block ATP from binding to the leading motor. This effect guarantees the two motor domains remain out of phase for many mechanochemical cycles and provides an efficient and adaptable mechanism for the maintenance of processive movement.

Members of the kinesin family of molecular motors are capable of taking over 100 steps on their microtubule track without dissociating, a feature that would be necessary for a transport motor that operates in isolation (1–6). A variety of kinetic, structural, and mechanical studies have revealed that this processive behavior requires that two motors of kinesin remain in different structural and enzymatic states during a processive run (7–10, 11, 12). This would ensure that, at any given time, at least one of the two heads would remain strongly attached to its track, preventing the motor from prematurely detaching. Such coordination requires a way for the two motor domains to communicate their structural states to each other while walking processively. Several lines of evidence suggest that this allosteric communication is mediated through the internal load generated when both heads attach to the microtubule (1, 13–16). As illustrated below in Fig. 1, kinesin initiates its mechanochemical cycle with its attached head (green) nucleotide free and its tethered head (magenta) containing ADP in the active site. ATP binding to the attached head reorients its neck linker (blue), which swings the tethered head forward to the next tubulin-docking site. ADP is then released from the new, weakly bound leading head (magenta) to produce an intermediate in which both heads are strongly bound to the microtubule. This situation would generate rearward strain on the neck linker of the leading head, depicted as a left pointing arrow, and forward strain on the corresponding structure of the trailing head, depicted as a right pointing arrow.

It has been proposed that this strain generates processivity by accelerating release of the trailing head (13, 17). In this mechanism, release of the ADP-containing trailing head would be very slow in the absence of forward strain and fast in its presence. In such a system, the greater that forward strain accelerates \( k_{\text{DPM}} \), the greater the degree of processivity. However, there is an internal inconsistency with this scheme. If kinesin’s processivity were dependent solely on this mechanism, the motor would dissociate from the microtubule after only a few steps. The reasoning behind this is illustrated in Fig. 1. We have recently shown (18) that the effective rate of trailing head dissociation \( (k_{\text{DPM}} \approx 50 \text{ s}^{-1}) \) is appreciably slower than that for ADP release \( (k_{\text{ADP}} = 170 \text{ s}^{-1}) \), this work, and Refs. 8, 19–21, and ATP hydrolysis \( (k_{\text{H}} = 100 \text{ s}^{-1}) \), Refs. 8, 19, 21, 22). This would lead to accumulation of a kinesin intermediate with both heads attached to the microtubule, with the leading head nucleotide free, and with ADP-P, in the active site of the trailing head. Given millimolar intracellular ATP concentrations and an apparent second order rate constant of \( >1 \mu\text{m}^{-1}\text{s}^{-1} \) (2, 8, 15, 19, 20, 23), ATP would then rapidly bind to the new leading head \( (>1000 \text{ s}^{-1}) \) and be hydrolyzed. This would generate an intermediate with both heads weakly bound, and dissociation would rapidly follow, as indicated in Fig. 1 by the red X. The fact that kinesin is highly processive (1–6) argues that there is a mechanism that prevents it from proceeding down this pathway.

An alternative possibility is that rearward strain on the leading head slows ATP binding and subsequent hydrolysis, insuring that the leading head would remain strongly attached until the trailing head had dissociated. ATP would then rapidly bind to the leading head and cause the trailing head to swing forward to the next tubulin-docking site. Processive movement would be favored, because the rate of this forward stepping movement, at \( \approx 800 \text{ s}^{-1} \), is nearly sixteen times faster than the rate of trailing head dissociation (18). Furthermore, blocking ATP binding to the forward head while it was experiencing rearward strain would prevent the motor from proceeding down the pathway marked by the red X in Fig. 1.

