Interacting Head Mechanism of Microtubule-Kinesin ATPase*

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Kinetic and equilibrium properties are compared for a monomeric kinesin construct (K332) and a dimeric construct (K379). MtK379 has a low affinity (5 × 10^4 M^-1) and a high affinity (5 × 10^9 M^-1) binding site for mant ADP while MtK332 has a single low affinity site (5 × 10^4 M^-1). Rate constants of dissociation of mant ADP are <1 s^-1 for the high affinity site and 75–100 s^-1 for the low affinity site for MtK379. For MtK332, the effective rate constant is 200–300 s^-1. It is proposed that the two heads of the dimer are different through the interaction with the microtubule, a strongly bound head with low affinity for 2'-3'-O-(N-methylanthraniloyl) adenosine 5'-di-phosphate (mant ADP), similar to the single strongly bound head of the monomer and a weakly bound or detached head with high affinity for mant ADP. Rate of binding of mant ADP to MtK379 showed an "S"-shaped dependence on concentration for MtK379 and a hyperbolic dependence for MtK332. Binding of K379-mant ADP dimer to microtubules releases only one mant ADP at a rate of 50 s^-1. The second strongly bound mant ADP is released by binding of nucleotides to the other head. Rates are 100 s^-1 for ATP, 30 s^-1 for AMPPPN or ATPγS, and 2 s^-1 for ADP. The rate of binding of mant ATP to MtK379 showed an "S"-shaped concentration dependence and limiting rate at zero concentration is <1 s^-1 while MtK332 gave a hyperbolic dependence and limiting rate of 100 s^-1. The limiting rate is determined by the rate of dissociation of mant ADP in the hydrolysis cycle. The evidence is consistent with an interacting site model in which binding of ATP to one head is required for release of ADP from the other head in the hydrolysis cycle. This model, in which the cycles are maintained partly out of phase, is an extension of the alternating site model of Hackney (Hackney, D. D. (1994) Proc. Nat. Acad. Sci. U. S. A. 91, 6865–6869). It provides a basis for a processive mechanism.

Kinesin is a processive motor which can travel a distance of micrometers before dissociating from the microtubule (1, 2). An average step of 8 nm for each ATPase cycle requires 60 cycles before dissociation and a rate constant of dissociation of less than 0.3 s^-1 in order to move 1 μm at a velocity of 0.5 μm s^-1. The motor must also satisfy the conflicting requirement that a single head detaches and reattaches with rate constant greater than the cycle rate of 50 s^-1 to couple movement steps to the ATPase cycle. To meet these requirements, it is likely that there is an interaction between heads, mediated by the constraints of binding to neighboring microtubule sites, which introduces a phase relation between the ATPase cycles of the two heads. An interaction between heads was not found in a comparison of monomeric and dimeric kinesins in constructs in the absence of microtubules (3, 4).

Hackney (5) showed that an interaction occurs for the dissociation of ADP since binding of a kinesin-ADP dimer to microtubules leads to the rapid dissociation of ADP from only one head of the dimer. On this basis, an alternating cycle mechanism was proposed for microtubule kinesin ATPase.

Previous kinetic studies of the microtubule kinesin dimer ATPase (6–8) have been analyzed by treating the heads as essentially identical and independent except for the dissociation of the dimer which requires the detachment of both heads. Although the kinetic scheme has been used to describe possible motility mechanisms, the interaction between the heads is an essential property of the system, and it is necessary to develop a mechanism which takes head interactions into account.

In this work, the kinetic schemes of monomeric kinesin K332 (3) and dimeric kinesin K379 (7) are compared to determine which steps in the cycle are affected by interaction between heads. The main difference is that the two heads of the dimer are not equivalent in their interactions with microtubule sites and substrates. Two equilibrium constants for ADP binding and two rate constants for ADP dissociation are observed which are equated with nucleotide binding to kinesin heads that are strongly versus weakly bound to microtubule sites. A mechanism is proposed in which the transition from strong to weak binding (or detachment) of one head is coupled to the transition from weak to strong binding of the other head. The scheme is similar to the alternating site model of Hackney (5), but a semiquantitative mechanism is developed based on measurements of the rate constants of the steps in the reaction. A preliminary report of the mant ADP displacement experiments has appeared (9).

