Monomeric Kinesin Head Domains Hydrolyze Multiple ATP Molecules before Release from a Microtubule*

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Wei Jiang and David D. Hackney‡

From the Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213

Transient kinetic analysis of microtubule-stimulated ATP hydrolysis by the monomeric kinesin motor domain DKH357 was performed to investigate the kinetic pattern of a monomer. Both ATP and ADP produced dissociation of the complex, microtubule (MT)-E, of microtubules with DKH357 at a maximum rate of $45\text{ s}^{-1}$ as determined by decrease in turbidity. The maximum dissociation rate was independent of the KCl concentration between 25 and 200 mM. At subsaturating levels of nucleotide, ATP was more effective than ADP in dissociating DKH357 from MT-E (1.6 and 0.4 $\mu\text{M}^{-1}\text{s}^{-1}$ for ATP and ADP, respectively, at 50 mM KCl). Addition of ATP to MT-E results in a burst of product formation with a maximum initial rate of $100\text{ s}^{-1}$ at saturating levels of ATP. This maximum hydrolysis rate of $100\text{ s}^{-1}$ is similar to the maximum steady state ATPase rate at saturating microtubules of $70\text{ s}^{-1}$, and thus hydrolysis is at least partially rate-limiting. When the MT lattice was highly occupied with bound DKH357, the amplitude of the burst was $2\text{ per DKH357 active site (superstoichiometric). The rate constant for the burst transient was }\approx 45\text{ s}^{-1}$, which is the same as the rate for dissociation of DKH357 from the microtubule and this suggests that dissociation and termination of the burst phase are coupled. The size of the burst increased with decreasing initial occupancy of the MT lattice with heads and is consistent with the value of $4\text{ ATP molecules predicted by previous steady state measurements (Jiang, W., Stock, M., Li, X., and Hackney, D. D., submitted for publication).}^{4}$

Dimers of kinesin head domains are capable of generating processive movement along MTs$^5$ for extended distances without dissociation (1–3). The detailed mechanism for generation of processivity and how it is coupled to ATP hydrolysis represents a considerable challenge. Analysis of the bimolecular rates for stimulation by MTs of steady state ATPase and ADP release, however, indicates that monomers DKH346, DKH357, and DKH365 hydrolyze $4\text{ ATP molecules during each cycle of net binding and release of kinesin from a MT.}^{5}$ This is equivalent to saying that the head domain has only a 1 in 4 probability of dissociating from the MT during hydrolysis of each ATP molecule. The value of $4\text{ ATP molecules is significantly less than the value of }50\text{ ATP molecules per head for dimers, but is still significantly greater than the value of }1\text{ ATP per head per MT binding cycle that would result if the monomer head always dissociated from the MT at least once during each ATPase cycle.}$

We now report transient kinetic results that directly demonstrate that hydrolysis of ATP by the MT-DKH357 complex is not only faster than dissociation of DKH357 from the MT, but also that dissociation does not even occur during most ATPase cycles. This results in a burst of product formation on addition of ATP to the rigor complex of kinesin with MTs that is larger than the concentration of kinesin active sites (superstoichiometric). The magnitude of this burst is $2\text{ per kinesin head at DKH357:MT ratios close to }1:1$, but increases at low initial occupancy of the MT lattice with heads and is consistent with the burst size of $4\text{ per head predicted by the steady state measurements. A preliminary report of these results has been made (17).}$

MATERIALS AND METHODS

All reactions were performed at 25 °C in A25 buffer (25 mM potassium acetate, pH 6.9, 2 mM magnesium acetate, 2 mM potassium EGTA, 0.1 mM sodium EGTA, and 1 mM 2-mercaptoethanol) supplemented with KCl as indicated. Taxol (3 $\mu\text{M}$) was included in reactions containing MTs. Tubulin was prepared and polymerized into MTs as described previously (13) except that unpolymerized tubulin was removed after polymerization by a final centrifugation and gentle resuspension. MT concentrations are reported as the concentration of tubulin heteropolymerization. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
erodimers. The concentration of DKH357 is reported as the concentration of ADP binding sites as determined by equilibration with [α-32P]ATP as described previously (18). This level of DKH357 binding sites was 90% of the molar level of total DKH357 determined by Bradford assay (19) using bovine serum albumin as standard. Pyruvate kinase and apyrase were obtained from Sigma.

