# Binding Manner of Actin to the Lysine-Rich Sequence of Myosin Subfragment 1 in the Presence and Absence of ATP†

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*Received December 16, 1988; Revised Manuscript Received March 20, 1989*

ABSTRACT: Actin was cross-linked to myosin subfragment 1 with a water-soluble carbodiimide both in the presence and in the absence of ATP, and the cross-linking of the N-terminal acidic sequence of actin to the lysine-rich sequence (--KKGGKKK--) at the junction between the 50K and the 20K fragments of subfragment 1 was studied. The cross-linked products were cleaved with elastase, and the yields of PTH-lysines in the lysine-rich sequence were compared between the resulting acto–22K fragment and the un-cross-linked 22K fragment by using a protein sequencer. It was found that, in the presence of ATP, a very small amount of cross-linked product was produced and, in the product, only one lysine residue which lies closest to the 50K fragment mainly decreased in its amount as compared to the corresponding lysine residue in un-cross-linked 22K. In the absence of ATP, on the other hand, the amounts of all five lysine residues in acto–22K were about 60% those of the corresponding residues in 22K. The results suggest that, in the so-called weakly binding state, the N-terminal acidic sequence of actin interacts infrequently and only at restricted sites of the lysine-rich sequence but it interacts fully over the whole length in the rigor state.

The subfragment 1 (S-1)[1] portion of myosin hydrolyzes ATP and interacts with F-actin to generate the force of muscle contraction. However, the mechanism by which S-1 converts the chemical energy of ATP into the sliding motion of F-actin is not known. To elucidate the mechanism, it is important to understand the nature of their interaction at the amino acid level. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC), the so-called zero-length cross-linker, cross-links actin to several sites on S-1 (Mornet et al., 1981; Sutoh, 1983; Yamamoto & Sekine, 1983, 1986). EDC cross-links the carboxyl group of one protein to the amino group of the other only when they are very close. Therefore, the cross-linked residues can be considered as ones participating in the electrostatic interaction of the two proteins. There are at least two sites on the S-1 heavy chain which are cross-linked to actin by EDC. Those sites are located in the regions which span 61K-69K and 76K-78K from the N-terminus (Sutoh, 1983). The latter region seems to interact with actin most strongly as judged from the amount of the cross-linked product (Chen et al., 1985).

Trypsin cleaves S-1 heavy chain (96K) at two sites and produces 26K, 50K, and 20K fragments in that order from the N-terminus (Balint et al., 1978; Lu et al., 1978). Binding of actin to S-1 inhibits the tryptic cleavage of the 50K–20K junction (Mornet et al., 1979; Yamamoto & Sekine, 1979a), and the cleavage of the 50K–20K junction lowers the affinity of S-1 for actin (Botts et al., 1982; Yamamoto & Sekine, 1979b). All these facts suggest that the site which interacts with actin most strongly exists at the 50K–20K junction. When the amino acid sequence of chicken skeletal muscle S-1 (Maita et al., 1987) is examined, it is noticed that there is a lysine-rich sequence, --KKGGKKK--, at the corresponding region. Since the cross-linked site on actin is suggested to be the N-terminal region which contains four acidic amino acids in series, Ac*DEDE*TT-- (Sutoh, 1983), the lysine-rich sequence located at the 50K–20K junction is a most probable candidate for the interaction site.

It is known that elastase cleaves S-1 and produces a larger C-terminal fragment (22K; Applegate & Reisler, 1983). This cleavage occurs even when actin is cross-linked to S-1 (Chen et al., 1985). I recently found that the site cleaved by elastase is very close to the lysine-rich sequence, upstream of the first lysine residue by only four residues. To know if the lysine-rich sequence is really cross-linked to actin, the 22K fragment cross-linked to actin was analyzed by a protein sequencer. Since the N-terminus of actin is acetylated, only the sequence of the 22K fragment can be analyzed. The yield of PTH-lysine in the cross-linked product was compared to that of un-cross-linked 22K. The Edman degradation is not blocked by the cross-linking of actin to the side chain of lysine, but, at the cross-linked site, the yield of PTH-lysine should decrease because the released derivative is no longer a PTH-lysine.