**MATERIALS AND METHODS**

The preparation of K332, K379, microtubules, and the substrates 2'-3'-O-(N-methylanthraniloyl) adenosine 5'-triphosphate and -diphosphate, respectively; 3'-deoxy,2'-mant ATP, 2'-O-(N-methylanthraniloyl-5'-O-(3-thiotriphosphate); ATPγS, adenosine 5'-O-(3-thiotriphosphate); PIPES, 1,4-piperazinediethanesulfonic acid; Mt, microtubule.

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1 The abbreviations used are: K332, K379, and K560, kinesin constructs of 332, 379, and 560 amino acid residues; AMPPPN, 5'-adenyl β,γ-imidodiphosphate; mant ATP and mant ADP, 2'-3'-O-(N-methylanthraniloyl) adenosine 5'-triphosphate and -diphosphate, respectively; 3'-deoxy,2'-mant ATP, 2'-O-(N-methylanthraniloyl), 3'-deoxyadenosine 5'-triphosphate; ATPγS, adenosine 5'-O-(3-thiotriphosphate); PIPES, 1,4-piperazinediethanesulfonic acid; Mt, microtubule.
RESULTS

Equilibrium Binding of mant ADP to Microtubule-Kinesin Monomer and Dimer Complexes—The extent of dissociation of mant ADP on mixing the monomer or dimer complex with microtubules was measured by the fractional decrease in fluorescence enhancement. Free mant ADP was separated from the protein-mant ADP complex by the centrifuge column method (4) before the addition of microtubules. The fluorescence enhancement decreased with microtubule concentration (Fig. 1). The dissociation was complete at 20 μM microtubule concentration for K332. The addition of excess ATP gave no further change in fluorescence. For K379, the decrease in fluorescence reached a plateau at 20 μM microtubules corresponding to approximately one-half of the total change in fluorescence. The addition of ATP to the microtubule-kinesin dimer complex released the remainder of the mant ADP.

In the experiments, the K379 site concentration is 2 μM; consequently, the free mant ADP concentration is 1 μM after dissociation of one mant ADP per dimer. This concentration appears to be sufficient to nearly saturate a high affinity ADP binding site. For concentrations of K379 of less than 1 μM, more than half of the mant ADP was released.

The rate of dissociation of this strongly bound mant ADP was determined by addition of apyrase (20 units/ml). The fluorescence decreased with a rate constant of 0.12 s⁻¹ (data not shown). The same concentration of apyrase added to a myosin subfragment 1-mant ADP complex at the same bound mant ADP concentration gave a rate constant of 0.5 s⁻¹ for mant ADP dissociation. Therefore, the apyrase concentration is sufficient to track mant ADP release from the MtK379 complex. The rate constant of dissociation of 0.12 s⁻¹, and an apparent second order rate constant for mant ADP binding of 1.5 μM⁻¹ s⁻¹ gives a binding constant of approximately 10⁷ M⁻¹ which accounts for the retention of the mant ADP by a high affinity site.

The results imply that the binding of mant ADP to the MtK379 dimer should fit two equilibrium constants. This inference was tested by fluorescence titration (Fig. 2). The data for the MtK332 monomer fitted a hyperbola with binding constant of 6 × 10⁴ M⁻¹. The enhancement of fluorescence for the MtK379 dimer was much larger than for MtK332 at low mant ADP concentrations. More than half of the maximum enhancement was attained at 2 μM free mant ADP compared to less than 10% of the maximum value for MtK332. The smooth curve was obtained by fitting the data to a model for binding to two independent sites. The calculated equilibrium constants are 5 × 10⁸ M⁻¹ and 7 × 10⁷ M⁻¹.

