The kinetic mechanism is analyzed for a monomeric human kinesin construct K332. In the absence of microtubules, the rate constants of the ATPase cycle are very similar to dimeric human kinesin K379 and whole kinesin from bovine brain. The microtubule-activated ATPase is 60 s⁻¹ at 20 °C; \( K_m(Mt) \) is 5 \( \mu M \); dissociation constants in the presence of ATP and ADP are 9 \( \mu M \) and 16 \( \mu M \), respectively. The values of dissociation constants are 5 times larger than for K379. Binding of K332 to microtubules increased the rate of the hydrolysis step from 7 s⁻¹ to greater than 200 s⁻¹ and the 2'-3'-(N-methylanthraniloyl) (mant) ADP dissociation step from 0.02 s⁻¹ to greater than 100 s⁻¹. At higher ionic strength, more than one ATP is hydrolyzed before dissociation of MtK332 (small processivity). Data are fitted to the kinetic scheme.

\[
\begin{align*}
Mtk + ATP & \rightarrow Mtk\cdot ATP \\
& \rightarrow Mtk\cdot ADP \cdot P \\
& \rightarrow k_4 Mtk\cdot ADP \\
Mtk\cdot ADP & \rightarrow Mtk + ADP \\
& \rightarrow k_{dis} Mtk \\
K\cdot ADP & \rightarrow Mtk
\end{align*}
\]

The detailed kinetic mechanism of microtubule kinesin ATPase has been investigated previously using dimeric kinesin constructs (1–4). The scheme has been analyzed in terms of an independent head model, but it is known that the heads interact at least in the ADP dissociation step (5).

A comparison of the kinetic behavior of a monomeric and a dimeric kinesin should allow us to determine which steps in the mechanism are affected by interaction between heads. Molecular weight measurements for various constructs showed that the dissociation constant of the dimer decreases with increasing chain length (6–8). The dimer is stabilized by a sequence of 30 to 40 amino acids between 340 and 380 which are believed to form a coiled-coil (9).

The present work is a study of a monomeric human kinesin construct of 332 amino acids (K332). Relative to the sequence at the C-terminal end, human K332 is equivalent to Drosophila 340, the first monomer to be studied (6). In the absence of microtubules, the kinetic properties of K332 are essentially the same as the K379 dimer or whole kinesin (1, 10). Therefore, it is suitable for comparison of the microtubule-activated ATPase of a monomer versus a dimer.

The rate constants of the steps in the kinetic mechanism of MtK332, ATP binding, the hydrolysis step, ADP dissociation, and dissociation of the MtK332 complex by ATP and ADP are quantitatively different for monomer and dimer. A simple kinetic scheme is proposed which accounts for most of the experimental results and provides a basis for comparison of the mechanisms. The dissociation of mant ADP is not explained by the simple model, and it is proposed that there are two ADP intermediate states.

**MATERIALS AND METHODS**

**Expression and Purification of Human Kinesin 332—Escherichia coli**

BL21(DE3) cells were transformed with a pET plasmid containing the truncated kinesin gene which was kindly provided by L. Romberg and used for expression of K332 protein. The construct was prepared from the human cDNA clone of Navone et al. (11), which was obtained from a placent cDNA library. The methods of cell culture and the purification of K332 were essentially the same as for K379 (1). The purified protein was dialyzed overnight against the standard buffer used in all experiments (25 mM PIPES, pH 6.9, 2 mM MgCl₂, 1 mM EGTA), plus 50 mM NaCl and 2 \( \mu M \) ATP and then clarified. Sucrose (10% final concentration) was added, and the solution was stored at −80 °C. The yield of K332 was about 85 mg from 1400 mg of crude protein. The preparations used in this work were at least 90% pure based on SDS-polyacrylamide gel electrophoresis. The content of nucleotide binding sites was determined by a centrifuge column method using \(^{3}H\)ATP (1). The protein concentration was measured by Bio-Rad Protein Assay reagent using calibration curves prepared with bovine serum albumin or myosin subfragment 1. The total bound nucleotide was 0.95 to 1.0 per 38-kDa molecular mass after correction for 10% impurities.