Determining whether processivity depends on the first mechanism, the second, or to some degree on both requires the ability to unambiguously measure the rates of key steps in the mechanochemical cycle and the effects of strain on these rates. These include the rates of trailing head dissociation, of ADP

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release from the tethered head after it attaches to the microtubule, and of ATP binding to the new, leading head. In this study, we apply the spectroscopic approaches developed in our previous study (18). K413BIO constructs served as a PCR template to amplify the kinesin insert with the following primers: upstream, 5'-AGATATCAGATGCGGACA-
CCTGACC-3'; downstream, 5'-AAGTTCAGTTGCTCCAGAAATTT-
CTATAAATCTCAAT-3'. The underlined sequences are the NdeI and 
XhoI restriction sites, respectively. The fragment was cloned into 
pcR2.1-TOPO (Invitrogen, Carlsbad, CA) and excised with NdeI and 
XhoI. The fragment was purified and then ligated into a pET-21 vector 
without an in-frame biotinyl transferase recognition peptide sequence 
at the XhoI (carboxyl terminus) site. The resultant construct was verified 
by sequence analysis.

**In Vitro Motility Studies of K413BIO**—Single molecule kinesin bead 
motility assays were performed essentially as described previously (15). 
The experiments in this study utilize a cysteine-light dimeric 
kinesin construct (K413), and it was necessary to establish that 
this kinesin construct is processive in this motor. Further-
more, the tethered biotin transferase recognition sequence 
was generated as described in our previous study (18). K413BIO was generated as described in our previous study (18). K413BIO is a 
construct in which K413W340F is fused at the carboxyl terminus to a 
coiled coil (neck linker), which throws the tethered biotin, was incorporated into the K413 sequence to allow for 
the attachment of the motor to streptavidin-coated beads used in motility 
assays in vitro.

1. **Stepping and Stretching of Kinesin**

The plasmid containing the K413W340F mutant kine-
sin construct served as a PCR template to amplify the kinesin insert with the following primers: upstream, 5'-AGATATCAGATGCGGACA-
CCTGACC-3'; downstream, 5'-AAGTTCAGTTGCTCCAGAAATTT-
CTATAAATCTCAAT-3'. The underlined sequences are the NdeI and 
XhoI restriction sites, respectively. The fragment was cloned into 
pCR2.1-TOPO (Invitrogen, Carlsbad, CA) and excised with NdeI and 
XhoI. The fragment was purified and then ligated into a pET-21 vector 
without an in-frame biotinyl transferase recognition peptide sequence 
at the XhoI (carboxyl terminus) site. The resultant construct was verified 
by sequence analysis.

2. **EXPERIMENTAL METHODS**

**Generation of K413W340FBIO**—K413W340F, a cysteine-light recombi-
nant kinesin construct with tryptophan 340 replaced by phenylalanine, 
consists of the first 413 amino-terminal residues of human kinesin, and 
was generated as described in our previous study (18). K413W340FBIO with 5-(((2-iodoacetyl)amino)ethyl)aminonaphthalene-1 
sulfonic acid or tetramethyl rhodamine maleimide was carried out as described previously (15, 18, 23). Labeling of phosphate-binding protein 
by MDCC was carried out as described previously (26). Transient ki-
netic measurements were made in an Applied Photophysics SX 18 MV 
stopped-flow spectrometer with instrument dead time of 1.2 ms as 
described previously (15, 18, 23). Unless otherwise described, complexes 
of microtubules and microtubules were formed with a 5- to 10-fold molar 
excess of microtubules over active sites. ADP was added to 3 mM 
in phosphate buffer containing 80 mM PIPES, pH 6.9, 50 mM potassium 
acetate, 4 mM MgCl₂, 2 mM dithiothreitol, 1 mM EGTA, 7 μM Taxol, 
and various ATP concentrations, and 2 mg/ml bovine serum albumin as a 
blocking protein. The beads were diluted to 80 fm, and final kinesin 
dilutions were chosen such that, on average, fewer than half of the 
beads moved (typically 1:500,000 to 1:1,000,000 from ~10 μm stock). An oxygen-scavenging system (37) was added to the kinesin:bead mix-
ture just prior to flow assay.

**Fluorescence Methodologies**—Labeling of K413W340F and 
K413W340FBIO with 5-(((2-iodoacetyl)amino)ethyl)aminonaphthalene-1 
acid or tetramethyl rhodamine maleimide was carried out as described 
previously (15, 18, 23). Labeling of phosphate-binding protein 
by MDCC was carried out as described previously (26). Transient ki-
netic measurements were made in an Applied Photophysics SX 18 MV 
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ture just prior to flow assay.