The titration curves provide direct evidence for two classes of binding sites in the MtK379 complex, but the values of the binding constants are approximate. At low concentrations, the calculation of the free mant ADP concentration is subject to error while at a high ratio of mant ADP to sites the signal is small. The binding constant of mant ADP to the MtK332 complex may also be overestimated because the complex is partially dissociated by ADP (dissociation constant, 15 μM in 10 mM NaCl), and mant ADP is very strongly bound to K332. The apparent binding constant obtained from the titration at 30 μM tubulin concentration was 30% smaller than at 20 μM tubulin. The values obtained from the titration curves are in reasonable agreement with values calculated from the rate constants of association and dissociation of mant ADP.

Kinetic Measurements of mant ADP Dissociation—It was shown previously that reaction of the K379-mant ADP dimer with microtubules plus ATP released both mant ADP molecules at a maximum rate of 30 to 35 s⁻¹ obtained by extrapolation of the rate constant versus microtubule concentration (7). The same experimental method gave a maximum rate of mant ADP dissociation of 110 s⁻¹ for the K332 monomer (3).

Reaction of K379-mant ADP with microtubules in the absence of ATP gave approximately half of the maximum fluorescence obtained with ATP present. The rate constant is 34 s⁻¹ at a microtubule site concentration of 15 μM (Fig. 3A, curve 1). This step is followed by a small further release at a rate less than 0.5 s⁻¹. The K379 site concentration is 0.5 μM, and the slow step corresponds to a small extent of dissociation from the high affinity site. The maximum rate for the first step obtained by extrapolation of the assumed hyperbolic dependence of the
rate constant on microtubule concentration is 50 s\(^{-1}\) (data not shown).

The K379-mant ADP complex mixed with microtubules plus a low concentration of ATP gave a biphasic signal. For curve 2, the concentration of ATP is 2.5 \(\mu\)M after mixing. The signal was fitted by rate constants of 30 s\(^{-1}\) and 5 s\(^{-1}\) and approximately equal amplitudes for the two steps. The rate constant of the second step increased linearly with ATP concentration over a low range of ATP concentrations. The variation in rate corresponds to an apparent second order rate constant of 2 \(\mu\)M\(^{-1}\)s\(^{-1}\) which is in the range expected for ATP binding to a MtK379 site.

At high concentrations of ATP, the data fitted a single exponential for the release of all of the mant ADP. For curve 3, the ATP concentration is 25 \(\mu\)M and the rate constant is 18 s\(^{-1}\) at this microtubule concentration. The value is half as large as the rate of dissociation of the first mant ADP in the absence of ATP at the same microtubule concentration.

The results show that after dissociation of one mant ADP from the dimer the second remains strongly bound and is released by a transition induced by binding of ATP to the other head in agreement with the findings of Hackney (5). Both mant ADP molecules are released if the K379-mant ADP dimer is mixed with microtubules plus AMPPNP. The maximum rate of mant ADP dissociation obtained by extrapolation versus microtubule concentration is 18 s\(^{-1}\) (data not shown). Therefore a hydrolysis step is not necessary for mant ADP release from the strongly bound site although the extrapolated maximum rate was twice as large for ATP compared to AMPPNP.

The observed rate of dissociation of the second mant ADP induced by nucleotide binding is affected by the rate of dissociation of the first mant ADP. Since it was shown that a MtK379 complex can be formed with one mant ADP bound at a high affinity site, this complex was used to measure the actual rate of mant ADP release by the binding of a nucleotide at the other site. The fluorescence signal for reaction of the complex with 1 \(\mu\)M ATP is shown in Fig. 3B (in 50 mM NaCl). The lag before release of mant ADP is 3 to 4 ms. Data collection is triggered 1.5 to 2 ms before flow stops in order to establish a baseline. The actual lag before mant ADP release is 2 ms.