I examined two types of cross-linked products which were produced under different conditions: one was produced in the absence of ATP, and the other was produced in the presence of ATP. It was found that the cross-linked amino acid residues of S-1 are different under these conditions.

## Materials and Methods

*Proteins and Reagents.* Myosin was prepared from rabbit skeletal muscle according to Kielley and Bradley (1956). Actin was extracted from the acetone powder of rabbit skeletal muscle and purified according to Spudich and Watt (1971). S-1 was prepared by digesting myosin with chymotrypsin according to Weeds and Pope (1977). Elastase and chymotrypsin were purchased from Sigma Chemical Co. EDC and *N*-[7-(dimethylamino)-4-methyl-3-coumarinyl]maleimide (DACM) were purchased from Dojin Chemical Co. and Wako Pure Chemical Co., respectively.

*Cross-Linking of S-1 and Actin.* S-1 (1.0 mg/mL) and actin (1.8 mg/mL) were cross-linked with 4 mM EDC in 10

† This work was supported by the special project fund of The University of the Air.

[1] Abbreviations: S-1, subfragment 1; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; DACM, *N*-[7-(dimethylamino)-4-methyl-3-coumarinyl]maleimide; MOPS, 3-(*N*-morpholino)propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; PVDF, poly(vinylindene difluoride).

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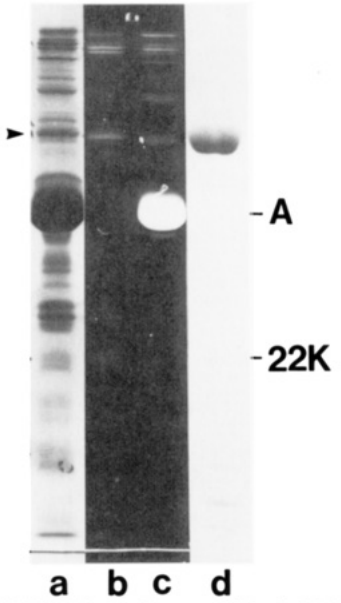


FIGURE 1: SDS–PAGE of acto–S-1 cross-linked with EDC and cleaved with elastase. A 12% acrylamide gel was used. The photograph of the gel was taken after staining with Coomassie brilliant blue (a) or it was taken under UV illumination before staining (b and c). In (b), the 22K fragment was selectively labeled with a fluorescent dye, DACM. In (c), actin was selectively labeled with the same dye. The position of 22K–actin is indicated by the arrowhead. The 22K–actin extracted from the band in the gel is shown in (d). The positions of actin and 22K are also indicated by "A" and "22K", respectively.

mM NaCl, 2 mM $MgCl_2$, and 20 mM 3-(*N*-morpholino)-propanesulfonic acid (MOPS), pH 7.0, in the absence of ATP for 30 min at 25 °C. The molar ratio of actin to S-1 was 5. At this molar ratio, the efficiency of cross-linking is maximum (Yamamoto & Sekine, 1986). Cross-linking in the presence of ATP was done according to Arata (1986): S-1 (0.69 mg/mL) and actin (0.13 mg/mL) were cross-linked with 4 mM EDC in 2 mM $MgCl_2$, 2 mM MgATP, and 20 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.0 at 20 °C, for 60 min. During the reaction, 0.02 volume of 0.1 M MgATP was added at an interval of 15 min. The reaction was stopped by 0.1 M 2-mercaptoethanol.

*Elastase Digestion.* EDC-treated acto–S-1 was digested by elastase at the weight ratio of elastase to S-1 of 1:10 and 25 °C for 20–60 min. The digestion was stopped by 2 mM phenylmethanesulfonyl fluoride.

*DACM Labeling.* Actin (6 mg/mL) in 5 mM MOPS, pH 7.0, was treated with 0.1 mM DACM for 15 h at 0 °C. S-1 (1.5 mg/mL) in 10 mM MOPS, pH 7.0, was treated with 0.01 mM DACM for 15 h at 0 °C. Both reactions were stopped by 2 mM 2-mercaptoethanol. The labeled site was not determined, but the 22K fragment of S-1 was selectively labeled by this procedure.

