

RAPID COMMUNICATIONS

STUDY OF THE MECHANISM OF VANADATE INHIBITION OF THE DYNEIN CROSS-BRIDGE CYCLE IN SEA URCHIN SPERM FLAGELLA

WINFIELD S. SALE and I. R. GIBBONS. From the Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii 96813

ABSTRACT

The effect of vanadate on the ATP-induced disruption of trypsin-treated axonemes and the ATP-induced straightening of rigor wave preparations of sea urchin sperm was investigated. Addition of ATP to a suspension of trypsin-treated axonemes results in a rapid decrease in turbidity (optical density measured at 350 nm) concomitant with the disruption of the axonemes by sliding between microtubules to form tangles of connected doublet microtubules (Summers and Gibbons, 1971; Sale and Satir, 1977). For axonemes digested to ~93% of their initial turbidity, 5 μM vanadate completely inhibits the ATP-induced decrease in turbidity and the axonemes maintain their structural integrity. However, with axonemes digested to ~80% of their initial turbidity, vanadate fails to inhibit the ATP-induced decrease in turbidity and the ATP-induced structural disruption of axonemes, even when the vanadate concentration is raised as high as 100 μM . For such axonemes digested to 80% of their initial turbidity, the form of ATP-induced structural changes, in the presence of 25 μM vanadate, was observed by dark-field light microscopy and revealed that the axonemes become disrupted into curved, isolated doublet microtubules, small groups of doublet microtubules, and "banana peel" structures in which tubules have peeled back from the axoneme. Addition of 5 μM ATP to rigor wave sperm, which were prepared by abrupt removal of ATP from reactivated sperm, causes straightening of the rigor waves within 1 min, and addition of more than 10 μM ATP causes resumption of flagellar beating. Addition of 40 μM vanadate to the rigor wave sperm does not inhibit straightening of the rigor waves by 2 μM –1 mM ATP, although oscillatory beating is completely inhibited. These results suggest that vanadate inhibits the mechanochemical cycle of dynein at a step subsequent to the MgATP^{2-} -induced release of the bridged dynein arms.

KEY WORDS cilia · ATPase · microtubule sliding · rigor waves · myosin

Much evidence now exists supporting a sliding microtubule model of ciliary and flagellar motion in which doublet microtubules slide relative to one another in a controlled manner such that bends form near the proximal end of the flagellum and

then propagate distally (20, 23, 24). A variety of recent data has indicated that the dynein arms play a major role in generating the shearing force that leads to sliding between adjacent doublet microtubules during normal axonemal beating (5, 6, 8, 24, 25). The arms have been hypothesized to function by a mechanochemical cycle in which the detachment of an arm from one site on the adja-

cent B-subfiber and its change in angle and reattachment to an adjacent site along the B-subfiber are associated with the binding and hydrolysis of a molecule of ATP. Such a mechanism of dynein arm function would be analogous to the role of myosin cross bridges in generating sliding between the thick and thin filaments in striated muscle. A variety of enzyme kinetic and structural evidence is available concerning the several steps in the myosin cross-bridge cycle (cf. reference 14), but evidence about the cross-bridge cycle of dynein is relatively limited (cf. references 6, 18, 24, 26).

It has recently been discovered that vanadate (vanadium in the +5 oxidation state) is a potent inhibitor of the motility of reactivated cilia and flagella and of the Mg^{2+} -activated ATPase activity of extracted dynein. This inhibition by vanadate can be reversed either by dilution to reduce the concentration of vanadate below the critical inhibitory level or by the addition of catechol or norepinephrine, which complexes the vanadate and reduces it to the +4 oxidation state (3, 11, 15).

Preliminary investigation of the steady-state kinetics of vanadate inhibition of the Mg^{2+} -ATPase activity of the latent form of dynein 1 has indicated that the inhibition is not competitive with ATP binding, but that the inhibition appears to be of the uncompetitive type, with a given concentration of vanadate causing approximately proportional changes in the K_m and the maximal velocity of ATP hydrolysis (11, 15). This result suggests that vanadate inhibits by binding to the dynein-ATP complex. To test such a mechanism of inhibition, we thought that it would be helpful to look at the effects of vanadate on ATP-induced disruption of trypsin-treated axonemes (24) and on the ATP-induced straightening of rigor wave sperm (6).

MATERIALS AND METHODS

Sperm were obtained from the sea urchin *Tripneustes gratilla* by injection with 0.5 M KCl. A concentrated stock solution of 0.1 M sodium metavanadate (Fisher Scientific Co., Pittsburgh, Penn.; 2X recrystallized from methanol/water) was prepared in water (~pH 8.3); subsequent, more dilute stock solutions were prepared by dilution with 10 mM Tris-HCl buffer, pH 8.1, to avoid formation of decavanadate. To compare the present results with our previous work (11), we tested vanadate dilutions for inhibition of the motility of demembrated sperm reactivated with ATP. Trypsin and soybean trypsin inhibitor were purchased from Worthington Biochemical Corp., Freehold, N. J. Vanadate-free ATP was obtained from Boehringer-Mannheim Corp., Indianapolis, Ind. Other materials were the same as described earlier (11).