The MT-E complex of DKH357 with MTs was formed in A25 buffer with 50 mM KCl by incubation of DKH357 with an excess of MTs in the presence of a low level of apyrase to hydrolyze the ADP that is released on binding of DKH357 to MTs. Control experiments indicated that aggregation of MTs was more likely with other buffers and salts and when DKH357 was in excess of MTs. Consequently the complex was always formed in this way and then mixed with solutions of different KCl concentration to give the desired final concentration during the reaction.

Stopped flow turbidity measurements were performed with an OLIS stopped flow spectrophotometer at 320 nm and 1.6 cm path length. The MT-E complex was dissociated by mixing with an equal volume of buffer containing MgATP or MgADP. Burst measurements were determined by quenched flow methods in a KinTek apparatus using either [α-32P]ATP or [γ-32P]ATP. Typically the MT-E complex was mixed with an equal volume of buffer containing [32P]ATP and then quenched by mixing with 2 N HCl to give a final concentration of 0.67 N HCl. The quenched reaction mixture was immediately neutralized by addition of 12 ml of cold 32 mM Tris containing 0.5 μmol each of carrier P, ADP, and ATP. A sample (1 ml) was counted to determine the total counts, and ATP. A sample (1 ml) was counted to determine the total counts, and 10 ml was chromatographed on a 1.5-ml column of AG MP-1 (Bio-Rad) generally as described previously (18). For analysis of [32P]Pi, derived from reaction of [γ-32P]ATP, the column was washed with water and then P was eluted with 10 mM HCl (eight fractions of 2 ml). The 10 mM HCl fractions were counted and the area of the peak of [32P]Pi was derived from reaction of [32P]ATP with 0.5 nmol each of ATP, with 0.5%. To correct for material in the 2N HCl fractions were counted and the area of the peak of [32P]Pi was determined. In each case the extent of hydrolysis was determined from the fraction of the total counts that were recovered as P, or ADP.

[32P]ATP was purchased from DuPont. In order to reduce the blank of counts that coelute with P and ADP, the [32P]ATP used in reactions was purified on AG MP-1. The [32P]ATP with 0.5 nmol each of carrier P, ADP, and ATP was adsorbed to 10 μl of AG MP-1; washed three times by centrifugation with cold 20 mM HCl; eluted with cold 160 mM HCl and immediately neutralized with Tris base. This typically reduced the blank level to < 0.5%. To correct for material in the [32P]ATP that is not hydrolyzable to P and ADP, a sample was exhaustively hydrolyzed by kinesin activated by MTs and the recovery as P, or ADP determined (see Ref. 18 for discussion of this problem).

RESULTS

Formation of MT-E Complex—Monomer heads bind to MTs with a stoichiometry of one head per tubulin heterodimer in the presence of AMP-PNP (13, 20). Addition to MTs of head domains with bound ADP results in the release of the ADP (E–ADP + MT ↔ MT–E + ADP), which is essentially complete at low head concentrations (6), but becomes reversible at higher head concentration where the level of released ADP approaches the Kd for ADP rebinding. A low level of apyrase can hydrolyze the released ADP, and this allows the binding of heads to the MT to proceed to completion as indicated in Fig. 1A. In the presence of apyrase, addition of DKH357 to MTs produces a linear increase in the turbidity (A280) up to the equivalence point of one head per tubulin heterodimer. The turbidity continues to increase above the equivalence point, but with a lower slope that is related to the weaker nonspecific binding observed in the presence of AMP-PNP (13). In the absence of apyrase, the initial increase at low concentrations of DKH357 is similar to that observed with apyrase, but further binding of DKH357 to the MTs is inhibited due to accumulation of free ADP. Control experiments at an initial ADP concentration of 10 μM indicate that the level of apyrase in Fig. 1A results in the hydrolysis of half of the ADP in 60 s. This is sufficient to remove free ADP during the several minutes required to load the drive syringes for stopped or quenched flow experiments, but not so fast as to interfere significantly during the short rapid mixing reactions.