*SDS–Polyacrylamide Gel Electrophoresis and Electroblotting of Protein Bands to Membrane.* SDS–polyacrylamide gel electrophoresis (PAGE) was done according to Laemmli and Favre (1973). Protein bands in gel were electroblotted to a poly(vinylidene difluoride) (PVDF) membrane (Immobilon, Millipore) according to Matsudaira (1987) using a semidry blotting apparatus (AE-6670P, Atto) at 4 mA/$cm^2$ for 90 min.

*Peptide Sequencing.* Proteins on PVDF membrane were stained with Coomassie Brilliant Blue, and the protein bands were cut out with clean scissors. Two to three of these bands were excised and placed on a polybrene-conditioned glass fiber filter in a cartridge block of a pulsed liquid-phase protein sequencer (Model 477A, Applied Biosystems) with an on-line PTH analyzer (Model 120A).

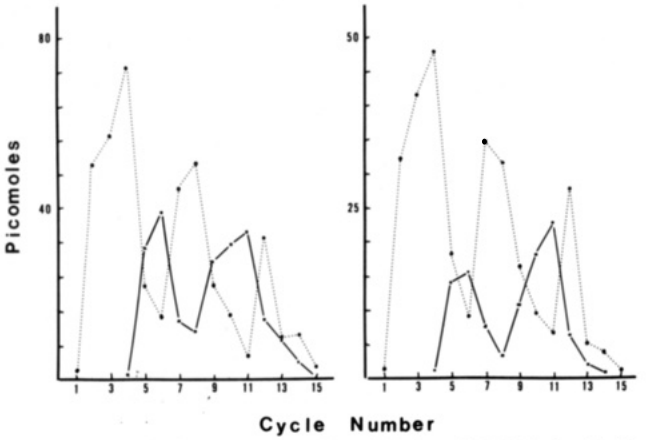


FIGURE 2: Yield of PTH-glycine (dotted line) and PTH-lysine (solid line) in each Edman degradation cycle of 22K (left) and 22K–actin (right). Since the efficiency of electroblotting and the initial coupling yield differ from sample to sample, it is difficult to start from the same amount. Therefore, the oridinates of these graphs are arranged to make comparison easy.

## RESULTS

*Isolation and Sequencing of 22K and 22K–Actin.* Figure 1 shows SDS–PAGE of the EDC-treated acto–S-1 cleaved with elastase. By comparing the fluorescence band patterns of digests in which 22K (Figure 1b) or actin (Figure 1c) was labeled with DACM specifically, it was deduced that a band with an apparent molecular weight of 67K is the cross-linked product of 22K and actin. For the sequencing, the bands of 22K and 22K–actin were cut out from the gel under UV illumination, and they were extracted separately from the gel by an apparatus which utilizes a glass filter modified specifically by the author (Yamamoto, 1987). Figure 1d shows the SDS–PAGE pattern of 22K–actin extracted in this way. Extracted 22K and 22K–actin were applied once again to SDS–PAGE on 14% and 9% gels, respectively, to separate them from other proteins having similar molecular weights. 22K and 22K–actin in the gel were electroblotted onto a PVDF membrane, and their bands were cut out and placed in a protein sequencer. It was found that 22K has the sequence EGGG*KKGGKKK*G--.

*Comparison of the Yield of PTH-Lysine in 22K–Actin with That in 22K.* The yield of PTH-glycine and PTH-lysine in each cycle of Edman degradation of 22K is plotted in Figure 2 (left panel). The same plot for 22K–actin is also shown in the right panel of Figure 2. This 22K–actin was extracted from the same gel plate from which 22K was extracted. It is almost impossible to start the sequencing from the same molar amount of proteins because the efficiency of electroblotting and the initial coupling yield of Edman degradation are very difficult to control. The ordinates of the left and right panels of Figure 2 are, therefore, not the same. However, it can be noticed that all five lysines decreased in their amounts in 22K–actin. To compare the difference between 22K–actin and 22K quantitatively, the data have to be normalized. I calculated the relative yields of PTH-lysines and PTH-glycines to the average yield of the first three PTH-glycines in the sequence, and these values were compared between 22K and 22K–actin (Table I). It is seen that the amounts of all five lysines ($K_1$–$K_5$) decreased in 22K–actin while those of the two glycines ($G_1$ and $G_2$) inserted between $K_2$ and $K_3$ remained almost the same as those in 22K. The amount of the last glycine ($G_3$) in 22K–actin exceeds that in 22K by about 10%. The decrease in $K_3$ is most prominent, and there is a tendency

