Transient State Kinetic Analysis of the ATP-induced Dissociation of the Dynein-Microtubule Complex*

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The kinetics of ATP-induced dissociation of dynein from the dynein-microtubule complex has been investigated by stopped flow light scattering methods. The addition of ATP to the dynein-microtubule complex induced a large, rapid decrease in light scattering followed by a smaller and much slower decrease. The fast light scattering change was shown to be a measure of the ATP-induced dissociation of dynein from the dynein-microtubule complex and was distinguished from microtubule disassembly by several criteria. (i) The fast reaction occurred over a period of milliseconds and the rate was a function of the ATP concentration, whereas, the slow reaction occurred over a period of several seconds and was independent of ATP concentration; (ii) the amplitude of the fast reaction was directly proportional to the amount of dynein bound to the microtubule lattice; and (iii) only the slow phase was inhibited by the addition of the microtubule-stabilizing drug, taxol. The rate of ATP-induced dissociation of dynein from the microtubule increased linearly with increasing ATP concentration to give an apparent second order rate constant for ATP binding equal to 

\[ k_1 = 4.7 \times 10^4 \text{ m}^{-1} \text{s}^{-1} \]

according to the following pathway:

\[ M \cdot D \rightarrow M \cdot D \cdot ATP \rightarrow M + D \cdot ATP \]

where \( M \cdot D \) represents the dynein-microtubule complex and \( D \) represents dynein. The loss of signal amplitude at high ATP concentration provided a minimum estimate for the rate of dissociation of the ternary complex \( (M \cdot D \cdot ATP) \) equal to \( k_2 > 1000 \text{ s}^{-1} \). Thus, the dynein-microtubule system is similar to actomyosin in that ATP induces an extremely rapid dissociation of dynein from the microtubule.

The dynein ATPase cross-bridges adjacent doublet microtubules in cilia and flagella and serves to couple the hydrolysis of ATP to the production of force for microtubule sliding (Brokaw and Benedict, 1968; Gibbons and Gibbons, 1972, 1974; Gibbons, 1965; Satir, 1968; Summers and Gibbons, 1971, 1973). Although the evidence that the hydrolysis of ATP is coupled to the cross-bridge cycle is quite convincing, the precise relationship between the mechanical cross-bridge cycle and the ATPase cycle has not been established. In general, data have been interpreted by analogy to the Lymn-Taylor (1971) actomyosin cycle (Sale and Gibbons, 1979; Satir et al., 1981), and although these interpretations are reasonable, there is no compelling evidence to determine alternative pathways. Namely, there has been no direct measurement of the kinetics of ATP binding and hydrolysis or the kinetics of association and dissociation of the dynein-microtubule complex. In addition, there is no convincing evidence that tubulin activates the dynein ATPase under physiological conditions in solution.

Several investigators have attempted to establish the reaction sequence indirectly by using either nonhydrolyzable ATP analogs or inhibitors of the dynein ATPase activity such as vanadate. Sale and Gibbons (1979) observed that the addition of ATP and vanadate to rigor wave sperm flagella or trypsin-treated axonemes was followed by relaxation of the waveform or peeling of the doublet, but reactivation of beating and sliding disintegration were completely inhibited. Later work by Okuno (1980) using stiffness measurements to assess relaxation demonstrated that MgATP\(^-\) could induce flagellar relaxation in the presence of vanadate, whereas MgADP\(^-\) could not. Mitchell and Warner (1980, 1981) and Satir et al. (1981) observed dissociation of dynein cross-bridges from the B-subfiber in the presence of MgATP\(^-\) and vanadate under conditions where sliding disintegration and steady state ATP hydrolysis were completely inhibited. However, in each of these experiments it was not possible to determine whether a single round of ATP hydrolysis had occurred prior to dissociation of the dynein from the microtubule following the addition of ATP in the presence of vanadate.

Interpretation of results obtained with nonhydrolyzable ATP analogs has also been controversial. Early work indicated that adenylyl-5'-yl imidodiphosphate could induce flagellar relaxation, but at concentrations much higher than that required with ATP (Penningroth and Witman, 1978). Subsequent work has shown the initial observation to be the result of ATP contamination (Okuno and Brokaw, 1981; Penningroth et al., 1980). Mitchell and Warner (1980) observed that adenylyl-5'-yl imidodiphosphate could dissociate dynein from B-subfibers; however, they presented no evidence to eliminate the possibility that the observed dissociation was due to a low concentration of contaminating ATP. Moreover, experiments with adenylyl-5'-yl imidodiphosphate do not establish the sequence of events that occur after the addition of ATP to dynein.