**RESULTS**

The experiments in this study utilize a cysteine-light dimeric 
kinesin construct (K413), and it was necessary to establish that 
this kinesin construct is processive. In our previous study, we 
provided enzymatic evidence that K413 is capable of undergo-
ing multiple enzymatic cycles per diffusional encounter with the 
microtubule, a prerequisite for processivity (18). Furthermore, 
attaching the biotin transferase recognition sequence 
onto the carboxyl terminus of K413 had no appreciable effect on k_cat.
lished that K413BIO was processive, we decided to use it to test
mean run length is reduced by a factor of 2
strain inhibits ATP binding to the leading head.

in vitro
decay revealed a mean run length of 276
and fitting data from all ATP concentrations to an exponential
lengths for K413BIO. Run length did not vary beyond experi-
B
laboratory (6, 27). Fig. 2

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in vitro
ior, we examined the
processivity are indirect. To directly assess processive behav-
imental error over the range of ATP concentrations examined,
fitting to an exponential decay revealed a mean run length of 276 ± 22 nm.

(20 ± 2 s⁻¹), K0.5,MT (0.30 ± 0.1 µM), or k0,ADP (1.28 ± 0.15
µM⁻¹s⁻¹) (data not shown). However, enzymatic measures of
processivity are indirect. To directly assess processive behavior, we examined the in vitro motility properties of K413BIO.

As with wild-type kinesin, average in vitro velocities showed a Michaelis-Menten dependence on ATP concentration, defining values of Vmax and Km,ATP of 703 ± 73 nm/s and 23 ± 9 µM, respectively (Fig. 2A). These compare with values of 650–800
nm/s and ~80 µM for wild-type squid kinesin measured in our
laboratory (6, 27). Fig. 2B illustrates the distribution of run
lengths for K413BIO. Run length did not vary beyond experimental
error over the range of ATP concentrations examined,
and fitting data from all ATP concentrations to an exponential
decay revealed a mean run length of 276 ± 22 nm. Thus, although in vitro velocities of K413BIO are similar to wild-type, mean run length is reduced by a factor of 2–3. Having established that K413BIO was processive, we decided to use it to test the two models of how kinesin uses strain to walk processively: that forward strain accelerates trailing head release or that rearward strain inhibits ATP binding to the leading head.

Evaluation of the Effect of Forward Strain on the
Trailing Head

Dissociation of K413BIO: Microtubule by ATP—If forward
strain on the trailing head accelerates its release, then we
would predict that release of the trailing head of a processively
moving kinesin dimer should be faster than that for a non-
processive monomeric construct, because the latter is incapable
of generating internal strain. The steps leading to ATP-induced
dissociation from the microtubule are summarized in Fig. 1. In
this scheme, the observed dissociation rate, λATP, depends on
the binding constant for ATP (KATP) and on the effective rate
constant for ATP-induced dissociation, kₑ. The value of kₑ
depends in turn on the values of the forward rate constants for
the two irreversible steps that lead to dissociation, ATP hydrol-
ysis (kₕ), and subsequent dissociation from the microtubule
(kd,MT). Under these conditions, λATP is defined by the following,

\[
\lambda_{ATP} = \frac{K_{ATP} \cdot [ATP]}{K_{ATP} + K_{ATP}} \cdot (k_e) (\text{Eq. 1})
\]

where \(k_e = \frac{(k_h k_{d,MT})}{(k_h + k_{d,MT})}\).

If strain accelerates trailing head dissociation, \(k_e\) should be
greater for K413BIO than for K349. We measured the rate of
trailing head dissociation for K413BIO and compared these
results to those for monomeric K349. For K413BIO, this was
accomplished using two spectroscopic probes, AEDANS and
TMR, whose use we have previously described (18), whereas for
K349 we utilized AEDANS and turbidity. The solid line in Fig.
3 depicts the fitting of the AEDANS data (open boxes) for
K413BIO to Equation 1, whereas the dotted line depicts the
corresponding fitting of the AEDANS data (open diamonds)
for K349. It is immediately apparent that \(\lambda_{ATP}\) is consistently
faster for monomeric kinesin than for trailing head dissociation
by dimeric kinesin, the opposite of what would be expected if
strain accelerated trailing head dissociation.