Table I: Comparison of the Yields of PTH-Lysines and PTH-Glycines Detected in the Sequence of 22K–Actin with Those in Free 22K

| amino acid | rel values[b] to yields of corresponding PTH-amino acids in free 22K in same digest for sample | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | average |
| $K_1$[a] | 0.819 | 0.495 | 0.446 | 0.710 | 0.618 ± 0.153 |
| $K_2$ | 0.543 | 0.508 | 0.656 | 0.565 | 0.568 ± 0.055 |
| $G_1$ | 1.08 | 1.11 | 0.824 | 1.12 | 1.03 ± 0.122 |
| $G_2$ | 1.07 | 1.07 | 0.846 | 0.866 | 0.963 ± 0.107 |
| $K_3$ | 0.576 | 0.558 | 0.354 | 0.554 | 0.511 ± 0.091 |
| $K_4$ | 0.631 | 0.421 | 0.651 | 0.813 | 0.629 ± 0.139 |
| $K_5$ | 0.852 | 0.662 | 0.654 | 0.954 | 0.781 ± 0.128 |
| $G_3$ | 1.35 | 1.14 | 0.725 | 1.22 | 1.11 ± 0.234 |

[a] $K_1$, $K_2$, ..., $G_3$ represent the 5th lysine, 6th lysine, ..., 12th glycine in the sequence of EGGGKKGGKKKG, respectively. [b] The yields of $K_1$–$G_3$ were expressed at first as relative values to the averaged value of the first three glycines in the sequence, and then these values were compared between 22K–actin and 22K.

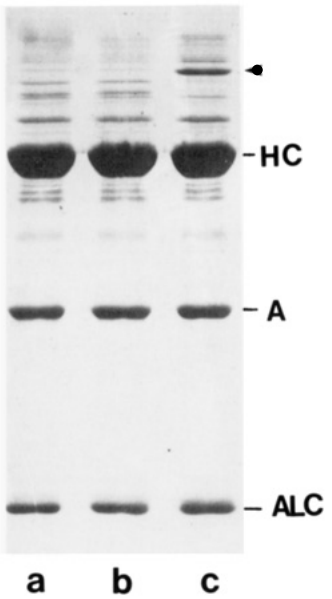


FIGURE 3: SDS–PAGE of EDC-treated S-1–actin in the presence of ATP. S-1 was mixed with actin which was treated with EDC for 30 min in the presence (a) or in the absence (b) of ATP. If the actin solution did not contain ATP, the S-1 solution contained ATP to make the final ATP concentration (5 mM) the same. The cross-linking reaction was allowed to proceed for 20 min. S-1–actin treated in the same way in the total absence of ATP is shown (c) for comparison. HC, S-1 heavy chain; A, actin; ALC, alkali light chain 1. The position of the cross-linked product is indicated by the arrowhead.

that the decrease at the both ends ($K_1$ and $K_5$) is less than that at the center. The total amounts of lysines decreased were about two out of five on average.

*22K–Actin Produced in the Presence of ATP.* The result mentioned above was obtained on 22K–actin which was produced when S-1 and actin were mixed at a molar ratio of 1 to 5 in the absence of ATP. It is known that, under certain conditions, S-1 does not dissociate from actin even when it binds ATP, thus forming a ternary complex, actin–S-1–ATP. I tried to cross-link such S-1–ATP to actin with EDC. S-1 was mixed with actin in the presence of ATP under the conditions described under Materials and Methods and centrifuged for 15 min at 240000*g* at 20 °C. The molar ratio of S-1 to actin in the pellet determined by SDS–PAGE and densitometry of the gel was about 0.5, suggesting that about 50% of actin forms the ternary complex. However, only a few percent of actin was cross-linked to S-1 with EDC under these conditions. It is conceivable that ATP reacts with EDC and