Quantitative measurements of the extent of trypsin digestion and of the degree of axonemal disruption induced by ATP were made by the turbidometric assay of Summers and Gibbons (25). Since the sperm heads scatter light to a greater extent than the axonemes, this procedure requires that all heads be removed from the preparation. Sperm were demembrated and broken by homogenization (Dounce type homogenizer) in a solution containing 1% (wt/vol) Triton X-100, 5 mM $MgSO_4$, 0.1 M KCl, 1.0 mM ATP, 1.0 mM DTT, 0.5 mM EDTA, 10 mM imidazole, pH 7.4 with NaOH, and the transversely fractured axonemes were isolated by differential centrifugation as described previously (12). After purification, the axonemal fragments were suspended at a concentration of ~0.25 mg protein/ml in Tris-Mg solution (2.5 mM $MgSO_4$, 0.25 mM EDTA, 0.1 mM dithiothreitol, 30 mM Tris-HCl buffer, pH 8.0). Digestion was carried out by addition of trypsin to the suspension of axonemes to give a trypsin:protein ratio of ~1:1500. The progress of digestion was monitored by the decrease in optical density at 350 nm measured in a 1-cm cuvette with a Zeiss PMQ2 spectrophotometer (Carl Zeiss, Inc., N. Y.). The initial optical density of the suspension (usually 0.4–0.5) was defined as 100%, and all subsequent changes are expressed as percentage changes relative to this initial value.

For most experiments, separate samples of a suspension of freshly isolated axonemal fragments in Tris-Mg solution were digested with trypsin to 93% and 80% of their initial turbidity, and then the digestion was stopped with about a 20-fold excess of soybean trypsin inhibitor. For conciseness, such preparations will be referred to as ⁹³axonemes and ⁸⁰axonemes, respectively. After addition of ATP or vanadate to a cuvette, the suspension was mixed gently, either by drawing it slowly into a pasteur pipette and releasing or by inverting the cuvette, before reading the turbidity. Changes in turbidity due to dilution and mixing were controlled by appropriate blanks. The morphological basis of the change in turbidity due to ATP-induced disintegration was studied by direct light microscope observation of individual axonemal fragments in a trough slide as a front of ATP diffused across the slide (24).

Sperm with their axonemes set into stationary rigor waves were prepared as described previously, except that 4 mM $CaCl_2$ and 0.02 mM ATP were added to the demembrating solution (6). Vanadate and ATP were added in various concentrations to suspensions of the rigor wave sperm in reactivation solution, and the effects on the rigor wave form were observed by dark-field light microscopy.

RESULTS

Trypsin-Treated Axonemes

In preliminary studies for this work, we confirmed our previous report (11) that the motility of demembrated sea urchin sperm reactivated

in 2 mM MgSO₄, 0.15 M KCl, 0.1 mM ATP, 1.0 mM DTT, 0.5 mM EDTA, 10 mM Tris-HCl, pH 8.1, was entirely inhibited by 4 μM vanadate. Having verified the critical inhibitory concentration of vanadate under these conditions, we proceeded to examine the effect of vanadate on ATP-induced disruption of trypsin-treated axonemes. Most further experiments were performed in the Tris-Mg medium described in Materials and Methods, but similar results were obtained in the reactivation medium given above.

The results of the turbidometric assay of the amount of disruption induced by addition of 0.25 mM ATP to digested axonemes in the presence and absence of 25 μM vanadate are shown in Table I. With no vanadate present, the ATP caused the same 38–42% decrease in turbidity at all degrees of digestion between 93% and 80%. Viewed by dark-field light microscopy, the majority of the digested axonemal fragments appeared as straight bright rods having the same uniform intensity and differing only in length (Figs. 1*a* and 2*a*). This appearance is consistent with the previous report (25) that axonemal fragments digested to 80% of their initial turbidity mostly retain the integrity of their 9 + 2 microtubule organization. Upon addition of ATP, the axonemal fragments disintegrated by smooth continuous sliding, resulting most often in a tangle of connected microtubules, but sometimes in a long slender structure ranging up to eight times the original length and much less bright than the original rods (Figs. 1*b* and 2*b*). The structures with a low uniform intensity in such dark-field micrographs have been shown to be individual doublet microtubules (19, 24, 25). In these figures, the doublet microtubules appear as thin threads emanating from the original axonemal fragment and are generally not disrupted into completely separate doublet microtubules. This appearance is characteristic of ATP-induced disintegration by sliding. It is important to note that the disintegration by sliding appears very similar in quantity and quality for both the ⁹³axonemes (Figs. 1*a* and 1*b*) and the ⁸⁰axonemes (Figs. 2*a* and 2*b*). This result agrees with the finding mentioned above that both degrees of digestion show the same percentage of turbidity decrease upon addition of ATP. Thus, in the absence of vanadate, varying the degree of digestion within the above limits appears to have no effect on the ATP-induced disintegration of the axoneme.