Human kinesin K349 monomer was prepared by the same method using a plasmid provided by R. D. Vale. The methods of preparation of microtubules, \(^{3}H\)K332, ATPase assays, stopped flow, and quench flow measurements were recently described (1, 2).

**Preparation of Nucleotide-free K332**—The strongly bound ADP was removed by the method described previously (1). Briefly, the protein was bound to a phosphocellulose column and nucleotide was removed by washing with 2 ml EDTA in standard buffer minus MgCl₂. The protein was eluted with 500 mM NaCl in standard buffer plus 20% glycerol. The content of nucleotide binding sites was at least 0.8 mol/mol of K332 assayed by the centrifuge column method or by titration of mant ADP.

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‡ This abbreviation is used for K332, K379, K349, and K401, kinesin constructs of 332, 379, 349, and 401 amino acid residues; AMPPNP, 5'-adenyl 

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**Kinetic Mechanism of a Monomeric Kinesin Construct**

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fluorescence enhancement. The nucleotide-free K332 was more stable than K379 which tends to aggregate with loss of binding activity. There was no significant loss of nucleotide binding sites during 2 weeks at −20 °C in the high salt-glycerol buffer. Bound ADP could also be removed by treatment with apyrase (Sigma Potato Apyrase, grade VII). The nucleotide-free K332 prepared by apyrase had essentially the same properties as the protein treated with EDTA, and it was used in some experiments.

Preparation of mant ATP—Preparation of the mixed isomer 2′-(3′)-O-(N-methylanthraniloyl) ATP (mant ATP) was described previously (1). The reaction was scaled down to prepare 2′-deoxy,3′-mant ATP and 3′-deoxy,2′-mant ATP from 10-mg amounts of 2′-deoxy- or 3′-deoxy-ATP. The yield was about 60%. The purity was checked by thin layer chromatography on silica plates in 1-propanol/NH₄OH/H₂O, 6:3:1 by volume plus 0.5 g/liter EDTA.

Molecular Weight of K332—The molecular weight was estimated by gel filtration on calibrated Sepharose CL6B and Sephacryl S-300 superfine columns as described previously (1). The elution position was also compared with K379 dimer and [3H]K349 monomer run on the same column. The calculated molecular mass was 35 ± 5 kDa, and the peak essentially coincided with the K349 peak. Concentrations were calculated using a molecular mass of 38 kDa obtained from the amino acid composition.

RESULTS

Kinetic Properties of K332

The rate of binding of mant ATP was measured by the enhancement of fluorescence. A typical recording is shown in Fig. 1A. The fluorescence increased to a maximum and decreased to about two-thirds of the maximum value. A biaphasic signal was also obtained with K379 and brain kinesin (1, 10).

mant ATP or mant ADP is a mixture of 2′ and 3′ isomers. The reaction of 2′-deoxy,3′-mant ATP with K379 gave a much larger decrease phase such that the signal fell almost back to baseline while the 2′-deoxy,3′-mant ADP gave a very small fluorescence enhancement (1). The fluorescence signal for the binding of 3′-deoxy,2′-mant ATP is shown in Fig. 1B. There is essentially no decrease phase. The 2′-deoxy- and 3′-deoxy-mant nucleotides are satisfactory substrates with similar maximum rates of the microtubule-activated ATPase. The fluorescent group in the 3′ position apparently detects a second step in the reaction which quenches the enhancement.

The signal for mant ATP was fitted to two exponential terms, and the observed rate constants are plotted in Fig. 2A. The fluorescence increase phase fitted a hyperbolic dependence with a maximum rate of 300 s⁻¹, and the initial slope gave an apparent second order rate constant of 9 μM⁻¹ s⁻¹ in 10 mM NaCl. The decrease phase is independent of substrate concentration as expected for a sequential process, and the rate constant is 7 s⁻¹. The corresponding values of the rate constants for K379 are 200 s⁻¹ and 9 s⁻¹ for the increase and decrease phases measured in 50 mM NaCl (1). The lower ionic strength was chosen because the MtK332 complex is partly dissociated by nucleotides at higher ionic strength which complicates the determination of the rate constants of the complex for comparison with K332. The larger values for the maximum rate and apparent second order rate constant of K332 versus K379 are partly a result of the lower ionic strength.