The fluorescence signal in Fig. 3B is fitted by a rate constant of 95 s\(^{-1}\). There is a small deviation from the fit to one exponential term which indicates a small contribution from a slow step. The amplitude of the slow phase increased with ionic strength which increases the degree of dissociation of the MtK379 complex. Any K379 which dissociates with mant ADP still bound must release the nucleotide by rebinding to the microtubule. In 25 mM NaCl, the dissociation of MtK379 by ATP is very small and no slow phase was detected at this ionic strength.

The rates of dissociation of the strongly bound mant ADP by various nucleotides are plotted in Fig. 4. ATP released the mant ADP at a rate of 100 s\(^{-1}\) with a half-maximum rate at 40
concentration as expected from the very high $K_m$ a hyperbola, maximum rate 210 s$^{-1}$. MtK332, 10°C, maximum rate 350 to 400 s$^{-1}$; zero concentration intercept 50 s$^{-1}$; zero concentration intercept 210 s$^{-1}$. Conditions: standard buffer, 50 mM NaCl.

![Graph](image)

**Fig. 5.** Rate of binding of mant ADP to MtK379 dimer and MtK332 monomer complexes. The fluorescence enhancement for the binding of mant ADP was fitted to one exponential term, and the apparent rate constants are plotted versus mant ADP concentration. ○, nucleotide-free MtK379 20°C, data fitted to a hyperbola for concentrations greater than 7.5 µM, maximum rate 280 s$^{-1}$; initial slope 1.5 µM$^{-1}$ s$^{-1}$; intercept at zero concentration is zero within experimental error. □, MtK379-ADP complex, one ADP bound per dimer, 10°C, data fitted to a hyperbola, maximum rate 210 s$^{-1}$; zero concentration intercept 50 s$^{-1}$, ▼, MtK332, 10°C, maximum rate 350 to 400 s$^{-1}$; zero concentration intercept 210 s$^{-1}$. Conditions: standard buffer, 50 mM NaCl.

µM which corresponds to the $K_m$ value of MtK379 ATPase. The maximum rate was even larger for 2′-deoxy-ATP. The rate is 90 s$^{-1}$ for GTP, but the half-maximum occurs at a much higher concentration as expected from the very high $K_m$ of MtK ATPase (10). AMPPNP and ATP$\cdot$S, which are nonhydrolyzed or slowly hydrolyzed nucleotides, gave maximum rates of 30–35 s$^{-1}$. AMPPNP does not dissociate the MtK379 complex, but the binding of mant AMPPNP does induce a first order transition with rate constant of 40 s$^{-1}$ (data not shown). Even ADP produces a slow rate of dissociation of 2–3 s$^{-1}$ which is still 20 times larger than the spontaneous rate of 0.1 s$^{-1}$.

Therefore, the binding of a nucleotide ligand to one head induces a transition of the other head to a state in which mant ADP can dissociate with a rate constant of more than 100 s$^{-1}$. The slower rates for ligands other than ATP indicate that a transition induced by these ligands is rate-limiting. Although the hydrolysis step is not necessary, the rate of mant ADP dissociation appears to approach the maximum value characteristic of the actual dissociation step only for hydrolyzed ligands. Even for ATP the observed rate could underestimate the actual rate constant of the ADP dissociation step. A different experiment to obtain an independent estimate of the rate constant of mant ADP dissociation is described below.

**Kinetics of mant ADP Binding to the MtK Complex**—The binding of mant ADP to MtK332 is relatively weak, and it was shown that the observed rate constant of binding, extrapolated to zero concentration, yielded a large apparent rate constant of mant ADP dissociation of up to 300 s$^{-1}$ (3). The binding of mant ADP to nucleotide-free MtK379 exhibited quite different kinetic behavior (Fig. 5). At low concentrations, the fluorescence signal fitted one exponential term, but the observed rate constant was small. At higher concentrations, the rate constant increased, but the plot shows upward curvature. The initial slope of the plot gives an apparent second order rate constant of 1.5 µM$^{-1}$ s$^{-1}$, and the line extrapolates to zero within experimental error ($\pm$1 s$^{-1}$). Therefore, the rate constant of mant ADP dissociation is very small as expected for binding to a high affinity site.