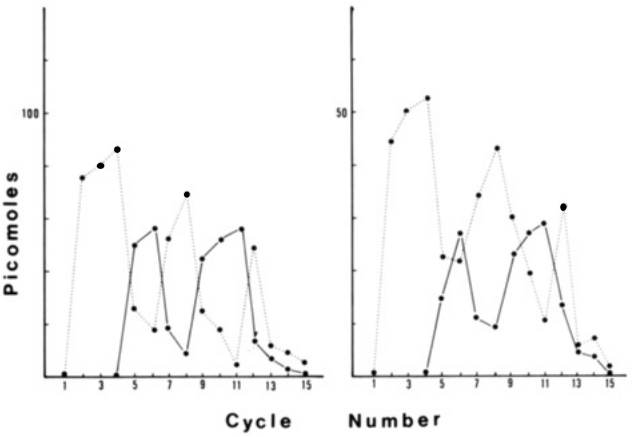


FIGURE 4: Yield of PTH-glycine (dotted line) and PTH-lysine (solid line) in each Edman degradation cycle of 22K (left) and 22K–actin (right).

Table II: Comparison of the Yields of PTH-Lysines and PTH-Glycines in 22K–Actin with Those of Free 22K[a]

| amino acid | rel values[c] to yields of corresponding PTH-amino acids in free 22K in same digest for sample | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | average |
| $K_1$[b] | 0.481 | 0.686 | 0.573 | 0.580 ± 0.084 |
| $K_2$ | 0.766 | 0.800 | 0.871 | 0.812 ± 0.044 |
| $G_1$ | 1.05 | 1.13 | 1.02 | 1.07 ± 0.046 |
| $G_2$ | 1.01 | 1.05 | 1.10 | 1.05 ± 0.037 |
| $K_3$ | 0.834 | 0.901 | 0.885 | 0.873 ± 0.029 |
| $K_4$ | 0.850 | 0.765 | 0.895 | 0.837 ± 0.054 |
| $K_5$ | 0.853 | 0.955 | 0.906 | 0.905 ± 0.042 |
| $G_3$ | 1.01 | 1.15 | 1.08 | 1.08 ± 0.057 |

[a] S-1 and actin were cross-linked with EDC in the presence of ATP. [b] Abbreviations are the same as those used in Table I. [c] The yields of $K_1$–$G_3$ were expressed at first as relative values to the averaged value of the first three glycines in the sequence, and then these values were compared between 22K–actin and 22K.

reduces the amount of EDC available for the carboxylate activation. Therefore, this effect of ATP was estimated by the following experiment. In two test tubes, actin was treated with EDC for 30 min in the presence or in the absence of ATP. To these solutions was added S-1 and allowed to react with actin for 20 min. If the actin–EDC solution did not contain ATP, ATP was added to the S-1 solution to make the final ATP concentration the same. As judged from the amount of cross-linked product seen in Figure 3, ATP does not affect the activation of carboxylate groups of actin by EDC. The amount of cross-linked product was very small even when actin was activated with EDC in the absence of ATP (Figure 3b), and the amount was almost the same as that produced when actin was activated with EDC in the presence of ATP (Figure 3a). S-1–actin treated with EDC in the same way but in the absence of ATP is shown in Figure 3c for comparison. The acto–S-1 cross-linked in the presence of ATP was cleaved with elastase and applied to SDS–PAGE. A small amount of 22K–actin was extracted from the gel and concentrated by the apparatus described previously. The 22K–actin thus obtained was examined by a protein sequencer. Figure 4 shows the yield of PTH-glycine and PTH-lysine in each step of the Edman degradation. As in Figure 2, the graph on the left shows the yield from 22K, and that on the right shows the yield from 22K–actin. Quantitative comparison of the yields of PTH-lysines and PTH-glycines in 22K–actin with those in the un-cross-linked 22K is shown in Table II. It is seen that the amount of the first lysine ($K_1$) decreased most prominently but the other lysines did not decrease so much. The total

amounts of lysines decreased were about one out of five.

## Discussion

This study is the first attempt to determine the S-1–actin interaction site by a protein sequencer. Fortunately, it was found that elastase cleaves a site on the S-1 heavy chain very close to the lysine-rich sequence, and this cleavage occurred even when actin was cross-linked to S-1 (Figure 1). The N-terminal sequence of the 22K fragment of rabbit skeletal S-1 is slightly different from the reported sequence of chicken skeletal S-1. The number of glycine residues preceding the lysine-rich sequence is 4 in chicken (E*GGGG*KKGGKKKG--; Maita et al., 1987) instead of 3 found in this study on rabbit skeletal S-1. I confirm the difference in the number by digesting both chicken and rabbit S-1's with *Staphylococcus aureus* $V_8$ protease (data not shown). $V_8$ protease produced a similar 22K fragment from both S-1's. It was found that the N-terminal sequence of 22K ($V_8$) of chicken S-1 was *GGGG*KKGGKKKG-- while that of the rabbit was *GGG*KKGGKKKG--.