The central question as to whether ATP hydrolysis precedes or follows the ATP-induced dissociation of dynein from the microtubule can only be answered by measuring the rates of ATP binding, hydrolysis, and product release and the rates of cross-bridge association and dissociation. Since the rate of
the entire cross-bridge cycle in an axoneme is extremely fast \( (\sim 35 \text{ s}^{-1}) \) (Brokaw and Gibbons, 1975), it is necessary to examine these reactions with the techniques of pre-steady state kinetics.

The addition of ATP to decorated \textit{Tetrahymena} axonemes causes cross-bridge dissociation, sliding disintegration, and fraying of outer doublets (Mitchell and Warner, 1980). To avoid these complications, we have used dynein-decorated brain microtubules to provide a simple, homogeneous model system and to apply these results to understanding the reactions in the ciliun. The data in the preceding paper (Porter and Johnson, 1983) showed that \textit{Tetrahymena} 30 S dynein\(^1\) binds to recycled brain microtubules by the ATP-sensitive site and the addition of ATP induced quantitative dissociation of the dynein from the surface of the microtubules. In this paper, we measure the rate of this reaction by stopped flow light scattering methods. Preliminary accounts of these data have been presented (Johnson and Porter, 1981, 1982a, 1982b; Porter and Johnson, 1981).

**MATERIALS AND METHODS**

**Preparation of the Dynein-Microtubule Complex—**Dynein and tubulin were prepared as described in the previous paper (Porter and Johnson, 1983). Microtubules were polymerized at a concentration of approximately 4–8 mg/ml by incubation at 35 °C for 15–20 min and then sonicated for 2 s at setting 4 with a Sonifier Cell Disruptor microprobe (Model 14D, Heat Systems-Ultrasonics, Inc., Plainview, NY). This sonication reduced the average microtubule length from 4–6 to 1–2 μm and was necessary to reduce the flow birefringence artifact in the stopped flow measurements (see below). In some experiments, the microtubules were first stabilized by the addition of an equimolar concentration of taxol (Schiff et al., 1979). The microtubules were then diluted into dynein that had been prewarmed to 28 °C and then incubated for 20 min. The ratio of dynein to tubulin was varied from 0.5 to 3 mg of dynein/mg of tubulin for some experiments, but, in general, the complex was formed by incubation for 20 min at 28 °C at concentrations of 1 mg/ml each for tubulin and dynein which corresponds to one-third occupancy of microtubule sites (Porter and Johnson, 1983).

**Stopped Flow Apparatus—**Light scatttering at 90° to the incident beam was measured using a stopped flow spectrofluorometer that was constructed in this laboratory based on a design similar to that described by Finlayson and Taylor (1969) with several modifications. The sample syringes, drive lines, and observation cell were thermostated using a refrigerated circulating water bath (Model RTE-9, Neslab Instruments, Inc., Portsmouth, NH). Syringes containing the two samples were driven by an air cylinder (Bimba Manufacturing Co., Monroeville, IL) that was controlled by a solenoid valve (Skinner & Creek, Inc., Jeffersonville, IN), which was driven by a microcomputer for data collection and analysis. In all cases, the data were adequately fit to a single exponential by the method of moments (Morton, 1971).

**Stopped Flow Measurements of ATP-induced Dissociation of the Dynein-Microtubule Complex—**All of the experiments reported here were performed at 28 °C in a buffer consisting of 50 mM PIPES and 4 mM MgCl₂ at pH 7.0. The dynein-microtubule complex was formed as described above, then diluted 5–10-fold with warm buffer, and immediately loaded into one syringe of the stopped flow apparatus. The reaction was initiated by mixing the dynein-microtubule complex 1:1 with ATP in the same buffer. All of the ATP concentrations reported are the concentrations after mixing unless specifically stated otherwise.