**Dissociation of MT-E by ATP and ADP**—The rate of dissociation of the MT-E complex by ATP in 50 mM KCl was determined from the decrease in turbidity as indicated in Fig. 1B. The A280 decrease for dissociation observed in Fig. 1B is consistent with the turbidity increase observed for association in Fig. 1A, indicating that dissociation is essentially complete on this rapid time scale. The observed rate constant for dissociation is 43 s\(^{-1}\), amplitude of 0.507, and offset of 0.0016 A280. C, dependence of dissociation rate constant and amplitude on concentration of ATP in 50 mM KCl. Dissociation rate constants (○) and amplitudes (△) were determined in 50 mM KCl as in Fig. 1B at indicated ATP concentrations. Theoretical line is fit at 48 s\(^{-1}\) and 29 μM for Kd\(_{ATP}\) and Kd\(_{ADP}\), respectively. D, dependence of maximum dissociation rate and Kd\(_{ADP}\) on concentration of KCl. MT-E complex was prepared in 50 mM KCl as in Fig. 1B and mixed with an equal volume of buffer containing MgATP and sufficient KCl to produce the indicated final KCl concentrations.

![Fig. 1. Turbidimetric analysis of interaction of DKH357 with MTs](image)

A. turbidimetric titration of MT with DKH357. MTs at 6.2 μM were titrated in A25 containing 50 mM KCl with DKH357 either in the presence (○) or absence (△) of apyrase (0.05 unit/ml). Corrected for A280 of DKH357 in absence of MTs. The arrow indicates the concentration of the MTs. B. dissociation of MT-E by ATP. MT-E complex was prepared by incubating 2.7 μM DKH357 with 3.1 μM MTs in the presence of apyrase in 50 mM KCl. The MT-E complex was mixed with an equal volume of 800 μM MgATP in 50 mM KCl and the turbidity decrease was determined. Final concentrations are 1.35, 1.65, and 400 μM for the concentration of ATP. The turbidity decrease is proportional to the concentration of ATP.

Kinesin ATPase

Approximately 90% of the molar level of total DKH357, dissociation of MT-E by ATP, and ADP, respectively. Detectable ammonium acrylamide was determined by variation of the final ATP concentration as in Fig. 1C. In some cases, the Kd\(_{ATP}\) value was estimated from more limited data at very high ATP concentrations without obtaining the additional data at low ATP concentrations needed for determination of Kd\(_{ADP}\).
The MT-E complex was mixed with an equal volume of $[^{32}\text{P}]\text{MgATP}$ in 50 mM KCl, and the formation of $[^{32}\text{P}]\text{IP}$ was determined. Concentrations of DKH357 and MTs after mixing were 2.25 and 3.1 μM, respectively. Concentration of DKH357 is indicated by the dashed line. Concentrations of ATP after mixing were 10.3, 24.7, 117, and 255 μM for $A-D$, respectively. Theoretical lines for 117 and 255 μM ATP were determined by nonlinear regression to a burst model using SigmaPlot. Values for the burst amplitude, burst rate constant, and steady state rate were 4.33 μM, 39.6 s⁻¹, and 5.1 μM/s for 117 μM ATP and 3.64 μM, 49.7 s⁻¹ and 7.0 μM/s for 255 μM ATP. The increase in the burst amplitude on increasing the MT concentration in Fig. 2, $A$ and $B$, detailed modeling was not attempted as the level of free ATP falls significantly during the time course due to conversion to product. At high ATP concentration in Fig. 2, $C$ and $D$, depletion of free ATP is less significant and they were fit to a model with a rapid initial burst followed by transition to a slower steady state rate. Fitting these curves to a burst model gives burst amplitudes of 1.9 and 1.6 per DKH357 site and burst rates of 40 and 50 s⁻¹, respectively, for $C$ and $D$. Thus the burst is superstoichiometric with more than one molecule of product formed per active site. This is incompatible with simple burst models in which the burst is due to stoichiometric or substoichiometric accumulation of product before a rate-limiting release step. The rate constant for completion of the burst phase of 40–50 s⁻¹ at high ATP is the same as the rate constant of ~45 s⁻¹ for dissociation of DKH357 determined turbidimetrically (Fig. 1C).