To compare the yields of PTH-amino acids obtained by sequence analysis of 22K–actin and 22K, the value relative to the averaged yield of the first three PTH-glycines was calculated and compared. Since the yields of two PTH-glycines ($G_1$ and $G_2$) which were inserted in the lysine-rich sequence were almost the same in 22K–actin as in 22K, the decrease in the yield of PTH-lysine in 22K–actin is not due to an indirect effect of cross-linking such as the binding of actin near the lysine-rich sequence simply lowers the efficiency of Edman degradation. To eliminate other possible artifacts caused by chemical reaction other than the cross-linking of actin to these lysine residues, the 22K fragment in the same digest was used as a standard in each analysis. Therefore, it can be considered that the decrease in the amounts of lysines in the lysine-rich sequence of 22K–actin is due to the cross-linking of actin to these residues.

In the absence of ATP, about two lysine residues out of five were cross-linked to actin nearly at random (Table I). The random cross-linking at two lysine residues suggests that the N-terminal acidic sequence of actin and the lysine-rich sequence of S-1 interact with each other as clusters of negative and positive charge, respectively, and they move with some freedom even in the rigor complex. It is known that one actin molecule can be cross-linked to only one site of S-1: either a site on the 22K fragment or a site on the 50K fragment (Sutoh, 1983; Chen et al., 1985). If all four acidic residues of actin interact as a unit with the lysine-rich cluster on 22K or a similar cluster on 50K, this mutually exclusive nature of the interaction can be explained. In the presence of ATP, on the other hand, the N-terminal acidic sequence of actin and the lysine-rich sequence seem to interact infrequently only at restricted sites: at a lysine residue which lies at one end of the lysine-rich sequence (closest to the 50K fragment, Table II). These results confirm Arata's suggestion that the cross-linked manner of S-1 to actin in the presence of ATP is different from that in the absence of ATP (Arata, 1986). The amount of cross-linked product was small in the presence of ATP. It was shown in Figure 3 that the effect of ATP is not due to its reaction with EDC, which can reduce the amount of EDC available for the carboxylate groups of actin. It seems, therefore, that there are other interaction sites which cannot be detected by the cross-linking with EDC because a considerable amount of S-1 cosedimented with actin under the conditions used.

If the cross-linked manners in the presence and in the absence of ATP represent so-called weakly and strongly binding states of S-1 and actin, respectively (Eisenberg & Greene, 1980), the results obtained in this study suggest that the interaction between the N-terminal acidic sequence of actin and the lysine-rich sequence of S-1 becomes strong when the binding state changes from weak to strong. Since this change in state is supposed to occur during the power stroke of the myosin head, the change in the binding site might be related to the force generation mechanism. In vitro motility assays strongly suggest that S-1 alone is capable of moving actin (Harada et al., 1987; Toyoshima et al., 1987). Therefore, the internal motions of S-1 are crucial for force generation. The lysine-rich sequence is located just at the junction between the 50K and the 20K domains. This region is considered to be a flexible loop protruded out from the rest because the site is very easily attacked by various proteases (Applegate & Reisler, 1983; Mornet et al., 1984; Yamamoto & Sekine, 1980). The binding of the N-terminal acidic sequence of actin to this flexible junction might be linked to the force generation mechanism by altering the spatial relationship between the 50K and the 20K domains.

## Acknowledgments

I thank Drs. H. Katsuki, H. Noda, and K. Maruyama for their encouragement and discussion.