The binding of mant ATP also gave a biaphasic signal. The two rate constants are plotted in Fig. 2B. The curve for the increase phase is “S”-shaped with an initial slope of 4 μM⁻¹ s⁻¹ and maximum rate of 350 s⁻¹. The decrease phase has a rate of 35 s⁻¹. The values for K379 are 200 s⁻¹ and 40 s⁻¹ for the increase and decrease phases.

It was shown previously, from the solution of the rate equations, that the upward curvature of a plot of rate constant versus mant ATP concentration can arise from a partial cancellation of the increase and decrease phases of the signal (1). To test this conclusion, rate constants were measured for the binding of 3′-deoxy,2′-mant ADP which has almost no decrease phase. A plot of rate constant versus concentration showed almost no curvature (data not shown). These findings for the monomer provide support for our previous conclusion that the “S”-shaped plot obtained for the K379 dimer was caused by the fitting procedure rather than interaction between heads.

The nucleotide-free K332 gave a phosphate burst phase with a rate constant of 8 s⁻¹ and amplitude of 0.88 mol per site, corrected for loss of binding sites (data not shown). The value for K379 is 9 ± 1 s⁻¹. The rate constant for the fluorescence decrease is equal to the rate of the phosphate burst within experimental error. As discussed previously for K379 (1), the fluorescence decrease phase may be determined by the effective rate of the step or steps leading to phosphate dissociation and formation of the K-ADP complex.

The steady state rate of K332 is 0.02 s⁻¹ at 20 °C which is equal to the rate of ADP or mant ADP dissociation determined from fluorescence enhancement. Therefore, the properties of K332 in the absence of microtubules are very similar to the K379 dimer.

Microtubule K332: Steady State Properties

The activation of K332 ATPase by microtubules is shown in Fig. 3 in which the ATPase rate is plotted versus microtubule
concentration expressed as tubulin dimer concentration. The dependence fits a hyperbola with maximum rate of $60 \text{s}^{-1}$ at $20^\circ\text{C}$ and concentration for half-maximum rate, $K_{\text{m}}(\text{Mt})$, of $5 \text{Mm}$ in $10 \text{mM} \text{NaCl}$. The $K_{\text{m}}(\text{Mt})$ increased markedly with increasing ionic strength.

The binding of K332 to microtubules in the presence of MgATP or MgADP is shown in Fig. 4. The measurements were made by the sedimentation method using $[3H]$K332. The smooth curves are hyperbolas fitted to data points. The extrapolated maximum binding is $0.8$ (not corrected for approximately $10\%$ impurities or inactive protein). The apparent dissociation constant is $9 \text{mM}$ in the presence of ATP and $16 \text{mM}$ for ADP. Concentration at half-maximum rate of $24 \mu\text{M}$ is an estimate of the dissociation constant of K332 mant ADP from the initial MtK332 mant ADP complex.

Dissociation constants were measured for complexes of MtK332 with ADP-BeF$_3$, ADP-AlF$_4$, and ADP-vanadate. The values are given in Table I. The binding of the ADP-BeF$_3$ complex is slightly stronger than in the presence of ATP which are 5 times smaller for the dimer at the same total ionic strength.

Nucleotide-free MtK332 was obtained by treatment with apyrase. The dissociation constant in $100 \text{mM} \text{NaCl}$ was $0.7 \mu\text{M}$. Assuming the same ionic strength dependence of dissociation constants found for K379, the dissociation constant in $10 \text{mM} \text{NaCl}$ is roughly $0.06 \mu\text{M}$. Therefore, the binding of ADP to K332 reduces the affinity for microtubules by about 250-fold.
is consistent with its being an analogue of an ATP state (12). However, the ADP-vanadate complex was more weakly bound than the ADP complex, while the ADP-AlF₄ complex was very strongly bound yet both complexes have been proposed to be analogues of an ADP-P state (12, 13). Further studies to characterize these complexes are in progress.