**Burst Kinetics at 200 mM KCl**—In order to better separate the burst and steady state phases, additional experiments were conducted at 200 mM KCl where the steady state rate is reduced, but the maximum rate for dissociation of the head remains constant at ~45 s⁻¹ (Fig. 1D). The burst amplitude is ~2.3 per DKH357 (Fig. 4, triangles) in 200 mM KCl at 2.25 μM DKH357 and 3.1 μM MTs, and there is good separation of the burst and steady state phases. For comparison, the $k_{\text{bi}}^{\text{ATPase}}$ and $k_{\text{bi}}^{\text{DAMP}}$ values were determined in 200 mM KCl for DKH357 as described previously (4) and are 0.046 and 0.0107 μM⁻¹ s⁻¹, respectively. Thus the $k_{\text{bi}}^{\text{DAMP}}$ for the average number of ATPs hydrolyzed is 4.3, and this is similar to the value of 4.0 for $k_{\text{bi}}^{\text{DAMP}}$ obtained in 120 mM potassium acetate.² Increasing the concentration of MTs to 10 μM at the same 2.25 μM concentration of DKH357 increases the burst amplitude to ~3.3 per DKH357 (Fig. 4, circles). The rate constant for the burst phase is 22 s⁻¹ at 3.1 μM MTs, and this is equal to the dissociation rate constant determined turbidimetrically at this subsaturating ATP level (the $k_{\text{bi}}^{\text{MT}}$ and $k_{\text{bi}}^{\text{ATP}}$ values in 200 mM KCl from Fig. 1D predict a rate constant of 22 s⁻¹ at 53 μM ATP).

As DKH357 only binds one nucleotide tightly, the superstoichiometric ADP that is generated in the burst phase must either be free, bound to a second site, or not really be stoichiometric due to an error in determination of the absolute concentration of DKH357 sites or the burst amplitude. In order to distinguish bound from free ADP, pyruvate kinase and P-enolpyruvate were included during the burst reaction as a trap for free ADP (Fig. 4, diamonds). The amount of pyruvate kinase used in Fig. 4 gave a $t_{1/2}$ for reaction with free ADP of 1 s in control reactions. This level of pyruvate kinase activity is sufficient to reduce the level of free ADP over a period of seconds, but not sufficient to immediately deplete any free ADP generated during the rapid burst phase. The results of Fig. 4 in the presence of pyruvate kinase indicate an overshoot and subsequent recovery back toward the stoichiometric level that is consistent with initial formation of substantial free ADP during the burst, followed by reduction in the free ADP level by pyruvate kinase at longer times. During steady state hydrolysis, DKH357 will be mainly dissociated from the MT, and only a stoichiometric level of bound ADP is expected (18).