When the digested axonemes were suspended in

TABLE I
ATP-Induced Turbidity Changes

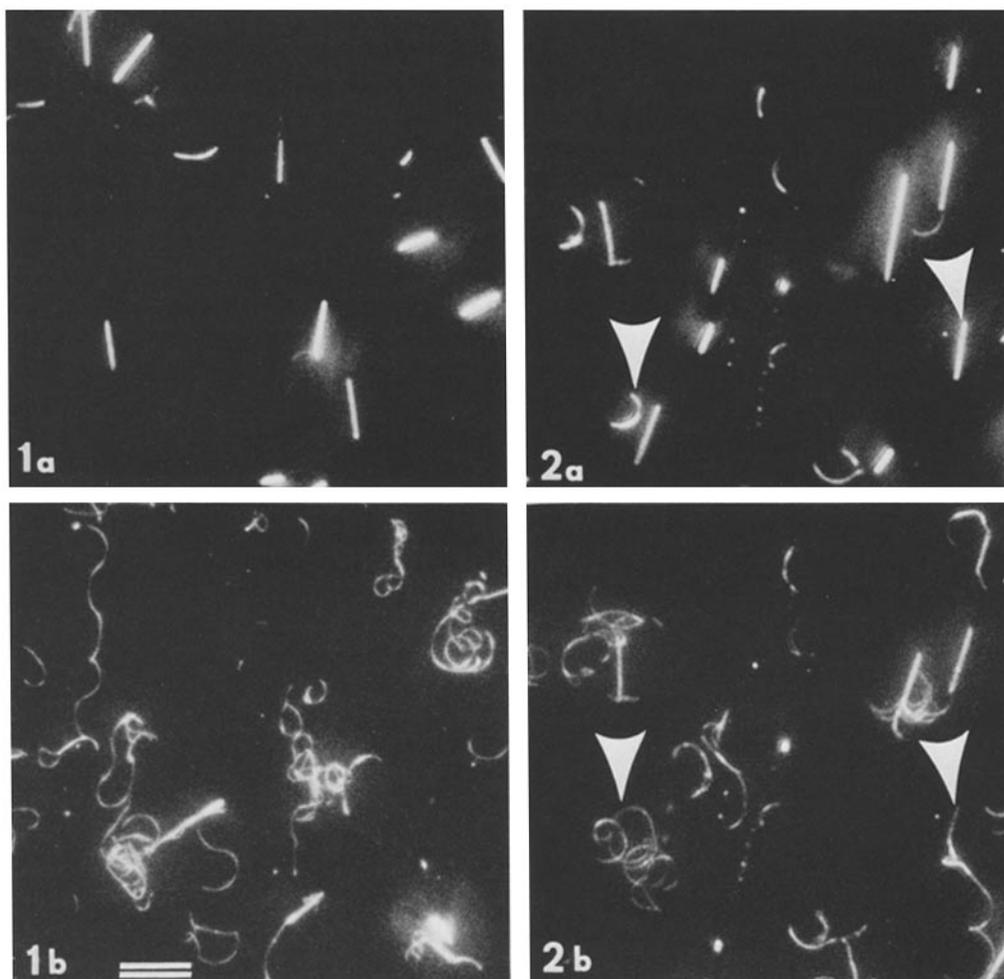
Axonemes digested to	Vanadate concentration	Decrease in turbidity* after ATP addition
%	μM	%
92–93	0	38–40
	25	0–3.8
78–80	0	39–41
	25	25–30

Axonemes were digested to various degrees of the initial turbidity described in the left column. ATP-induced changes in turbidity were measured in the presence or absence of 25 μM vanadate. Decrease in turbidity, the right column, was calculated by subtracting the final turbidity, after ATP addition, from the turbidity of the sample just before ATP addition, dividing by the initial turbidity and multiplying times 100.

* Conditions: 2.5 mM MgSO₄, 0.25 mM ATP, pH 7.8.

Tris-Mg solution containing vanadate, the change in turbidity induced by subsequent addition of ATP depended greatly on the degree of digestion. The presence of 25 μM vanadate almost completely inhibited the ATP-induced decrease in turbidity of ⁹³axonemes (Table I), whereas with ⁸⁰axonemes the ATP-induced decrease in turbidity was nearly as great as in the absence of vanadate. In other experiments with different concentrations of vanadate, we found that as little as 4 μM vanadate almost completely inhibited the ATP-induced decrease in turbidity of ⁹³axonemes. For ⁸⁰axonemes, there was a roughly linear increase in inhibition of ATP-induced turbidity drop for increasing vanadate concentrations: an ATP-induced decrease of ~30% in 25 μM vanadate was reduced to 15–25% in 200 μM vanadate. For vanadate concentrations of 200 μM–1 mM, the ATP-induced drop in turbidity was ~15%. (Optical density measurements at vanadate concentrations >200 μM were performed at a wavelength of 550 nm to avoid interference from the absorbance of a vanadate-DTT complex at 350 nm.)