Mixing microtubules or the dynein-microtubule complex with buffer in the stopped flow apparatus produced a small change (10%) in light scattering that can best be described as a flow birefringence artifact. The intense scattering of light by microtubules makes this artifact noticeable under conditions where no change is observed with actin or actomyosin. As shown in Fig. 6 (upper curve), there was a small, rather noisy decrease in light scattering during the first 50 ms after mixing that was followed by an increase to the original level over the period of approximately 2 s. This can be understood as follows. The microtubules initially enter the observation cell in turbulent flow and are randomly oriented. After flow stops, the turbulence continues for a few milliseconds, but as the turbulence ceases, the flow in small eddies becomes laminar and tends to orient the microtubules leading to a decrease in light scattering. With time, the microtubules randomize, leading to a subsequent increase in light scattering. The time dependence of the increase in light scattering over the period of a few seconds corresponded to rates expected for rotational relaxation times for long rods (Tanford, 1967).

It was important to maximize the signal due to dynein dissociation and to minimize the noise associated with the light scattering of the microtubules themselves. First, we sonicated the preassembled microtubules to reduce their length without altering the monomer-polymer equilibrium. This decreased the light scattering change associated with the flow birefringence artifact. We also formed the dynein-microtubule complexes at high protein concentrations (1 mg/ml of dynein and 0.5–1.0 mg/ml of tubulin) for at least 20 min at 28 °C to ensure that the binding reaction had gone to completion (Porter, 1982). The complexes were then diluted 5-fold with warm buffer to obtain a final concentration of 0.2 mg/ml of tubulin in the stopped flow before mixing. As shown in Fig. 6 (lower curve), the decrease in light scattering during the first 50 ms after mixing was due to the flow birefringence artifact relative to the change seen upon dissociation. The major factor in our ability to measure dynein dissociation without a noticeable contribution of the flow artifact was the extremely large signal resulting from the dissociation. The flow birefringence artifact placed a limit on the rate of reaction that could be accurately measured due to the loss of signal amplitude at the faster rates.

**RESULTS**

Light Scattering is a Measure of Dissociation of the Dynein—In the previous paper (Porter and Johnson, 1983), we showed that the change in turbidity was a quantitative measure of the association and dissociation of dynein at periodic sites on the microtubule. Since there was no cross-bridging of microtubules detectable by electron microscopy, it was concluded that the decrease in light scattering was not due to the disaggregation of bundles of microtubules. However, some of the light scattering change might have been due to the depolymerization of microtubules. This is a major concern in measuring the kinetics of ATP-induced dissociation of the dynein from the microtubule, since dynein stabilizes the microtubule.

\(^1\) The faster sedimenting dynein isolated from \textit{Tetrahymena} cilia has been referred to as the 30 S particle based upon the original description by Gibbons (1965). Our recent measurements by analytical ultracentrifugation have given a value of \(s_{20,w} \) equal to 22 S for the faster sedimenting species in our preparations (D. Clutter and K. A. Johnson, manuscript in preparation) which is close to the value reported by Mitchell and Warner (1980). There is evidence to suggest that the 30 S particle described by Gibbons (1965) may have been an aggregate of the 22 S particle, but further work is required to establish definitively whether there is a single species. We will continue to use the name 30 S dynein to refer to the faster sedimenting species of dynein, since that name has been so widely used in the literature.

\(^2\) K. A. Johnson, manuscript in preparation.

\(^3\) The abbreviation used is: PIPES, 1,4-piperazinediethanesulfonic acid.
microtubules against depolymerization and nearly all of the experiments were performed below the critical concentration for microtubule assembly.

A dynein-microtubule complex was formed by incubation of polymerized bovine brain microtubules with *Tetrahymena* dynein at concentrations of approximately 1-2 mg/ml each for 20 min at 28 °C. This dynein-microtubule complex was then diluted 3-5-fold with warm buffer and loaded into one syringe of the stopped flow apparatus. Buffer or solutions containing ATP were then loaded into the other syringe. Fig. 1 (bottom curve) shows the change in light scattering as a function of time following the mixing of the dynein-microtubule complex with 22 μM ATP. First, there was a rapid decrease in light scattering in a reaction that was complete in less than 100 ms. This decrease can be fit by a single exponential with a rate constant of 56 s⁻¹. The rapid decrease was followed by a smaller slow decrease over a time course of several seconds.

The most reasonable explanation of these results is that the rapid phase of the light scattering decrease is due to the dissociation of the dynein from the microtubules and that the slower phase is due to microtubule depolymerization following the release of the dynein. We used three criteria to test this postulate. (i) The addition of taxol should eliminate or markedly reduce the amplitude of the light scattering change that is due to microtubule depolymerization; (ii) the amplitude of the light scattering change due to dynein dissociation should be proportional to the ratio of dynein to tubulin in the preformed complex up to the point where the microtubule surface is saturated with dynein (3 mg of dynein/mg of tubulin); and (iii) the rate of the light scattering change due to dynein dissociation should depend upon the ATP concentration, whereas, the rate of microtubule disassembly should be independent of ATP concentration.