The increase in the burst amplitude on increasing the MT level (Fig. 4, circles) could be due to the increased absolute concentration of MTs or to the decrease in the DKH357:MT ratio. This distinction is illustrated by the scheme of Fig. 5. In case I, the MT lattice is close to fully occupied with head domains and interaction between heads may influence the rates of hydrolysis and the rate of dissociation of heads in the presence of ATP. This corresponds approximately to the trian-
unoccupied MTs is added with the ATP, corresponding to the initial population of MTs is highly occupied, but an excess of MTs before mixing with ATP. In case III, an initial population of MTs is highly occupied, but an excess of unoccupied MTs is added with the ATP, corresponding to the

diamonds of Fig. 5, A and B. The absolute concentration of MTs is the same in cases II and III, but the distribution of heads differs. The results of Fig. 5, A and B, indicate that the burst amplitudes in cases I and III are both similar to each other and considerably smaller than the burst in case II. Thus the fractional occupancy of the MT lattice has an influence on the burst amplitude that is independent of the absolute concentration of MTs. Specifically, the burst amplitude increases with decreasing occupancy of the MT lattice.

Burst Kinetics with DKH340—Steady state measurements indicate that DKH340 hydrolyzes a significantly greater number of ATP molecules during each productive encounter with a MT in 120 mM potassium acetate (26 versus −4 for other monomer heads). The $k_{bi}^{ADP}$ and $k_{bi}^{ATP}$ values were determined for DKH340 in 200 mM KCl and are 1.11 and 0.086 μM$^{-1}$ s$^{-1}$, respectively. Thus the $k_{bi}^{ratio}$ for the average number of ATP molecules hydrolyzed is 12.8. Transient kinetic analysis of ATP hydrolysis by MT-DKH340 (Fig. 6) indicates that the burst amplitude is 5.5 per DKH340 even when the MT lattice is largely saturated. This value is significantly higher than that for DKH357, in agreement with the expectation based on the higher $k_{bi}^{ratio}$ value of DKH340.

**Discussion**

With monomeric DKH346, DKH357, and DKH365, MTs stimulate steady state ATP hydrolysis with a bimolecular rate constant that is approx. 10-fold greater than that for stimulation of the rate of ADP release from E-ADP. The most direct explanation of this difference is that kinesin monomers hydrolyze 4 ATP molecules during each productive encounter with a MT, although more complex kinetic explanations are also possible. This is strikingly different from the case of actomyosin in which dissociation is much more rapid than hydrolysis and the corresponding ratio is 1 (Ref. 21 and see Ref. 16). The transient kinetic studies presented here now directly demonstrate that hydrolysis is faster than dissociation even for monomeric DKH357 and that multiple ATP molecules are hydrolyzed before kinesin diffusional separates from a MT. A burst size of -2 per site is observed with DKH357 at both 50 and 200 mM KCl (Figs. 2 and 4). Inclusion of pyruvate kinase and P-enolpyruvate as a trap for released ADP (Fig. 4) indicates that a significant part of the ADP that is produced during the burst phase is not bound to kinesin. This release of ADP during the burst is consistent with hydrolysis of multiple ATP molecules per kinesin because the ADP that is produced by the first

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**Fig. 4.** Time course of ATP hydrolysis by MT-E in 200 mM KCl. Conditions were as the same as in Fig. 2 with MT-E complex prepared in 50 mM KCl, but mixed with ATP in 350 mM KCl and with use of [γ-32P]MgATP and analysis for [γ-32P]MgADP. Final concentrations were 200 mM KCl, 2.25 μM DKH357, and 53 μM MgATP. MTs were present at 3.1 μM (○, ○) or 10 μM (○). For ○, the syringe containing [γ-32P]ATP also contained pyruvate kinase (80 μg/ml) and P-enolpyruvate (4 mM). The theoretical fits for ○ and ○ are for burst amplitudes and rates of 5.2 and 7.2 μM and 22 and 18 s$^{-1}$, respectively.