The magnitude of the ATP-induced decrease in turbidity of ⁸⁰axonemes in the presence of 25 μM vanadate was notably sensitive to the degree of mixing and agitation in the cuvette. When the ATP was added to the cuvette and gently mixed by pasteur pipette, the decrease in turbidity was ~20%. Further gentle agitation of the suspension by drawing in and out of a pasteur pipette caused a further decrease of up to 10% in turbidity, for a total decrease of ~30% (Table I). Similar results were obtained when mixing was carried out by



FIGURES 1 and 2 Dark-field light micrographs of trypsin-treated axonemes before (Figs. 1 *a* and 2 *a*) and after (Figs. 1 *b* and 2 *b*) addition of 0.2 mM ATP. Axonemes were photographed while attached to the coverglass. Bar, 20 μ M. \times 470.

FIGURE 1 Axonemes digested to 92% of the initial turbidity.

FIGURE 2 Axonemes digested to 80% of the initial turbidity. Arrows depict explicit examples of axonemes from the same field before (2 *a*) and after (2 *b*) ATP-induced sliding disruption.

inversion of the cuvette. This sensitivity of the turbidity to mild agitation was observed only with axonemes digested to <90% of the initial turbidity and suspended in Tris-Mg solution to which first vanadate and then ATP were added; identical agitation applied to 80 axonemes in the presence of ATP alone or of vanadate alone, or applied to 93 axonemes in the presence or absence of ATP and/or vanadate, had no significant effect on the turbidity. (Interpretation of the turbidity decrease must be made with caution since total turbidity decrease is the sum of that due to ATP-induced

structural disruption of the axoneme and that due to ATP-induced solubilization of axonemal proteins [9].)

When 93 axonemes were mixed with 10 μ M vanadate and observed in a trough slide by light microscopy, no visible disruption of the axonemal structure occurred upon addition of ATP; this is in agreement with the fact that little change in turbidity occurs under these conditions (Table I). Similar light microscope observations of 80 axonemes in the presence of vanadate revealed that ATP caused the axonemes to disrupt by dou-

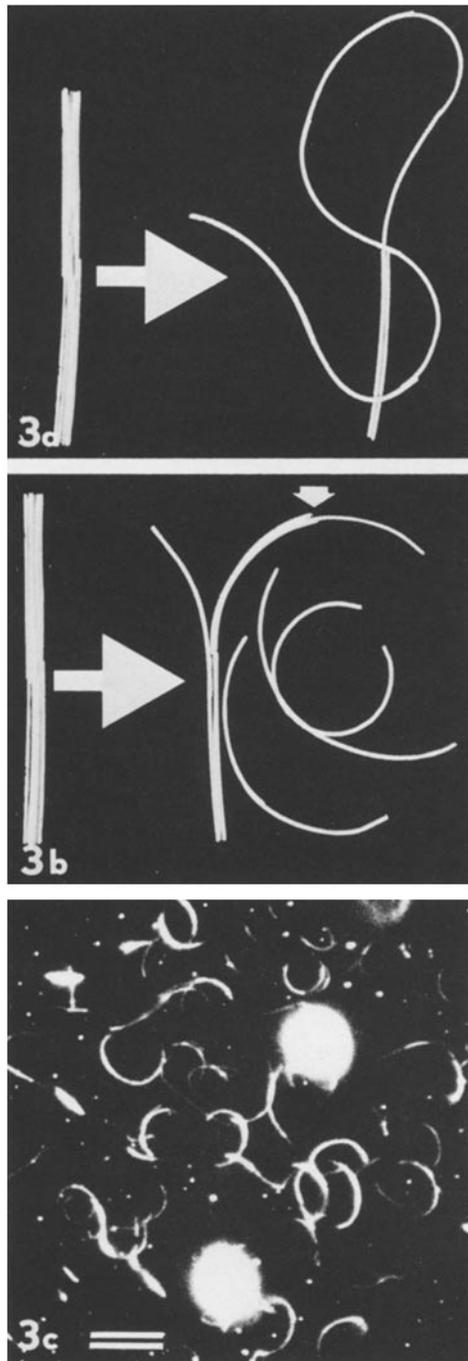


FIGURE 3 (a) Model of ATP-induced sliding disruption of ^{80}S axonemes suspended in buffer. (b) Model of ATP-induced peeling disruption of a vanadate-treated ^{80}S axoneme suspended in buffer. (c) Examples of the C-shaped structures, presumably single or small groups of doublet microtubules, resulting from ATP-induced disruption of a suspension of vanadate-treated ^{80}S axonemes.