For the experiment shown in the top curve of Fig. 1, the microtubules were stabilized by the addition of a 2-fold molar excess of taxol (Schiff *et al.*, 1979) prior to incubation with dynein to form the dynein-microtubule complex. The rate (65 s⁻¹) and amplitude of the large, rapid light scattering change following the addition of ATP were unaffected by taxol, whereas the slow phase was reduced. This suggests that the fast light scattering change is due to the dissociation of the dynein from the microtubules and the slow phase is due to microtubule depolymerization. Presumably, dynein stimulates microtubule initiation (Haimo *et al.*, 1979) and inhibits microtubule disassembly in a manner analogous to that described for other microtubule-associated proteins (Murphy *et al.*, 1977), and the ATP-induced dissociation removes this stabilizing effect.

The amplitude of the signal due to the ATP-induced dissociation of dynein from the microtubule should be proportional to the concentration of dynein in the dynein-microtubule complex when the microtubule sites are in excess. The titration experiment described in the previous paper (Porter and Johnson, 1983) indicated that the microtubule surface lattice was saturated at a ratio of 3 mg of dynein/mg of tubulin and that the change in turbidity was linear up to the point of saturation. We therefore prepared dynein-microtubule complexes at varying ratios of dynein to tubulin in the preincubation mixtures. Each complex was then mixed with ATP in the stopped flow, and the time course of the light scattering change was recorded to obtain the results shown in Fig. 2. The amplitude of the ATP-induced change in light scattering increased linearly with increasing dynein/tubulin ratio (Fig. 2, inset). Moreover, the pseudo-first order rate constant for dissociation was independent of the dynein/tubulin ratio. The rate of dissociation was 100 ± s⁻¹ at all ratios examined at an ATP concentration of 30 μM.

![Fig. 1](image1.png)

**Fig. 1.** Light scattering changes induced by ATP addition to dynein-microtubule complexes. Dynein (1.2 mg/ml) was preincubated with polymerized microtubules (1.5 mg/ml) in the presence and absence of taxol (33 μM) for 20 min at 28 °C. Samples were then diluted 3-fold and loaded into one syringe of the stopped flow apparatus and mixed 1:1 with 44 μM ATP. The change in light scattering was followed for 100 ms and then for an additional 15 s. The smooth curve shows the best fit to a single exponential for the fast phase. Upper curve, no taxol; middle curve, added taxol.

![Fig. 2](image2.png)

**Fig. 2.** Amplitude of ATP-induced dissociation as a function of the dynein-tubulin ratio. Dynein was preincubated with polymerized microtubules at different dynein-tubulin (D/T) ratios. Samples were then loaded into the stopped flow apparatus and mixed 1:1 with 60 μM ATP (30 μM final concentration). The ATP-induced decrease in light scattering was followed over 60 ms. Top curve, 2 mg of dynein/mg of tubulin; middle curve, 1.5 mg of dynein/mg of tubulin; bottom curve, 1.0 mg of dynein/mg of tubulin. The rate of ATP-induced dissociation, shown by the fit to a single exponential, was 103 s⁻¹ in each trace. Inset, amplitude of the light scattering decrease plotted as function of the dynein-tubulin ratio.
The observed dependence of the rate and amplitude of the ATP-induced change in light scattering exhibited all of the properties expected for a signal that is a function of the dissociation of dynein from the microtubule under conditions where the tubulin lattice sites are in excess (see “Discussion”). The rate was independent of the dynein concentration and was determined only by the concentration of ATP (see below) under the conditions for pseudo-first order kinetics with ATP in excess.

Kinetics of Dissociation with Different Dynein Preparations—Due to the limitations of the quantities of material available for these transient state kinetic studies, it was necessary to perform most of the experiments with the column-purified dynein preparation which was composed of 80% 30 S dynein and 15% 14 S dynein. Moreover, this enabled us to perform all experiments within 24 h of the preparation of the protein. In the previous paper (Porter and Johnson, 1983) it was shown that 14 S dynein did not bind to microtubules and did not interfere with the binding of 30 S dynein to microtubules as assayed by electron microscopy or co-sedimentation experiments. However, the possibility still remained that the 14 S dynein might alter the rate at which the 30 S dynein dissociated from the microtubule following the addition of ATP.