The AEDANS probe monitors motor-microtubule associa-
tion, and results with this probe for K349 are nearly identical
to those using turbidity (data not shown). For K413BIO, the
TMR probe monitors neck linker-neck linker reassociation,
a step whose rate we have shown is kinetically controlled by
dissociation of the trailing head (18). We would therefore
predict that results using the TMR probe (closed triangles)
should be superimposable on those using the AEDANS probe
(open boxes), and Fig. 3 confirms this. Table I summarizes the
values of \(K_{ATP}\) and \(k_e\) for both probes. Nearly identical results were
seen at 10 mM KCl (data not shown).

Phosphate Release by K413BIO-Microtubule—Phosphate re-
lease occurs concomitantly with trailing head dissociation (1, 2,
20). Therefore, measuring its release kinetics should provide an
independent measure of \(k_e\). We accomplished this by mixing a
complex of K413BIO-microtubules with a range of ATP concentrations in the presence of a fluoroscently labeled phosphate-binding protein (28) and compared our results to K349. The resulting fluorescence transient consisted of an initial exponential, burst phase, followed by a linear increase, which could be described by the following relationship,

\[ F(t) = A \exp(\lambda_p t) + k_p t \]  
(Eq. 2)

where \( F(t) \) is the time-dependent fluorescence, \( \lambda_p \) is the rate of phosphate release in the burst phase, and \( k_p \) is the steady-state rate at the microtubule concentration achieved after mixing in the stopped flow. If phosphate release were tightly coupled to dissociation, values of \( \lambda_p \) and \( \lambda_{ATP} \) should be nearly identical. This is confirmed in Fig. 3 for both K413BIO (open circles) and K349 (closed circles). Furthermore, the extrapolated value of \( \lambda_p \) at infinite [ATP], \( \lambda_{ATP} \), should be essentially identical to \( k_p \). As Table I indicates, this is the case for both monomeric and dimeric kinesin constructs. Finally, our results with phosphate release kinetics confirm the results from turbidity and fluorescence, namely that the effective rate constant for trailing head dissociation is not accelerated by forward strain.

**Dissociation of K413BIO-Microtubule by ADP—**In the absence of added nucleotide, kinesin attaches to the microtubule via only one motor domain (7, 8, 29, 30). This is illustrated in Fig. 1 by the upper left kinesin-microtubule complex. If ADP is added, it will bind to the empty catalytic site of the attached (green) head and dissociate the complex (17). This reaction would occur in the absence of internal strain, because ADP does not induce forward stepping and strong attachment of the other, tethered (magenta) head. We would, therefore, predict that the kinetics of K413BIO and K349 dissociation from the microtubule should be identical. This is confirmed by comparing the solid (K413BIO) and dashed (K349) curves in the inset of Fig. 4. ADP-induced dissociation was monitored by turbidity and by the AEDANS probe. Data for turbidity (closed symbols) and AEDANS (open symbols) are nearly identical and showed a hyperbolic dependence on [ADP] for both K413BIO and K349, defining maximum rates and apparent dissociation constants of 201 ± 27 s⁻¹ and 154 ± 58 μM and 219 ± 16 s⁻¹ and 123 ± 19 μM, respectively.

ADP dissociates wild-type kinesin from the microtubule at a rate of 12 s⁻¹ (17, 31). This is considerably slower than \( k_{cat} \) under processive conditions, under conditions where strain would be present, and this finding has been used to support the argument that trailing head dissociation is accelerated by forward strain (17). If processivity depended solely on this mechanism, it would follow that any mutation in kinesin that accelerates the rate of ADP-induced dissociation in the absence of strain should reduce average run length proportionally. Nevertheless, our data show that, although the rate of ADP-induced dissociation is nearly 19-fold larger than for wild-type, mean run length is only reduced 2- to 3-fold (Fig. 2).

**Evaluation of the Effect of Rearward Strain on the Leading Head**

We next set out to examine the effect of rearward strain on the leading head by measuring the kinetics of 2'-deoxy-mant-ATP (2'dmT) binding to a K413BIO-microtubule complex. Binding of 2'dmT was monitored by FRET from kinesin tyrosine residues to the mant fluorophor, as previously described (18), and the experimental design is illustrated in Fig. 5A.