blets peeling away from the axonemal backbone, either singly or in small groups, and then often floating off as curved thin filaments. The same preparation of ^{80}S axonemes tested in the absence of vanadate disintegrated by sliding upon addition of ATP as described above (Fig. 2b). Examples of axonemes which had disintegrated by sliding were generally easy to photograph because the same ATP-induced sliding occurred with axonemes attached to the coverglass as with the axonemes freely suspended in the medium (Figs. 1 and 2 were taken of attached axonemes). However, the mechanical constraint of being attached to the coverglass prevented the ATP-induced disruption of ^{80}S axonemes in the presence of vanadate. For this reason, observations of the effects of ATP on ^{80}S axonemes were necessarily made on axonemes suspended in the medium. Photography of such freely suspended axonemes was unsatisfactory because of their Brownian movement and drifting and because of the limited depth of focus. Our observations must, therefore, be presented and described with the help of models.

Fig. 3a shows a model of the typical events observed when freely suspended ^{80}S axonemes were treated with ATP in the absence of vanadate. Disruption appears to occur by a sliding in which doublet microtubules smoothly extend along one another, generally coiling as extension proceeds, and eventually form a tangle in which most of the tubules remain connected at their ends (24). When the same preparation of ^{80}S axonemes is suspended in a medium containing $25\ \mu\text{M}$ vanadate, addition of ATP causes the axoneme to curl into a circular arc while groups of doublet microtubules peel away from each other to form C-shaped structures (Fig. 3b). It is likely that the C-shaped structures are the side-on view of curved doublet microtubules or fractional parts of helically coiled doublet microtubules. (See references 17 and 31 for dark-field micrographs of extensively coiled doublet microtubules.) Only occasional structures were observed that may have resulted from sliding, and these were less than two times the length of the original axonemal fragment (small arrow, Fig. 3b). It is unlikely that small amounts of sliding could be directly observed by dark-field light microscopy. To obtain an objective assessment of our

The structures were allowed to settle before photography, and attachment to the surface has caused some distortion of the uniform conformation and diameter of the C-shaped structures. Under the same conditions of added vanadate and ATP, ^{89}S axonemes maintain their integrity. Bar, $10\ \mu\text{M}$. $\times 940$.

ability to distinguish the mode of ATP-induced disruption of suspended axonemes in the presence or absence of vanadate, we performed a blind test with eight samples that contained either no vanadate or 25 μM vanadate. The test resulted in 100% correct identification by dark-field observations. The final result of ATP-induced disruption of ^{80}S axonemes in the presence of vanadate, after the fragments have settled, is shown in Fig. 3c. Because of the distortion that tends to occur upon attachment, this image is not perfectly representative of the suspended C-shaped structures which are usually more uniform in diameter. The inhibition of ATP-induced sliding by 5–100 μM vanadate, like the inhibition of motility of undigested sperm flagella (11), could be reversed by addition of 1 mM catechol.

Rigor Sperm

Sperm with their flagellar axonemes bent into stationary waves (“rigor waves”) were produced by abruptly depleting reactivated sperm of ATP (6). Such flagellar rigor waves can be straightened by addition of 1–5 μM ATP which is too low to support normal oscillatory bending; in 1 μM ATP, this straightening takes ~ 10 min for completion. Rigor wave sperm regain normal motility upon addition of 10 μM or more ATP.

The presence of vanadate did not inhibit ATP-induced straightening of the rigor waves. With 40 μM vanadate added to rigor wave sperm, 2 μM ATP caused straightening in ~ 10 min, and 10 μM or 1 mM ATP caused straightening in less than the time required for mixing and resumption of observation, but did not cause resumption of flagellar beating. In the presence of 1 mM vanadate, straightening of the rigor waves by 10 μM or 1 mM ATP was usually incomplete with the bend angles decreasing only to about half of their initial values. We conclude from these observations that a concentration of vanadate as high as 40 μM , which is ten times that necessary to inhibit flagellar beating, under the same conditions of salt and pH, has little effect on the ATP-induced straightening of rigor waves; the partial inhibition of straightening by 1 mM vanadate may be due to secondary effects at this very high concentration.

DISCUSSION

The results described in this paper indicate that the ATP-induced sliding of trypsin-treated axonemes is inhibited by about the same concentration of vanadate as that required to inhibit the

motility of demembrated sperm under the same conditions. In the case of the more highly digested axonemes, exposure to ATP in the presence of vanadate still causes the axonemes to disintegrate but they appear to do so by a peeling apart of the doublet microtubules rather than by sliding. In addition, vanadate does not inhibit ATP-induced straightening of rigor wave axonemes. These data taken together suggest that vanadate does not block ATP-induced detachment of an arm from the adjacent B-subfiber. To provide a framework for discussion of the mechanism of vanadate inhibition, it is convenient to postulate a model of dynein cross-bridge activity (Fig. 4).