In the experiment shown in Fig. 3, four different dynein preparations (column-purified dynein, 30 S dynein, 14 S dynein, and a mixture of 30 and 14 S dynein) were each separately incubated with polymerized microtubules for 20 min, diluted with buffer, and then loaded into one syringe of a stopped-flow spectrophotometer. Buffer or a solution of ATP was loaded into the other syringe, the two solutions were rapidly mixed, and the change in light scattering was recorded. Mixing a column-purified dynein-microtubule complex with 10 μM ATP induced a very rapid decrease in light scattering (Fig. 3A). The reaction was complete within 150 ms, and the curve could be fit to a single exponential with a rate of 39 s⁻¹.

Identical results were obtained with purified 30 S dynein (Fig. 3B) and with a mixture of 30 dynein and 14 S dynein (Fig. 3D). No light scattering change was observed when purified 14 S dynein was used. This was expected since our previous studies indicated that the 14 S dynein did not bind to the microtubules under these conditions (Porter and Johnson, 1983). Since the kinetics of the ATP-induced dissociation were virtually identical whether the experiment was performed with the column-purified dynein, the 30 S dynein, or the mixture of 30 and 14 S dynein, we conclude that the 14 S dynein does not alter the rate of dissociation.

Kinetics of Dissociation As a Function of the ATP Concentration—Having established that the decrease in light scattering observed following the addition of ATP to the dynein-microtubule complex is due to the dissociation of the 30 S dynein, we then examined the rate of dissociation as a function of ATP concentration. When the dynein-microtubule complex was mixed with 5 μM ATP, dissociation occurred with a pseudo-first order rate constant of 23.6 s⁻¹ (Fig. 4A). When the ATP concentration was increased to 40 μM, dissociation was complete within 25 ms and was fit to a single exponential with a rate constant of 179 s⁻¹ (Fig. 4B). At ATP concentrations up to 60 μM, the dissociation kinetics could be fit to a single exponential.

The rate of dissociation increased linearly with increasing ATP concentration as shown in Fig. 5. The slope of this line provides a measurement of the apparent second order rate constant for the ATP-induced dissociation (k1 = 4.7 × 10⁶ M⁻¹ s⁻¹ (see Equation 1)) which equals the rate of ATP binding. If the intrinsic rate of dissociation of the dynein-microtubule complex becomes rate-limiting at high ATP concentrations, one expects to observe a plateau in the rate at
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Fig. 4. Kinetics of dissociation at two ATP concentrations. 
A, dynein-microtubule complex mixed 1:1 with 10 μM ATP as described in the legend to Fig. 1. Dissociation was complete within 200 ms with a rate constant of 23.6 s⁻¹. B, complex mixed 1:1 with 80 μM ATP and dissociated within 20 ms with a rate constant of 179 s⁻¹. The dissociation fit a single exponential at all concentrations of ATP investigated.

saturating ATP. At ATP concentrations greater than 60 μM, dissociation rates greater than 300 s⁻¹ were observed and the reaction became too fast to accurately measure due to loss of signal amplitude. Thus, in the time scale accessible in the stopped flow apparatus, it was not possible to directly measure the intrinsic rate of dissociation.

It is possible to obtain a minimum estimate for the intrinsic rate of dissociation of the dynein-microtubule complex by examination of the signal amplitude at high ATP concentration. At concentrations of ATP greater than 500 μM, the dissociation reaction was essentially complete in the time required to mix the reactants in the stopped flow (1–2 ms) as shown in Fig. 6. The loss of signal amplitude in relationship to the known dead time of the instrument provides a minimum estimate of the rate of dissociation. The total signal amplitude, as the observed change in light scattering at low ATP concentration (5 μM), was 1.05 units (see Fig. 6, middle curve). At 500 μM ATP, only a small change in light scattering was observed with an amplitude of approximately 0.1 units (Fig. 6, lower curve). The observed change in light scattering places an upper limit upon the amplitude of the signal remaining in 1–2 ms after mixing, the dead time of the instrument. This provides a minimum estimate of the rate of reaction to be 1200–2400 s⁻¹ (see “Discussion”). Conservatively, we estimate the rate constant to be greater than 1000 s⁻¹.