In the absence of microtubules, binding of 2'dmT to nucleotide-free K413BIO produced a fluorescence increase characterized by a single phase (Fig. 5B, -microtubules). The rate depended hyperbolically on [2'dmT], defining a maximum of 1033 ± 153 s⁻¹ (Fig. 5B, inset, dotted curve). By contrast, mixing a 1:10 K413BIO-microtubule complex with 2'dmT produced a fluorescence increase that occurred in two distinct phases of similar amplitudes, separated by a lag (Fig. 5B, +microtubules). The rate of the first phase showed a hyperbolic dependence on 2'dmT concentration, defining a maximum rate of 457 ± 56 s⁻¹, an apparent affinity of 80 ± 49 μM, and an apparent dissociation rate constant of 107 ± 50 s⁻¹ (Fig. 5B, inset, solid curve). The amplitude of this phase is approximately half of that for an equal concentration of K413 in the absence of microtubules. Repeating these experiments in the presence of microtubules alone produced no fluorescence change (data not shown). These findings led us to conclude that the first phase in this transient is due to 2'dmT binding to the attached, nucleotide-free head. The rate of the second rising phase also showed a hyperbolic dependence on [2'dmT], defining a maximum rate of 39 ± 4 s⁻¹ and an apparent affinity of 39 ± 10 μM (Fig. 6, inset, dotted curve). Given that the amplitudes and the apparent affinities of the two phases of the fluorescence transient are similar, we propose that the second phase in the transient is due to binding of 2'dmT to the leading head of a doubly attached kinesin-microtubule complex.

Why is the rate of ATP binding to the leading head so much higher than the rate of ADP binding to the trailing head?
slowly than that for the trailing head? One possibility is that it is rate-limited by the dissociation of bound ADP. To determine if this is the case, we measured the rate of 2'dmD dissociation from the tethered head by mixing a complex of K413BIO-2’dmD plus a 10-fold molar excess of microtubules with varying concentrations of ATP in the stopped flow. The resulting fluorescence transient consisted of a single falling phase whose rate depended hyperbolically on ATP concentration, defining a maximum rate constant of 170 ± 17 s⁻¹ (Fig. 6, inset, solid curve). This is over four times faster than the rate of binding of 2’dmT to this head (Fig. 6, inset, dotted curve). Thus, nucleotide binding to the leading head of a doubly attached kinesin-microtubule complex is rate-limited by some process other than ADP release, and we propose that this process consists of a rearward strain imposed on this head.

We can test our hypothesis that ATP binding to the leading head is strain-inhibited by examining the effect of AMPPNP on ADP-induced kinesin dissociation. Adding AMPPNP to a kinesin-microtubule complex induces the two neck linkers to separate from each other, in a manner similar to what is seen when kinesin takes a forward step with ATP binding (18). This occurs hand-in-hand with an acceleration of ADP release from the tethered head (8, 19) and leads to strong binding of both heads to the microtubule (29, 30) and to immobilization of both neck linkers (18). Furthermore, at equilibrium, the stoichiometry of nucleotide binding is 1 mol of AMPPNP:2.4 mol of active sites (32). Taken together, these results indicate that AMPPNP binding to the tethered head causes both heads to bind strongly, with the trailing head containing AMPPNP, with the leading head nucleotide-free, and with both heads under strain. This is illustrated in the left half of Fig. 7A. Furthermore, adding ADP to this system will cause one of the two heads to dissociate, leaving one head strongly bound, as illustrated in the right half of Fig. 7A (29, 30). If rearward strain inhibits nucleotide binding to the leading head, we would predict that mixing a kinesin-microtubule complex plus 1 mM AMPPNP in the stopped flow with ADP will dissociate from the leading head very slowly when compared with ADP-induced dissociation in the absence of AMPPNP (Fig. 4).

We measured the kinetics of leading head dissociation by mixing a complex of 1:10 AEDANS-labeled K413BIO-microtubule plus 1 mM AMPPNP with a range of ADP concentrations, as illustrated in Fig. 7A. An example of the fluorescence transient produced by mixing with 400 μM ADP is depicted as the red jagged curve in the figure. Its rate demonstrated a hyperbolic dependence on ADP concentration, defining a maximum of 0.28 s⁻¹, nearly three orders of magnitude slower than seen in the absence of AMPPNP (Fig. 4, inset). The apparent second order rate constant for this process, at 0.016 μM⁻¹ s⁻¹, compares to a value of 1.31 μM⁻¹ s⁻¹ in the absence of AMPPNP (Fig. 3, inset). Similar results were also seen using the rhodamine probe (data not shown).