Kinetic Studies of the ATPase Mechanism of MtK332

The mant ATP Binding Step—The rate of binding of mant ATP was measured at a high microtubule concentration and a low ionic strength such that the complex remains associated. The fluorescence signal fitted a single rate constant at low concentrations, but the rate was large, 150 s⁻¹ at 5 μM mant ATP. At higher concentrations, the signal became biphasic, an increase followed by a decrease. The two rate constants are plotted in Fig. 5. The concentration dependence of the fluorescence increase, fitted to a hyperbola, had a maximum rate of about 700 s⁻¹ and an intercept at zero concentration of approximately 100 s⁻¹. The decrease phase was independent of concentration with a rate constant of 30 s⁻¹.

The Hydrolysis Step—The phosphate burst in 10 mM NaCl had a very large rate constant and small amplitude (Fig. 6). The system reached a steady state in 3 to 5 ms and the inter-cept, which defines the size of the phosphate burst, is 0.4 to 0.5 mol per site. Under these conditions, the system is largely associated and the steady state rate of 53 s⁻¹ is nearly equal to the maximum value. The rate constant of the transient phase is too large to measure but it is greater than 250 s⁻¹. The rate constant is determined by the rate of the hydrolysis step plus the rate of product release in reaching the steady state. To satisfy the equations for the steady state rate, burst size, and transient rate (2), the hydrolysis step must be at least 200 s⁻¹.

The transient phase of phosphate formation was also measured in 75 mM NaCl so that the complex is largely dissociated at the end of the transient. The results of a burst experiment are also plotted in Fig. 6. The steady state rate is 15 s⁻¹, and the complex is 80 to 90% dissociated in the steady state based on a sedimentation measurement. The transient phase has a rate constant of 50 s⁻¹. However, the size of the burst was greater than 1.

Variation of the ATP concentrations from 55 to 150 μM gave at most a 20% increase in the rate constant of the transient. The rate also did not increase more than 10% for variation in the microtubule concentration from 20 to 40 μM. These differences are within the error of fitting to one exponential plus a linear term. It is concluded that the maximum rate is in the range 50 ± 5 s⁻¹.

The amplitude of the burst ranged from 1.1 to 2.0 in six experiments. A burst larger than 1 can only be explained by a processive mechanism.

Dissociation of MtK332 by ATP and ADP—The rate of dissociation of MtK332 by ATP and ADP was measured by the small decrease in light scattering. Mixing MtK332 with buffer generated a slow change with a rate in the 5 to 8 s⁻¹ range and an amplitude equal to 25% of the change for complete dissociation of the complex. Measurements were limited to high substrate concentrations such that the rate of dissociation was much larger than the artifact. It was still necessary to use signal averaging to reduce the noise level. Light scattering transients are shown in Fig. 7 (in 50 mM NaCl). The maximum rate of dissociation is 65 to 90 s⁻¹ by ADP and 40 to 50 s⁻¹ by ATP. The rate increased slightly with ionic strength.

Although the errors in rate constants are large, the experiments make two important points. First, the rate of dissociation by ADP is significantly larger than the rate of dissociation by ATP. Second, the rate of dissociation by ATP is approximately equal to the rate of the transient phase of phosphate formation at the same ionic strength. This relatively slow dissociation prolongs the transient phase, and more than one ATP is hydrolyzed before the steady state is attained. Thus, the MtK332 monomer has a small processivity.