The experiments were repeated using the MtK379-ADP complex with the high affinity site occupied by ADP. In this case, a small amplitude signal with a large rate constant was obtained at low mant ADP concentrations. Because part of this small signal is lost in the dead time, the process was slowed by measuring the rate of binding at 10°C (Fig. 5). The rate constant is 85 s$^{-1}$ at 5 µM mant ADP which is the lowest concentration that gave a measurable signal. The data extrapolate to a value of 50 s$^{-1}$. The binding of mant ADP to MtK332 at 10°C is included in the figure for comparison. The extrapolated value is about 200 s$^{-1}$.

Therefore, MtK379 has a high affinity site for mant ADP (small rate constant of mant ADP dissociation). The extrapolated value of the rate constant of $<1$ s$^{-1}$ corresponds to spontaneous dissociation from this high affinity site (0.1 s$^{-1}$ based on treatment with apyrase). If this site is already occupied, the mant ADP binds to a low affinity site on the other head (large rate constant of mant ADP dissociation). The large rate constant corresponds to dissociation from a strongly bound head since it is similar to the rate constant of dissociation from the strongly bound head of the MtK332 monomer complex. The rate constant of approximately 75 s$^{-1}$ at 20°C is also similar to the rate of release of mant ADP by the binding of ATP. The correlation suggests that ATP induces a transition of the ADP-containing head from a weakly bound state with high affinity for ADP to a strongly bound state with low affinity for ADP.

The “S”-shaped plot of the rate constant versus mant ADP concentration could provide further evidence for head interactions. However, mant ADP is a mixture of 2′ and 3′ isomers, and the S-shaped dependence might be explained by differences in the fluorescence signals of the isomers (3, 4). Experiments were done using the 3′-deoxy,2′-mant ADP isomer which does not give a biphasic fluorescence signal (3). An S-shaped dependence was still obtained for the rate of binding to MtK379 and a hyperbolic dependence for K379 alone (data not shown).

Therefore, the S-shaped dependence requires different apparent rate constants for binding to the two heads. Some upward curvature of the plot is generated by a model in which the sites have binding constants of $5 \times 10^6$ M$^{-1}$ and $5 \times 10^5$ M$^{-1}$ but do not interact. The displacement of mant ADP from the strongly bound site by ADP binding to the other head (Fig. 4) indicates that there is interaction between heads, and this effect can increase the upward curvature of the rate constant plot.

**Kinetics of mant ATP Binding to MtK332 and MtK379**—It has been shown that the dependence of the rate constant of mant ATP binding to MtK332 extrapolated to 100 s$^{-1}$ at zero concentration (3) while for MtK379 the intercept was zero within experimental error and the concentration dependence was S-shaped (7). The experiments were repeated with 3′-deoxy,2′-mant ATP to eliminate possible complications from the use of mixed isomers. The fluorescence signal fitted a single exponential term, and the concentration dependence of the rate constants is plotted in Fig. 6. The rate plot has a large intercept for MtK332 and an essentially zero intercept and S-shaped dependence for MtK379. Qualitatively, the results show the presence of two kinds of sites for MtK379 and a single site for MtK332. The analysis of the kinetics is more complex for the mant ATPase reaction than for mant ADP binding, and the evidence is treated under “Discussion.”