To be sure that the fluorescence changes detected with the AEDANS probe are indeed due to the effects of ADP binding, we directly measured the kinetics of 2’dmD binding to a 1:10 kinesin-microtubule complex in the presence of 1 mM AMPPNP as described above (Figs. 5 and 6). As shown in Fig. 7B, the rate of the fluorescence rise produced by mixing with 400 μM 2’dmD (final concentration), at 0.29 s⁻¹, was nearly identical to the rate of the fluorescence decrease seen with the AEDANS probe.

DISCUSSION

The most significant finding of this study is that strain appears to affect one discrete step in the kinesin mechanochemical cycle: binding of ATP to the leading head. This conclusion is supported not only by direct evidence from 2’dmT binding kinetics (Figs. 5 and 6) but also from the effect of AMPPNP on nucleotide binding and nucleotide-induced dissociation (Fig. 7). If rearward strain effectively blocks ATP bind-

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**Fig. 5.** Kinetics of nucleotide binding to a processively moving kinesin dimer. A, depiction of the expected changes in mnt fluorescence emission produced by mixing a 1:10 K413BIO-microtubule complex with 2’-deoxy-mant-ATP (2’dmT) in the stopped flow. The fluorescent nucleotide is depicted by the magenta labeling, and the mnt fluorescence emission is represented by the blue rays emanating from each motor domain. B, fluorescence transients produced by mixing a 1:10 K413BIO-microtubule complex (red transient) or an equimolar concentration of nucleotide-free K413BIO (green transient) with 100 mM 2’dmT (final concentration) in the stopped flow. Although the transient for K413BIO could be fit to a single-exponential process with a rate constant of 362 s⁻¹, which in the presence of microtubules consisted of two distinct phases separated by a lag. The smooth curve is the fit to two exponential processes plus a lag, with rate constants of 328 and 27 s⁻¹ and an amplitude ratio of 0.58:0.42. Inset, plot of the rate of the faster phase (closed boxes and solid curve) for K413BIO-microtubules versus 2’dmT, compared with corresponding data for K413BIO alone (open boxes and dotted curve).

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**Fig. 6.** Comparison of kinetics of 2’dmD release to 2’dmT binding to the leading head. The fluorescence transient produced by binding of 2’dmT to processively moving kinesin is reproduced from Fig. 6 as the green transient in this figure. This is compared with the fluorescence transient associated with 2’dmD release from the leading head. This reaction fits a single-exponential decay whose rate constant showed hyperbolic dependence on [ATP] (inset and solid curve), defining a maximum rate of 170 ± 17 s⁻¹. Fitting of the data for 2’dmT binding (green transient) to two exponential terms plus a lag demonstrated that the slower phase also fits a hyperbola, defining a maximum rate of 39 ± 4 s⁻¹ (inset and dotted curve).
gether, these results suggest that the effect of rearward strain makes the catalytic site relatively inaccessible to nucleotide. Furthermore, we have shown that kinesin:nucleotide is an equilibrium mixture of two states (15). Taken together, these results suggest that the effect of rearward strain is to drive an equilibrium distribution of catalytic site conformations to favor one that is relatively "closed" and inaccessible to nucleotide binding.

Our results also provide a critical test of a recently proposed "inchworm" model of kinesin movement (33). In this model, the leading motor is always leading, the trailing motor is always trailing, and one motor remains enzymatically inactive throughout a processive run. Our data show that binding of 2'dmT occurs in two distinct phases (Fig. 5B, red transient), and the rates of both of these phases are considerably faster than kcat. This means that a processive kinesin moving on a microtubule reaches the steady state after two nucleotide-binding events. This is both consistent with and required by a hand-over-hand mechanism, such as the one depicted in Fig. 1. However, it is inconsistent with an inchworm mechanism, which would predict only one nucleotide-binding event before the steady state is reached.