Fig. 5. ATP dependence of the dissociation rate constant. The rate of ATP-induced dynein dissociation is plotted as a function of the final ATP concentration. The slope of the line gives the apparent second order rate constant of 4.7 × 10⁶ M⁻¹ s⁻¹.

Fig. 6. Signal amplitude as function of ATP concentration. The dynein-microtubule complex was mixed 1:1 with buffer (top curve), 10 μM ATP (middle curve), or 1000 μM ATP (bottom curve), and the time course of dissociation was followed over 200 ms for the first 200 data points and then over 1 s for the last 50 data points. The small fluctuations in light scattering in the top curve are probably due to the flow birefringence of the microtubules after mixing (see “Materials and Methods”).
The data presented in this paper show for the first time that ATP induces an extremely rapid dissociation of dynein from the dynein-microtubule complex. The decrease in light scattering after ATP addition can be adequately fit to a single exponential, indicating that the ATP-induced dissociation of dynein from the microtubule occurs as a single step kinetically. The simplest interpretation of this result is that we are looking at the dissociation of a single cross-bridge upon the addition of ATP. Structural analysis of the dynein molecule has shown that there are three independent heads that interact with the microtubule to form the ATP-sensitive complex (Johnson and Wall, 1983). The data presented here at relatively high ATP concentration are similar to that obtained for actomyosin (Lynn and Taylor, 1971; Taylor, 1977; White and Taylor, 1976) in that the dissociation kinetics does not show the kind of cooperativity that one expects if two or more molecules of ATP are required to induce the dissociation of a single dynein molecule. We are currently investigating the dissociation kinetics at lower concentrations of ATP to more closely examine the reaction under conditions where such cooperativity would be more apparent.

The linear increase in rate with increasing ATP concentration implies that the dissociation of dynein from the microtubule is not limited by reactions occurring subsequent to ATP binding, such as ATP hydrolysis or the dissociation reaction per se. Therefore, the observed rate of dissociation of the dynein is a direct measure of the rate of ATP binding to the dynein-microtubule complex. We have placed a minimum estimate on the rate-limiting step in the dissociation pathway by analysis of the apparent amplitude at high ATP concentration (500 \( \mu \)M). The observed amplitude (A) should be related to the total reaction amplitude (A_o) and the dead time of the stopped flow (t) by the equation: 

\[
A = A_o e^{-kt} 
\]

where \( k \) is the rate of the reaction. The amplitude observed at high ATP concentration (Fig. 6) provided a minimum estimate for \( k \) equal to 1200–2400 s\(^{-1}\) for the dead time of 1–2 ms. If the rate were to continue linearly up to 500 \( \mu \)M ATP, one would expect a rate of 2400 s\(^{-1}\). Conservatively, we place a lower limit of 1000 s\(^{-1}\) for the intrinsic rate of dissociation of dynein from the microtubule following ATP binding. Thus, the rate observed at low ATP concentrations is limited by the rate of ATP binding, not by the rate of dissociation or the rate of ATP hydrolysis. This sequence is diagrammed below:

\[
M.D + ATP \xrightarrow{k_1} M.D-ATP \xrightarrow{k_2} M + D-ATP
\]

where \( M.D \) represents the dynein-microtubule complex, \( k_1 = 4.7 \times 10^7 \) M\(^{-1}\) s\(^{-1}\), and \( k_2 > 1000\) s\(^{-1}\).

The very rapid dissociation of dynein following the binding of ATP is significant for the coupling of ATP hydrolysis to the cross-bridge cycle. The rigor state of muscle, for example, is formed at the end of the power stroke, after the myosin has undergone the presumed change in conformation to produce the force of sliding. This rigor state must have a short lifetime to preclude the attached myosin from inhibiting the sliding due to other cross-bridges. The rapid ATP-induced dissociation ensures that the lifetime of the attached rigor state will be short. At physiological ATP concentration in the cilium (1 mM), the lifetime of the dynein rigor state will be less than 0.7 ms. Models of dynein-induced sliding and wave propagation in flagella described by Brokaw (1975) require this fast dissociation.

Whether ATP hydrolysis precedes or follows the cross-bridge dissociation is a separate question. This can only be determined by direct measurement of the pre-study state rate of ATP hydrolysis under the same conditions. The results of these experiments will be described in a subsequent paper.²

REFERENCES