**Discussion**

The comparison of the kinetic properties of MtK332 monomer and MtK379 dimer complexes showed significant differences which are explained by an interacting head mechanism. The MtK332 monomer binds mant ADP weakly ($5 \times 10^4$ M$^{-1}$), and the effective rate of mant ADP dissociation is 100 s$^{-1}$ to 200 s$^{-1}$ (3). MtK379 has a high affinity mant ADP site ($5 \times 10^6$ M$^{-1}$) and a low affinity site similar in value to the monomer complex. The rate constant of dissociation of mant ADP is 0.1 s$^{-1}$ for the high affinity site and 50 s$^{-1}$ to 100 s$^{-1}$ for the low
FIG. 6. Rate of binding of 2'-mant ATP to MtK379 dimer and MtK332 monomer complexes. The fluorescence enhancement was fitted to one exponential term, and the apparent rate constants are plotted versus nucleotide concentration. MtK332 data (\(\bullet\)) are fitted to a hyperbola, maximum rate 700 s\(^{-1}\), zero concentration intercept, 100 s\(^{-1}\). MtK379 data (\(\bigtriangleup\)) are fitted to a hyperbola for concentrations >3 \(\mu\text{M}\), maximum rate 280 s\(^{-1}\); initial slope is 1 \(\mu\text{M}^{-1}\) s\(^{-1}\) for linear region and zero intercept is zero within experimental error. Conditions: 12.5 \(\mu\text{M}\) tubulin dimer, 2–10 \(\mu\text{M}\) nucleotide-free K332, 1–8.5 \(\mu\text{M}\) nucleotide-free K379, standard buffer, 50 mM NaCl.

affinity site.

A possible explanation of these results is that the K379 dimer is bound to the microtubule by only one head. Mant ADP would bind weakly to this head with values similar to the single strongly bound head of the monomer. The detached head would be equivalent to free kinesin and bind mant ADP with high affinity.

Various lines of evidence suggest that this explanation is too simple and that the second head is weakly bound. The rate of dissociation of mant ADP from the high affinity site is 4–5 times larger than the rate for free K332 or K379. The rate of dissociation of the MtK379 complex by ATP or ADP is 5 times slower than for the monomer even if the high affinity site is occupied by ADP.

The dissociation constants of K379 and its nucleotide complexes from microtubules as well as the \(K_m\) for microtubule activation of the ATPase are at least 5 times smaller than for K332. If the binding constant of a single head is the same for both, then \(K_{\text{dimmer}} = 2K_{\text{mono}}(1 + K_2)\) where \(K_2\) is the effective equilibrium constant for binding of the second head. Because \(K_{\text{dimmer}}\) is larger than \(2K_{\text{mono}}\) the second head is weakly bound but the value calculated for \(K_2\) is only 2 to 4; consequently, the second head is detached part of the time. If the microtubule lattice is saturated, binding by one head (one kinesin dimer per tubulin dimer) can still be more favorable than binding by both heads (one dimer per two tubulin dimers). Unless both heads were attached, at least transiently, it would be very difficult to explain the rapid dissociation of ADP from one head by the binding of ATP to the other head.

A better comparison would be between monomeric and dimeric forms of the same construct. This was not possible for K379 because of the small dimer dissociation constant but human K413 was reported to have a much larger dissociation constant and the dimer form is more strongly bound to microtubules than the monomer (11).

The reaction of mant ADP with the MtK332 monomer was described by a two-step mechanism (3)

\[
\text{Mt} + \text{K-ADP} \rightleftharpoons \text{Mt,K-ADP} \rightleftharpoons \text{Mt,K-ADP} \rightleftharpoons \text{Mt,K + ADP}
\]

\text{REACTION 1}

In the initial complex, kinesin is weakly bound to Mt and ADP is strongly bound. A transition to a state in which kinesin is more strongly bound and ADP is weakly bound is followed by rapid dissociation of ADP. The same mechanism is proposed for the dimer but with the restriction that when one head is very strongly bound in the nucleotide-free state the other head containing nucleotide is blocked in the weakly bound or detached state.