**Registry No.** ATP, 56-65-5.

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# ADP Release Is Rate Limiting in Steady-State Turnover by the Dynein Adenosinetriphosphatase†

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*Received July 12, 1988; Revised Manuscript Received November 10, 1988*

ABSTRACT: The kinetics of the product release steps in the pathway of ATP hydrolysis by dynein were investigated by examining the rate and partition coefficient of phosphate–water $^{18}O$ exchange under equilibrium and steady-state conditions. Dynein catalyzed both medium and intermediate phosphate–water oxygen exchange with a partition coefficient of 0.30. The dependence of the rate of loss of the fully labeled phosphate species on the concentration of ADP was hyperbolic, with an apparent $K_d$ for the binding of ADP to dynein of 0.085 mM. The apparent second-order rate constant for phosphate binding to the dynein–ADP complex was 8000 $M^{-1}$ $s^{-1}$. The time course of medium phosphate–water oxygen exchange during net ATP hydrolysis was examined in the presence of an ATP regeneration system. The observed rate of loss of $P^{18}O_4$ was comparable to the rate observed at saturating ADP which implies that ADP release is rate limiting for dynein in the steady state. Product inhibition of the dynein ATPase was also examined. ADP inhibited the enzyme competitively with a $K_i$ of 0.4 mM. Phosphate was a linear noncompetitive mixed-type inhibitor with a $K_i$ of 11 mM. These data were fit to a model in which phosphate release is fast and is followed by rate-limiting release of ADP, allowing us to define each rate constant in the pathway. A discrepancy between the total free energy calculated compared to the known free energy of ATP hydrolysis suggests that there is an additional step in the pathway, perhaps involving a change in conformation of the enzyme–ADP state preceding ADP release.

Dynein couples the energy of ATP hydrolysis to drive the sliding of adjacent microtubules in the axoneme. This sliding is coordinated to produce the motive force for the beating of eukaryotic cilia and flagella. In previous work on the pathway for ATP hydrolysis by dynein (Scheme I), the rates of ATP binding, $k_1$, and hydrolysis, $k_2$, were determined by chemical quench flow studies to be $4.7 \times 10^6$ $M^{-1}$ $s^{-1}$ and 55 $s^{-1}$, respectively (Johnson, 1983), and indicated that product release was rate limiting in the reaction pathway. The kinetics of vanadate inhibition of the dynein ATPase suggested that phosphate release preceded ADP release (Shimizu & Johnson, 1983a), and it was implied, without proof, that phosphate release was rate limiting.

Scheme I

$$D + ATP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} D\text{–}ATP \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} D\text{–}ADP\text{–}P_i \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} D\text{–}ADP \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} D + ADP$$

In the microtubule–dynein ATPase pathway, the binding of ATP induces the dissociation of the microtubule–dynein complex. ATP hydrolysis occurs on the free dynein. Microtubule activation of the dynein ATPase was first described by Omoto and Johnson (1986) and shown to be due to the effect of microtubules in increasing the rate of product release. In order to complete our thermodynamic description of the pathway, it is important to determine whether the rate-limiting step during steady-state turnover is phosphate release or ADP release. Moreover, it is likely that the slow step may also be the site of regulation of the ATPase cycle in the axoneme.

We have examined the product release steps of the dynein ATPase, using the techniques of phosphate–water $^{18}O$ exchange. Dynein has been shown to catalyze both medium and intermediate exchange reactions (Barclay & Yount, 1972; Kuleva et al., 1983; Holzbaur & Johnson, 1986). In this report, the rate and extent of medium exchange catalyzed by dynein were examined under both equilibrium and steady-state conditions. The data fit a model in which phosphate release is fast, and ADP release is the slow step. We further tested this hypothesis by examining the exchange reactions under conditions in which the dynein ATPase is both activated and inhibited.

Dynein from *Tetrahymena* cilia has three heads and three ATP binding sites per molecule (Johnson & Wall, 1983; Johnson, 1983; Shimizu & Johnson, 1983b). Because it is possible that there is more than one mechanism for ATP turnover, the process of intermediate exchange was examined in an attempt to distinguish multiple pathways for ATP hydrolysis in solution. The observed isotopic distribution of the product phosphate resulting from the hydrolysis of [$\gamma$-$^{18}O$]ATP was compared to theoretical models in order to evaluate the

† This work was supported by NIH Grant GM26726. K.A.J. was supported by an Established Investigatorship from the American Heart Association with funds contributed in part by the Pennsylvania Affiliate.
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0006-2960/89/0428-5577$01.50/0 