The mant ADP Dissociation Step—The rate of mant ADP dissociation from a MtK332-ADP complex was measured by reacting the K332-mant ADP complex with microtubules plus a high concentration of ATP to block re-binding. The fluorescence signal for mant ADP dissociation fitted a single exponential term, and the values are plotted versus microtubule concentration in Fig. 3. Extrapolation, assuming an hyperbolic dependence on microtubule site concentration, gave a rate of 110 s⁻¹ for the dissociation of mant ADP from the MtK-mant ADP

![Table I: Rate and dissociation constants of MtK332](image-url)
complex. The concentration at half-maximum rate is 24 mM in 10 mM NaCl which is 50% larger than the equilibrium dissociation constant. For a two-step reaction,

\[ \text{Mt} + \text{K} \cdot \text{ADP}^* \rightleftharpoons \text{MtK} \cdot \text{ADP}^* \rightleftharpoons \text{MtK} + \text{ADP} \]

**REACTION 1**

where the asterisk denotes a state of enhanced fluorescence, a hyperbola would fit the results if the first step were in rapid equilibrium relative to the second step. This condition is probably not met because of the large rate constant of dissociation of the mant ADP. A Scatchard plot exhibited some curvature indicating that the maximum rate may be overestimated. However, the observed rate is as large as 80 s\(^{-1}\), and the extrapolated value is at least 100 s\(^{-1}\). The corresponding experiment for MtK379 gave a value of 30 to 35 s\(^{-1}\) (2). The rate of dissociation of mant ADP is about 50% larger than the maximum steady state rate of MtK332 and the same correlation holds for MtK379 and also for microtubule-ncd (nonclaret disjunction) (14).

**Binding of mant ADP to MtK332**—The binding of mant ADP to MtK332 gave a signal of much smaller amplitude at low nucleotide concentrations compared to mant ATP and a very large rate of increase in fluorescence (>300 s\(^{-1}\) at 5 mM mant ADP). The transient fitted one exponential term. The rate constant increased with mant ADP concentration (Fig. 8, in 10 mM NaCl) and appeared to be reaching a maximum value of about 700 s\(^{-1}\). The limiting rate at zero concentration is approximately 300 s\(^{-1}\). For a one-step reaction,

\[ \text{MtK} + \text{ADP} \rightleftharpoons \text{MtK} \cdot \text{ADP} \]

**REACTION 2**

with rate constants \(k_1\) and \(k_2\) in the forward and reverse directions, the intercept as [ADP] approaches zero is \(k_2\), the rate constant of ADP dissociation. The value is significantly larger than the rate constant determined in the mant ADP release experiment (Fig. 3).

At higher ionic strength (50 mM NaCl), the fluorescence transient fitted two exponential terms, a very large rate of increase followed by a slower increase in fluorescence of 10 to 20% of the total, after correction for loss of the fast signal in the dead time. In this case, the complex is partly dissociated by
mant ADP. The second step reached a rate of 60 to 70 s\(^{-1}\) which is equal to the rate of dissociation measured by light scattering. Therefore, the additional signal arises from dissociation of KADP which binds the substrate with much higher affinity.

A second type of experiment supported the conclusion that the rate constant of mant ADP dissociation is very large. At a high microtubule concentration, MtK332 is largely associated in the presence of mant ADP. The dissociation constant of mant ADP in this complex is expected to be 20 to 30. More than half of the amplitude of the slow process, with a rate constant of 13 s\(^{-1}\), arises from dissociation of mant ADP. The fluorescence transient is shown in Fig. 9 (20 \(\mu\)M K332, 20 \(\mu\)M mant ADP, 75 \(\mu\)M tubulin dimer, concentrations before mixing one to one with 1 mM ATP). More than half of the amplitude of the signal (corrected for signal loss during the dead time) corresponds to a very fast step with rate constant of 400 s\(^{-1}\). The slower process, with a rate constant of 13 s\(^{-1}\), is attributed to the presence of some dissociated K332-mant ADP which must bind to microtubules before release of the mant ADP. The experiment demonstrates that at equilibrium, at least part of the bound mant ADP is present in a complex with a very large rate constant of dissociation.

**DISCUSSION**

The objective of this study is to formulate a kinetic scheme for a monomeric kinesin (K332). In the absence of microtubules, the rate constants for K332 are very similar to dimeric K379 and whole kinesin prepared from bovine brain (1, 10). The removal of the 47 amino acid residues, which stabilize the motor as a dimer complex, appear to have little or no effect on the steps of substrate binding, hydrolysis, and release of reaction products.