**Fig. 5.** Dependence of burst amplitude on the occupancy of the MT lattice. Scheme: three cases for distributions of kinesin heads on MT lattice. Monomeric kinesin head domains are indicated by circles and MTs are indicated by solid bars. See text for explanation. Reaction conditions were the same as in Fig. 4 with 50 mM MgATP and 2.25 μM DKH357 as final concentrations in all cases. For ○ in A and B, the MT-E complex was formed with 4.5 μM DKH357 and 6.2 μM MTs and mixed with ATP. For ○, the complex was formed with 4.5 μM DKH357 and 20 μM MTs (A) or 24 μM MTs (B) and mixed with ATP. For ○, the complex was formed with 4.5 μM DKH357, 6.2 μM MTs and mixed with ATP containing 13.8 μM MTs (A) or 17.8 μM MTs (B) to give the same final concentration of MTs as in ○. A and B are the results of independent experiments performed at different times. Control experiments indicated that MTs hydrolyze negligible ATP in absence of DKH357.

**Fig. 6.** Time course of ATP hydrolysis by MT-DKH340 in 200 mM KCl. The reaction was performed the same as in Fig. 4 except with DKH340. Final concentrations were 200 mM KCl, 0.67 μM DKH340, 1 μM MTs, and 53 μM MgATP. The dashed line indicates concentration of DKH340. The theoretical line was determined by nonlinear regression to a burst model. Values for the burst amplitude, burst rate constant, and steady state rate are 3.7 μM, 12.5 s$^{-1}$, and 0.3 μM/s, respectively.
The rate-limiting step is ADP release (15). In the absence of MTs, Pi release is fast, and the rate-limiting step is ADP release (15). Thus, Pi and ADP release are sequential as indicated on the lower line. In the presence of MTs, the relationship of these two steps is not established, but they are indicated on the upper line as sequential with the same order for purposes of discussion only.

The observed burst stoichiometry of ~2 for MT-E complexes in which the MTs are largely saturated with DKH357 is lower than the stoichiometry of ~4 per site predicted by the steady state measurements, but this disparity is likely due to differences in the DKH357:MT ratio for the two cases. The steady state measurements are performed with the MTs in large excess of DKH357, and most of the lattice of kinesin binding sites on the MT is unoccupied. Because of experimental limitations, the transient kinetic studies must start with a MT lattice with a significant fraction of the sites occupied with kinesin head domains. The MT concentration cannot be further increased without introduction of a large blank absorbance in the turbidity measurements or introduction of a high steady state rate in the burst measurements. Conversely, the DKH357 concentration cannot be reduced greatly without the magnitude of the turbidity change or fractional ATP hydrolysis becoming too close to the blank value in the absence of DKH357. High occupancy of the MT lattice can potentially lead to steric crowding that may accelerate the rate of diffusional separation of DKH357 after addition of ATP, particularly if conformational changes occur that bring a head into closer contact or overlap with heads on neighboring MT sites. Additionally, the rate of ATP hydrolysis may be sensitive to the fractional occupancy of the MT lattice. It is also possible that kinesin head domains transiently dissociate from one MT site and reattach to a new MT site, and thus the occupancy of neighboring sites by other kinesin head domains would prevent rebinding and result in more rapid net diffusional separation. The results of Figs. 4 and 5, A and B, indicate that the stoichiometry of the burst reaction does increase as the occupancy of the MT lattice is reduced, although it is not possible to go to very low DKH357:MT ratios. This increase in burst amplitude at low MT occupancy is consistent with the burst amplitude approaching the value of 4 per site observed by ATPase measurements in the limit of low occupancy of the MT lattice with DKH357. An additional complication that results from the rate constants being sensitive to the occupancy of the MT lattice is that the transients observed here should not be strictly first order, although the deviations from first order behavior would be difficult to detect.