The kinetic evidence is consistent with the mechanism

\[
\text{Mt(K1,K-ADP) + L} \rightleftharpoons \text{Mt(K1L,K-ADP) = Mt(K1L,K) + ADP}
\]

\text{REACTION 2}

where L is a nucleotide ligand. The effective rate of ADP release depends on the ligand which affects the rate of the second step (Fig. 4). For ATP, the rate of dissociation of mant ADP of 100 s\(^{-1}\) is similar to the effective rate of mant ADP dissociation from the strongly bound state with low affinity for mant ADP (approximately 75 s\(^{-1}\), Fig. 5).

It was shown previously that the rate of mant ADP dissociation is 30–35 s\(^{-1}\) obtained from the reaction of the K379-mant ADP dimer with microtubules plus ATP (7). In the absence of ATP, the rate for the release of the first mant ADP was 50 s\(^{-1}\) while the second was dissociated by the action of ATP at approximately 100 s\(^{-1}\). The average rate for two steps in sequence is 33 s\(^{-1}\) which agrees with the previous result.

Further evidence for interaction between heads is given by the S-shaped concentration dependence of the rate of binding of mant ADP to the MtK379 dimer.

Head Interactions in the ATPase Cycle—The discussion of the evidence for interaction between heads is based on experiments on the binding and dissociation of ADP. The kinetics of binding of mant ATP provides evidence that head interactions also occur in the ATPase cycle. The limiting rate constant for the binding of mant ATP, extrapolated to zero concentration, is approximately 100 s\(^{-1}\) for the MtK332 monomer and less than 1 s\(^{-1}\) for the MtK379 dimer (Fig. 6). An analysis of the kinetic scheme is necessary to interpret this result. For the monomer the minimum scheme is

\[
\text{MtK + ATP} \rightleftharpoons \text{MtKATP} \rightleftharpoons \text{MtK-ADP} \rightleftharpoons \text{MtK + ADP + P}
\]

\text{SCHEME 1}

where \(k_i\) is the effective rate constant for the dissociation of both products, and the asterisk denotes states of enhanced fluorescence. The analytic solution of this mechanism showed that the intercept at zero concentration is approximately equal to \(k_2\) (7).

The value of 100 s\(^{-1}\) for the MtK332 monomer is consistent with the value expected from the rate constants and the maximum steady state rate. If the heads of the MtK379 dimer were independent, the intercept would be 30–40 s\(^{-1}\). Because the intercept is essentially the effective rate of dissociation of ADP, the value of <1 s\(^{-1}\) means that at a low concentration mant ATP binds primarily to the weakly bound or detached head and it is hydrolyzed to mant ADP which remains bound to high affinity site. Dissociation of the mant ADP requires the binding and possibly the hydrolysis of ATP on the other head. At low mant ATP concentrations, the rate of this process is linear in ATP concentration (\(k_3\) of 1–2 \(\mu\text{M}^{-1}\) s\(^{-1}\)) and the limiting rate extrapolates to nearly zero.

The positive curvature of the plot of rate constant versus concentration (Fig. 6) provides further evidence for the interaction between heads. Binding of mant ATP to the second head increases the rate of mant ADP release and thereby increases
the observed rate constant. The ATPase results make the important point that the MtK379-mant complex generated by hydrolysis behaves in the same way as the complex formed by binding mant ADP to MtK379.

The Interacting Site Mechanism—Hackney (5) proposed an alternating site mechanism for the ATPase cycle. Although the ratio of steady state rates for MtK332 and MtK379 is a factor of 2 (50–60 s⁻¹ versus 25–30 s⁻¹), this correlation is probably a coincidence. The cycle on one head is delayed at the state in which ADP is strongly bound. If the other head had to bind ATP and complete most of a cycle, the release of ADP would be delayed by 15–20 ms, but the lag in mant ADP release is less than 2 ms in the experiments (Fig. 3B); thus, the coupled step must occur early in the cycle. The scheme is better described as a cycle in which the two heads are partly out of phase.