The data are analyzed in terms of the simple kinetic scheme proposed for dimeric K379 (Scheme 2). The first step in mant ATP or mant ADP binding, the formation of a rapid equilibrium complex, is omitted for simplicity; \(k_1, k_2\), and \(k_{-1}, k_{-2}\) are the first order rate constants for the transition induced by nucleotide binding.

Measurements were made at 10 mM NaCl to obtain rate constants for transitions between MtK states because the MtK332 complex is partly dissociated at higher ionic strengths. The maximum observed rates for the first order transition for the binding of mant ATP are approximately 350 s\(^{-1}\) for K332 and 700 s\(^{-1}\) for MtK332. In part, the increase in rate constant arises from an increase in \(k_1\), but the value of \(k_1\) is probably larger than \(k_1\). The maximum rate for mant ADP binding is also twice as large for MtK332 compared to K332, and, as discussed below, the increase in rate is caused mainly by the large rate of dissociation of mant ADP. The rate of hydrolysis (step 2) is increased from 7 s\(^{-1}\) to at least 200 to 250 s\(^{-1}\) by binding to the microtubule and the rate of dissociation of mant ADP is increased from 0.02 s\(^{-1}\) to greater than 100 s\(^{-1}\).

The irreversible steps 3 and 4 are equivalent to an effective rate \(k_d = k_3 + k_4\). To obtain a steady state rate of 50 to 60 s\(^{-1}\), the values assigned to the rate constants of the first two steps require \(k_d\) to be 70 to 100 s\(^{-1}\). The intercept at zero concentration for the rate of binding of mant ATP is a measure of \(k_9\), and the value is approximately 100 s\(^{-1}\) (Fig. 5). A difficulty arises in assigning values to the individual rate constants \(k_3\) and \(k_4\). The only direct measurement of \(k_9\) is the report that the rate of phosphate dissociation for a dimeric kinesin complex (Drosophila K401) is comparable to the steady state rate (4).

Two kinds of experiments were done to estimate \(k_d\), but the results did not agree. The extrapolated maximum rate of mant ADP dissociation from the reaction of K-ADP with microtubules is 110 s\(^{-1}\). The rate of mant ADP binding to MtK extrapolated to zero concentration (Fig. 8), and the dissociation of mant ADP from an equilibrium MtK-ADP complex (Fig. 9) gave approximately 300 s\(^{-1}\). For the simple kinetic scheme, both experiments should measure \(k_d\), and the discrepancy is larger than expected from experimental errors.

A reasonable explanation is that there are two MtK-ADP states. The initial complex formed by KADP with the microtubule is weakly bound, but it undergoes a transition to a more strongly bound state followed by rapid dissociation of ADP. This complication will be discussed later. In terms of the simple scheme, the effective rate of ADP dissociation in the cycle lies between the values obtained in the two experiments or roughly 200 s\(^{-1}\). Rate constants \(k_3\) and \(k_4\) are similar in magnitude in the scheme.

**Pathway of Dissociation**—The important problem is to determine which state or states dissociate in the reaction cycle. The simple scheme with one MtK-ADP state is satisfactory for the discussion of this question. The rate of dissociation of the MtK332 complex by ATP is 40 s\(^{-1}\) in 50 mM NaCl and probably smaller in 10 mM NaCl while the rate of the hydrolysis step is at least 200 s\(^{-1}\). Therefore, the reaction reaches the MtK-ADP-P state before dissociation. The dissociation constant and the rate of dissociation for the MtK-ADP-P state are not known. The state formed with ADP-AlF\(_4\) has been considered to be an analogue of ADP-P (12), and the binding is much stronger than in the presence of ADP (13, 14 and Table 1). The ADP-avanadate state may be a better analogue of ADP-P in the

---

**Scheme 2**

![Scheme 2](image-url)
case of myosin (15), and it is more weakly bound than the MtKADP state. Further studies are needed to determine the significance of the findings with these analogues. The ADP state (or states) dissociate with an effective rate constant of 75 ± 10 s⁻¹.