Since there is relatively little detailed evidence available regarding the kinetics and structural changes of the dynein cross-bridge cycle, we have based our model in large part upon analogy to that proposed for myosin cross-bridge activity in

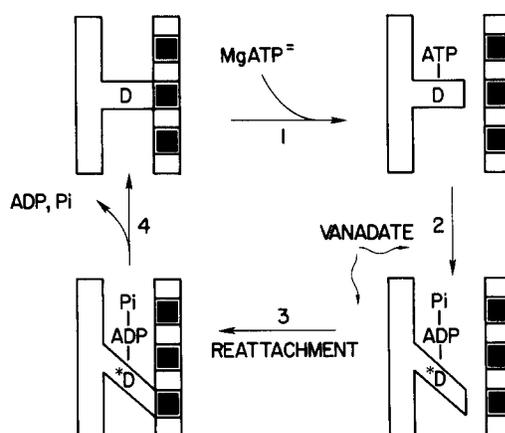


FIGURE 4 Postulated dynein cross-bridge cycle which is based on the model proposed for actomyosin by Lynn and Taylor (16). In the model, dynein ATPase activity kinetics are combined with possible structural changes. In step 1 of the cycle, binding of MgATP^{2-} to the dynein (D) initiates detachment. Step 2 is a conformational change in the dynein (*D) arm that is associated with hydrolysis of ATP. Step 3 is the reattachment of the dynein arm to the next site along the adjacent B-subfiber, and step 4 is the release of the products, ADP and inorganic phosphate, linked with the power stroke of the dynein arm. Our data indicate that vanadate inhibits at either step 2 or 3 in this model. The tilted arm, *D, of Fig. 4 is the usual edge-on image of dynein arms viewed by negative contrast electron microscopy (19, 22, 26, 29). Other conformations have been identified (21), but the relationship of structural changes, observed by electron microscopy, to the formation and straightening of rigor waves is not clear at this time. See Satir (21) for further discussion.

contracting striated muscle by Tonomura et al. (27) and by Lynn and Taylor (16). The general features of this model for myosin are supported by a variety of enzyme kinetic, x-ray diffraction, and electron microscope data (cf. reference 14). As will be discussed below, we believe that our model for the dynein cross-bridge cycle is consistent with the evidence currently available regarding the properties and function of the dynein arms in producing shear between adjacent doublet tubules, and as such it provides a suitable basis for discussion. However, it must be emphasized that the information available about the subunit structure and kinetic properties of the dynein arms is far from complete, and alternative models of their activity are also possible.

The steps in our model are described in the legend of Fig. 4. The stable bridge conformation of the dynein arms (upper left of Fig. 4) presumably represents the position of the arms in the rigor state (i.e., in the absence of $MgATP^{2-}$). The presence of fixed arm cross-bridges in rigor is supported by studies on the physical properties of rigor wave forms and supported by the finding that rigor wave forms are maintained after digestion with trypsin (6). Ultrastructural studies of axonemes that have been fixed for electron microscopy in the absence of ATP show that the arms form bridges between the adjacent microtubules (10, 21, 28, 30). However, these ultrastructural studies must be interpreted cautiously since Warner and his colleagues have discovered that the presence of dynein cross-bridges visible by electron microscopy is also dependent on the divalent cation concentration and buffer composition during fixation (28, 30). It is not clear how closely the Mg^{2+} -dependent bridged arms observed by electron microscopy are related to the cross bridges that are responsible for maintaining wave forms in the absence of ATP, since the stationary rigor wave forms can be preserved by abruptly lowering the concentration of Mg^{2+} in the reactivating medium (7) just as well as by lowering the concentration of ATP (6, 18). Be this as it may, the observations of Warner are generally compatible with the model proposed in Fig. 4 in that the percentage of bridged dynein arms decreases upon addition of ATP (28).

Step 1 of the cycle, arm detachment, occurs upon the binding of nucleotide to the dynein and, as drawn, implies that ATP-dependent detachment does not require hydrolysis of the nucleotide. This step is supported by the observations of Pen-

ningroth and Witman (18) that the nonhydrolyzable ATP-analogue adenylyl imidodiphosphate (AMP-PNP), which is a competitive inhibitor of ATP hydrolysis, causes straightening of rigor wave sperm. Step 1 is also supported by the observation that ATP induces dissociation of 30S dynein from the B-tubules of doublet microtubules of *Tetrahymena* (26). Further, our results indicate that vanadate does not inhibit ATP-induced detachment of bridged arms. Therefore, we suggest that vanadate inhibits the cycle either at step 2 by binding to the dynein-ATP complex and preventing hydrolysis, or at step 3 by binding to the dynein-ADP-inorganic phosphate(Pi) complex and preventing reattachment to the B-subfiber. This mechanism of vanadate inhibition is consistent with the reports that vanadate is an uncompetitive inhibitor of dynein ATPase activity (11, 15), for binding of an inhibitor to the enzyme-substrate complex represents a basis for uncompetitive kinetics (4). It seems unlikely that vanadate blocks the dissociation of products at step 4, since the rebinding of the arm to the B-subfiber at step 3 would presumably prevent relaxation of rigor waves.