A processive run of the motor begins with the binding of the KADP dimer to the microtubule followed by the release of ADP from one head. The Mt symbol is omitted in the scheme since we consider transitions between associated states.

\[
\begin{align*}
\text{(1)} & \quad \text{KADP} \\
\text{(2)} & \quad \text{KADP} \xrightarrow{250 \text{ s}^{-1}} \text{KADP-P} \xrightarrow{150 \text{ s}^{-1}} \text{K + ADP}
\end{align*}
\]

In the first step, ATP binds to the complex in which the other head is occupied by ADP. This step corresponds to the conditions used in the mant ADP release experiments shown in Fig. 4. Rate constants are intended to illustrate the scheme and are approximate values based on monomer and dimer experiments. The rate constant of phosphate dissociation was not measured, but a large value is necessary to account for the steady state rate of the MtK332 monomer. For simplicity, the ADP and phosphate release steps are given the same rate constant in step 3. Otherwise, an additional state would have to be included. A simulation using this set of rate constants was fitted by a steady state rate of 43 s⁻¹ for a “monomer” and 30 s⁻¹ per head for a dimer. The lag in ADP release is 3 ms for the binding of ATP to the nucleotide-free head, and the rate of release of the ADP gives an approximate fit to one exponential of 80 s⁻¹.

Step 2 in which the heads interchange strongly and weakly bound states probably includes additional transitions which are not specified in the preliminary mechanism. However, the mechanism is highly processive if the transitions of the two heads are coupled such that one head of the dimer is always in a strongly bound state. It is proposed that efficient coupling is necessary for high processivity. Step 2 in the scheme may include an intermediate in which both heads are weakly bound and dissociation of the complex competes with the transition to a strongly bound state.

The pathway of dissociation of the dimer is still unclear. In the monomer case, it was proposed that dissociation of KADP is the main step and this pathway accounts for the small processivity of the monomer (3). The rate of dissociation of MtK379 by ATP of 10 s⁻¹ is too large to be compatible with high processivity (7).

The K560 dimer (9) has a much smaller rate of dissociation consistent with the high processivity of this construct (2), and this system is better suited to an investigation of the pathway of dissociation.

Relation of the Kinetic Scheme to Structural Studies and Motility—Image reconstruction of the microtubule-kinesin complex has provided evidence for two structural states. The distal part of the kinesin monomer tilts toward the plus end of the microtubule for the nucleotide-free or AMPPNP complex.

while in the presence of ADP the distal part is more nearly perpendicular (12, 13). A similar correlation of the orientation in the presence or absence of ADP has also been observed for the actin-smooth muscle myosin subfragment 1 complex (14).

There are more than two biochemical states and some of them may not be distinguishable at the resolution of the reconstructions. Also, if there are two ADP states, the more stable state at equilibrium under the conditions of sample preparation is likely to be observed. The cartoon (Fig. 7) represents a possible correlation of our biochemical scheme with structural proposals, but a number of cartoons can be drawn (5, 13).

The strongly and weakly bound states are assigned to tilted and more nearly perpendicular structural states. The model bears an obvious similarity to actomyosin models (14–16). Net motion in one direction requires an asymmetry in the interaction of the two heads with the microtubule lattice sites. It is introduced by assumption because it is determined by structure rather than biochemistry. Heads in a strong-weak state in the order shown in the figure are assumed to have a lower strain energy than a weak-strong state. The coupled transition of strong-weak to weak-strong requires dissociation of the trailing head and preferential rebinding to the next available lattice site in the positive direction.

In this type of model, the coupling of a positive step to the ATPase cycle is less than one to one because there is a non-zero probability for the heads to be on the same pair of tubulin dimer sites after completion of a cycle (a zero step). Also, the motor can stall at a finite ATPase cycle rate. The increase in strain energy for a positive step against an external force increases the probability of taking zero or negative steps which appears to be a property of the system (17).

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