The finding of a phosphate burst of more than 1 mol/mol of sites provides evidence for the dissociation pathway. The size of the phosphate burst is 0.4 to 0.5 for conditions such that the system remains associated which agrees with the value calculated from the rate constants and steady state rate (equations are given in Ref. 2). At higher ionic strength (75 mM NaCl) under conditions that the system is largely dissociated in the steady state, the rate constant of the burst is only 40 to 50 s⁻¹ which is equal to the rate of dissociation by ATP, and the size of the burst was as large as 2 mol per site. The excess of the burst over the value expected from the hydrolysis step is termed the extra burst.

The significance of the extra burst is made clear by considering two possible reaction pathways. In the first case, the rate of dissociation of MtKADP-P is assumed to be much smaller than the rate of phosphate release (k₃); consequently, only the MtKADP state dissociates in the cycle. The rate constants assigned to steps 1 through 4 and k₃ define a model which can be simulated using KINSIM. In the limit of nearly complete dissociation in the steady state, the extra burst is approximately k₃/k₄₃₄₅₆ and, since the ratio of the rate constants is approximately 2, this pathway accounts for the extra burst. Furthermore, the rate of dissociation of MtK by ATP is equal to the rate of the extra burst.

In the second case, the rate of dissociation of MtKADP-P is taken to be large compared with k₃. In the limit of complete dissociation, there is no extra burst. It is concluded that the main pathway is dissociation (and rebinding) of the MtKADP state. Some dissociation of MtKADP-P may occur, but this step reduces the size of the extra burst. Thus, even a monomer shows a small processivity in that more than one ATPase cycle can occur before reaching the steady state level of dissociation. Gilbert et al. (4) obtained a burst of at least 2 mol per site for a monomeric kinesin and also concluded that the size of the extra burst is a measure of processivity.

Gilbert et al. (4) considered two kinetic pathways. Although their studies were made with a dimeric kinesin, the kinetic scheme is presented in terms of a single head. They favor a mechanism in which the dissociation of MtKADP-P is the rate-limiting step and phosphate dissociation occurs from the KADP-P state. Because this model cannot account for the extra burst, it is incorrect when applied to a monomer. Their second model makes phosphate dissociation from MtKADP-P the rate-limiting step, and the model includes two MtKADP states even though evidence for two states was not given. Thus, their second model is similar to the one proposed here.

Problem of Two MtKADP States—The rate constants for a two-step reaction are not uniquely determined by the data.

Simulation using KINSIM gave an approximate fit to the results for the two ADP release experiments and the rate of dissociation of MtK by ADP for the following scheme:

\[
\begin{align*}
K \cdot \text{ADP} + \text{Mt} & \xrightarrow{300 \text{ s}^{-1}} \text{Mt}_3 \text{K} \cdot \text{ADP} \xrightarrow{150 \text{ s}^{-1}} \text{Mt}_2 \text{K} \cdot \text{ADP} \xrightarrow{300 \text{ s}^{-1}} \text{Mt}_1 \text{K} + \text{ADP}
\end{align*}
\]

**REACTION 3**

Two different rate constants in the two experiments would only be observed if the equilibrium between ADP states favors MtKADP. The two-step fluorescence signal for the binding of mant ADP to K332 and other kinesin constructs provides some evidence for two ADP states. Simulation of the ATPase cycle with two ADP states showed that the value of k₃ of 100 s⁻¹ was consistent with the rate constants assigned to the ADP release steps and a phosphate dissociation rate of 150 s⁻¹ to 200 s⁻¹. Two ADP states have also been reported for myosin S-1 and actomyosin (17, 18).

The microtubule kinesin mechanism has been described as alternating between a weak and a strongly bound state much like the actomyosin mechanism (13). The weakly bound MtKADP state has a dissociation constant of 20 μM based on the concentration dependence of the rate of mant ADP release (Fig. 3). The dissociation constant of the more strongly bound state is not known, but it is expected to be much larger than the value for nucleotide-free kinesin. The proposal that a weak to strong transition is necessary for ADP release is relevant to the problem of head interactions in the microtubule-kinesin dimer complex (19).

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