It has been shown that the integrity of axonemes digested to below 90% of the initial turbidity is maintained primarily by dynein arms, whereas the integrity of the ⁹³axonemes is maintained, in addition to dynein arms, by residual undisrupted interdoublet links (nexin) and spoke connections (see Fig. 8 in reference 25). Presumably, the remaining interdoublet links and spoke connections of the ⁹³axonemes are sufficient to inhibit ATP-induced splitting disruption of the axoneme but not sufficient to resist active sliding disruption. The ⁸⁰axonemes have essentially no remaining interdoublet links or spokes and are, therefore, subject to splitting disruption upon dynein arm detachment. It is reasonable that the ATP-induced splitting or peeling disruption of the ⁸⁰axonemes is a relatively passive process compared to active sliding disruption, and, therefore, requires outside forces, such as the coiling tendency of the doublet microtubules and the agitation of mixing, for the tubules to separate from one another.

An efficient cyclic transduction of the chemical energy of ATP hydrolysis into the mechanical energy of sliding requires that the rate of at least one step of the hydrolysis pathway be slow except when an ATPase molecule (dynein) on one component of the sliding system is correctly located to interact with its effector on the second sliding

component (the B-subfiber). In the case of myosin ATPase, this rate-limiting step is thought to be the dissociation of ADP and Pi from the enzyme, which is slow in myosin alone, and is greatly accelerated when the myosin can interact with actin (cf. reference 16). A similar coupling of ATPase activity appears to occur in flagella, where ~70–80% of the total ATPase activity of dynein is dependent upon motility (1, 2, 5). When dynein 1 is extracted under appropriate conditions, it has a low latent ATPase activity that can be increased about sixfold by rebinding to dynein-depleted axonemes. The latent ATPase activity of soluble dynein 1 can be increased up to about tenfold by exposing the enzyme to Triton X-100, to mild heat, or to SH-binding reagents (13). The latent form of dynein 1 ATPase appears to have about a fivefold greater affinity for vanadate than the activated form (11) of dynein 1, which could result from a greater concentration of dynein-ATP or dynein-ADP-Pi complexes during steady-state hydrolysis by latent dynein 1 as compared to that present during hydrolysis by activated dynein 1. However, it is also possible that the decrease in vanadate affinity after activation results directly from an altered conformation of the dynein molecule.

In summary, vanadate apparently does not inhibit ATP-induced detachment of dynein arms but rather binds dynein at a succeeding step to inhibit completion of the cross-bridge cycle.

We are indebted to Dr. Barbara Gibbons for extensive assistance throughout, and we thank Frances Okimoto for her patient help in preparing the manuscript. We also thank Dr. P. Satir and Dr. F. Warner for sending us copies of their manuscripts before publication.

This work was supported by grant HD 06565 from the National Institute of Child Health and Human Development and by a Helen Hay Whitney Foundation Fellowship to W. S. Sale.