Our model explains how processive movement by kinesin can be both efficient and adaptable. By preventing ATP binding to the lead head, internal strain guarantees that this head will remain strongly attached to the microtubule at the moment that the trailing head dissociates. ATP would then bind rapidly to the leading head (>1000 s⁻¹), but hydrolysis and subsequent dissociation would still be relatively slow (k_d = 48–55 s⁻¹, Table I). This disparity would give the trailing head time to swing forward and associate with the microtubule, because we have shown (18) that this process is very rapid (~800 s⁻¹). Processivity would therefore result from two features of the mechanochemical cycle: blocking of ATP binding to the leading head by strain, and very rapid forward stepping of the trailing head and its concomitant docking to the microtubule surface (Fig. 1). A particular advantage of this arrangement is that, if the tethered head were to come across an obstacle during its forward swing, the entire kinesin molecule would dissociate at a rate defined by k_d. This feature would enable kinesin to sidestep an obstruction, diffuse to another microtubule, and continue on with its journey.

Does forward strain have any effect on the trailing head? A variety of mechanical studies have suggested that it accelerates trailing head dissociation (1, 9, 29). However, our data with K413BIO does not support this. The effective rate constant for dissociation, k_d, was in fact slower for dimeric kinesin than for a monomeric construct. As we have shown (Equation 1), k_d is a composite rate constant and depends on the rates of ATP hydrolysis (k_h) and microtubule dissociation (k_DMT). Direct measurements using chemical quench methods have consistently shown that, although k_h is ~100 s⁻¹ for dimeric constructs, it is considerably faster for monomers, with estimates placing it at >250 s⁻¹ (2, 20, 22). On the other hand, our previous studies with K349 show that the rate of docking of the neck linker places an upper limit on k_h of ~800 s⁻¹ (23). We have performed fitting to the data in Fig. 3 to obtain values of k_DMT for monomeric and dimeric kinesins, using values of k_h of 100 s⁻¹ for K413 and the limiting values of 300 s⁻¹ and 800 s⁻¹ for K349. These reveal values of k_DMT of 122 ± 27 s⁻¹ for K413 and 143 ± 16 s⁻¹ (k_h = 300) and 111 ± 10 s⁻¹ (k_h = 800) for K349. Thus, even when correcting for differences in the kinetics of ATP hydrolysis between monomeric and dimeric constructs, we find that k_DMT is relatively unaffected by forward strain.

However, K413BIO is a mutant construct that has eliminated all the surface-reactive cysteines. Hence, it may still be possible that forward strain has some effect on the processivity of wild-type kinesin. Our kinetic characterizations of K349 and K413 have shown that only one step in the mechanochemical cycle is affected (18, 23, and this work). This is the rate of ADP-induced dissociation, which is accelerated 19-fold com-

![Fig. 7. Effect of AMPPNP on kinetics of ADP binding to the leading head.](image-url)
pared with wild-type (Fig. 4). Furthermore, although K413BIO is processive and has near wild-type in vitro velocities, its mean run length is reduced ~2- to 3-fold (Fig. 2). Thus, it is possible that forward strain may accelerate trailing head dissociation in wild-type kinesin. However, even if this were the case, the degree of acceleration would be relatively small, amounting to no more than a factor of 2 or 3. This degree of acceleration is almost identical to the value predicted by Uemura et al. (29) using unbinding force measurements. Thus, our data with K413BIO clearly shows that, although a forward strain-induced dissociation mechanism may modulate the length of a processive run, it is not required for processivity.

In summary, this study has shown that the internal strain generated by kinesin during its mechanochemical cycle provides a mechanism that supports processivity. The major effect of strain is to markedly slow ATP binding to the leading head, an effect that guarantees that the two motor domains remain out of phase of each other during multiple mechanochemical cycles. Although strain may also accelerate dissociation of the trailing head, our results show that this effect is not necessary for processive movement. Finally, the strain-dependent mechanism that we describe may have more general applicability. Other molecular motors, such as myosins V and VI are also processive (34–36). Like kinesin, these motors need a mechanism to keep their individual motor units out of phase enzymatically to prevent premature dissociation from actin. Several of the methods developed in this study are directly applicable to these motors and may be useful in future studies to elucidate the mechanisms underlying their processivity.

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