At both 50 and 200 mM KCl the rate constant for termination of the burst phase equals the rate constant for dissociation of DKH357 from the MT as determined turbidimetrically. This indicates that diffusional separation of DKH357 from the MT is responsible for termination of the burst phase or the kinetic equivalent in which a conformational change leads to both termination of further ATP hydrolysis and to rapid dissociation from the MT. The analysis of this process is best illustrated by reference to the scheme of Fig. 7. In the absence of MTs, the lower pathway is followed with ADP release via step 4 being rate-limiting (15). Addition of MTs stimulates the rate of steady state ATP hydrolysis by allowing more rapid ADP release to occur via steps 8 (reverse) and 4 (6, 15) to generate the more complex MT-E. Addition of ATP to MT-E results in rapid hydrolysis with a rate constant of ~100 s⁻¹ for k₂ at saturating ATP (Figs. 2 and 3) without indication of a lag phase. Subsequent passage through steps 3 and 4 likely occurs at rates comparable with or greater than 100 s⁻¹, as there is no indication of a delay between hydrolysis of the first and subsequent ATP molecules during the burst phase (particularly evident for DKH340 in Fig. 6) and because steady state hydrolysis is almost as fast at ~70 s⁻¹. This suggests that ATP hydrolysis itself is the major rate-limiting step. As multiple ATP molecules are hydrolyzed before DKH357 diffusionally separates from the MT, all of the states on the top line of the scheme are more likely to proceed to the right with completion of a cycle of ATP hydrolysis than they are to proceed to the lower line via dissociation of DKH357. Thus k₂ must be greater than k₆ and similarly for k₇ and k₈ and for k₉ and k₆. All that can be said is that, on average, there are two to four sequential passages through steps 1–4 before dissociation occurs. The rigor binding of DKH357 to MTs is strong, and thus little dissociation is expected via step 5, but it is not known whether the bulk of the dissociation occurs via step 6, 7, or 8.

Most previous transient studies of kinesin have been performed with dimeric species and do not directly relate to the kinetics of individual kinesin head domains. Dimeric species are known to move processively along a MT (1–3) with hydrolysis of many ATP molecules per encounter (4) and to react with alternating head kinetics (6). Thus the observation that initial hydrolysis of ATP by a dimer is faster than dissociation from the MT (9, 11) is expected based on the processive nature of dimeric constructs and does not itself indicate whether hydrolysis is faster than dissociation for individual head domains. One factor complicating analysis of transient kinetic studies with dimers is that the tethered head can have different kinetics from that of the attached head. An additional factor is that tightly bound ADP dissociates from the tethered head in a MT-dimer complex in a slow process that is not part of the normal rapid ATPase cycle (6). This altered state would have time to form during the loading of the drive syringe in transient kinetic studies, but in the work to date, the nature of the MT-dimer at the start of the reaction has not been determined.

Moyer et al. (14) have also investigated the kinetics of two monomeric kinesin constructs. Their transient kinetic analysis of ATP hydrolysis did detect an initial burst of product formation, but the amplitude was substoichiometric and not supers-
toichiometric as reported here. This is at least in part due to different experimental conditions. In the work by Moyer et al. (14), the ionic strength and concentration of MTs was such that dissociated heads would rapidly reattach to the MT and continue the MT-stimulated reaction. Thus a larger burst is not observed, because the reaction continues at close to its maximal rate with no sharp transition to a slower steady state phase. In the experiments presented here, initial dissociation of DKH357 terminates its MT-stimulated phase, because conditions were chosen so that rebinding to MTs was slow.

The failure of monomer head domains to dissociate from the MT during each cycle represents partial uncoupling of the ATPase reaction from motility, unless monomer heads have some mechanism to slide along the MT without net diffusional separation during each ATPase cycle. In contrast, the individual head domains of dimers likely do efficiently dissociate and move to a new site during each ATPase cycle, because the ATPase of $\frac{40}{s}$ per head equals the dissociation rate of $\frac{40}{s}$ per head needed to produce the observed sliding velocities with steps of 8 nm (see Ref. 6 for discussion). One possible mechanism for this interaction in dimers is that the movement of the detached head toward the MT, as it releases ADP, could induce strain in the trailing attached head that accelerates the rate of dissociation of the attached head from the MT.

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