Received for publication 14 February 1979, and in revised form 2 April 1979

REFERENCES

- BROKAW, C. J., and T. F. SIMONICK. 1976. CO₂ regulation of the amplitude of flagellar bending. In *Cell Motility*. R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y. 933–940.
- BROKAW, C. J., and T. F. SIMONICK. 1977. Mechanochemical coupling in flagella. V. Effects of viscosity on movement and ATP-dephosphorylation on triton-demembrated sea urchin spermatozoa. *J. Cell Sci.* **23**:227–241.
- CANDE, W., and S. M. WOLNIAK. 1978. Chromosome movement in lysed mitotic cells is inhibited by vanadate. *J. Cell Biol.* **79**:573–580.
- DIXON, M., and E. C. WEBB. 1964. *Enzymes*, 2nd edition. Academic Press, Inc., N. Y. 324.
- GIBBONS, B. H., and I. R. GIBBONS. 1972. Flagellar movement and adenosine triphosphatase activity in sea urchin sperm extracted with Triton X-100. *J. Cell Biol.* **54**:75–97.
- GIBBONS, B. H., and I. R. GIBBONS. 1974. Properties of flagellar "rigor waves" formed by abrupt removal of adenosine triphosphate from actively swimming sea urchin sperm. *J. Cell Biol.* **63**:970–985.
- GIBBONS, B. H., and I. R. GIBBONS. 1978. Formation of flagellar rigor waves by abrupt removal of Mg²⁺ from actively swimming sea urchin sperm, and the lack of inhibition by vanadate of the relaxation of rigor waves by MgATP. *J. Cell Biol.* **79**(2, Pt. 2):285 a. (Abstr.)
- GIBBONS, B. H., and I. R. GIBBONS. 1979. Relationship between the latent adenosine triphosphatase state of dynein 1 and its ability to recombine functionally with KCl-extracted sea urchin sperm flagella. *J. Biol. Chem.* **254**:197–201.
- GIBBONS, I. R. 1965. An effect of adenosine triphosphate on the light scattered by suspension of cilia. *J. Cell Biol.* **26**:707–712.
- GIBBONS, I. R. 1975. The molecular basis of flagellar motility in sea urchin spermatozoa. In *Molecules and Cell Movement*. S. Inoué and R. E. Stephens, editors. Raven Press, N. Y. 207–232.
- GIBBONS, I. R., M. P. COSSON, J. A. EVANS, B. H. GIBBONS, B. HOUCK, K. H. MARTINSON, W. S. SALE, and W.-J. Y. TANG. 1978. Potent inhibition of dynein adenosine triphosphatase and of the motility of cilia and sperm flagella by vanadate. *Proc. Natl. Acad. Sci. U. S. A.* **75**:2220–2224.
- GIBBONS, I. R., and E. FRONK. 1972. Some properties of bound and soluble dynein from sea urchin sperm flagella. *J. Cell Biol.* **54**:365–381.
- GIBBONS, I. R., and E. FRONK. 1979. A latent adenosine triphosphatase form of dynein 1 from sea urchin sperm flagella. *J. Biol. Chem.* **254**:187–196.
- HUXLEY, H. E. 1976. Introductory remarks: the relevance of studies on muscle to problems of cell motility. In *Cell Motility*. R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y. 115–125.
- KOBAYASHI, T., T. MARTENSEN, J. NATH, and M. FLAVIN. 1978. Inhibition of dynein ATPase by vanadate, and its possible use as a probe for the role of dynein in cytoplasmic motility. *Biochem. Biophys. Res. Commun.* **81**:1313–1318.
- LYMN, R. W., and E. W. TAYLOR. 1971. Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochemistry*. **10**:4617–4624.
- MIKI-NOUMURA, T., and R. KAMIYA. 1976. Shape of microtubules in solutions. *Exp. Cell Res.* **97**:451–453.
- PENNINGROTH, S. M., and G. B. WITMAN. 1978. Effects of adenyllyl imidodiphosphate, a nonhydrolyzable adenosine triphosphate analog, on reactivated and rigor wave sea urchin sperm. *J. Cell Biol.* **79**:827–832.
- SALE, W. S., and P. SATIR. 1977. The direction of active sliding of microtubules in *Tetrahymena* cilia. *Proc. Natl. Acad. Sci. U. S. A.* **74**:2045–2049.
- SATIR, P. 1968. Studies on cilia. III. Further studies on the cilium tip and a "sliding filament" model of ciliary motility. *J. Cell Biol.* **39**:77–94.
- SATIR, P. 1979. Basis of flagellar motility in spermatozoa: current status. In *The Spermatozoon: Maturation, Motility, and Surface Properties*. D. W. Fawcett and J. M. Bedford, editors. Urban and Schwarzenberg, Baltimore.
- SATIR, P., and W. S. SALE. 1977. Tails of *Tetrahymena*. *J. Protozool.* **24**:498–501.
- SHINGYOJI, C., A. MURAKAMI, and K. TAKAHASHI. 1978. Local reactivation of Triton-extracted flagella by iontophoretic application of ATP. *Nature (Lond.)*. **265**:269–270.
- SUMMERS, K. E., and I. R. GIBBONS. 1971. Adenosine triphosphate-induced sliding of tubules in trypsin-treated flagella of sea urchin sperm. *Proc. Natl. Acad. Sci. U. S. A.* **68**:3092–3096.
- SUMMERS, K. E., and I. R. GIBBONS. 1973. Effects of trypsin digestion on flagellar structures and their relationship to motility. *J. Cell Biol.* **58**:618–629.
- TAKAHASHI, M., and Y. TONOMURA. 1978. Binding of 30S dynein with the B-tubule of the outer doublet of axonemes from *Tetrahymena pyriformis* and adenosine triphosphate-induced dissociation of the complex. *J. Biochem.* **84**:1339–1355.
- TONOMURA, Y., H. NAKAMURA, N. KINOSHITA, H. ONISHI, and M. SHIGEKAWA. 1969. The pre-steady state of the myosin-adenosine triphosphate system. X. The reaction mechanism of the myosin-ATP system and a molecular mechanism of muscle contraction. *J. Biochem. (Tokyo)* **66**:599–618.
- WARNER, F. D. 1978. Cation-induced attachment of ciliary dynein cross-bridges. *J. Cell Biol.* **77**:R19–R26.
- WARNER, F. D., and D. R. MITCHELL. 1978. Structural conformation of ciliary dynein arms and the generation of sliding forces in *Tetrahymena* cilia. *J. Cell Biol.* **76**:261–277.
- ZANETTI, N. C., D. R. MITCHELL, and F. D. WARNER. 1979. Effects of divalent cations on dynein cross bridging and ciliary microtubule sliding. *J. Cell Biol.* **80**:573–588.
- ZOBEL, C. R. 1973. Effect of solution composition and proteolysis on the conformation of axonemal components. *J. Cell Biol.* **59**:573–594.