Light chain exchange in 4.7 M NH&l was used to hybridize the essential light chain of cardiac myosin with the heavy chain of fast muscle myosin subfragment 1, S-1. The actin-activated ATPase properties of this hybrid were compared to those of the two fast S-1 isoenzymes, S-1(A1), fast muscle myosin subfragment 1 which contains only the alkali-1 light chain, and S-1(A2), fast muscle myosin subfragment 1 which contains only the alkali-2 light chain. This hybrid S-1 behaved like S-1(A1). At low ionic strength in the presence of actin, this hybrid had a maximal rate of ATP hydrolysis about the same as that of S-1(A1) and about one-half that of S-1(A2), while at higher ionic strengths the actin-activated ATPases of these three S-1 species were very similar.

Light chain exchange in NH&l was also used to hybridize the essential light chains of fast muscle myosin with the heavy chains of cardiac myosin and to hybridize the essential light chains of cardiac myosin with the heavy chains of fast muscle myosin. In 60 and 100 mM KCl, the actin-activated ATPases of these two hybrid myosins were very different from those of the control myosins with the same essential light chains but were very similar to those of the control myosins with the same heavy chains, differing at most by one-third.

Fast twitch muscle myosin contains two pairs of low molecular weight subunits. One pair, molecular weight 19,000, can be partially dissociated from myosin by treatment with 5,5'-dithiobis(2-nitrobenzoic acid) (1). These DTNB light chains are not essential for ATP hydrolysis or actin binding (1, 2); however, they may be involved in calcium-dependent modulations of actin interactions (3, 4). The other pair of light chains are generally thought to be essential as they cannot be removed without loss of ATPase activity (5-7). Fast muscle myosin has two different essential light chains, alkali-1 with a molecular weight of 21,000 and alkali-2 with a molecular weight of 16,500 (8). Both types of alkali light chains are present in a single muscle cell (9) and are often part of the same myosin molecule (10-12).

Myosin subfragment 1, S-1, can be fractionated into two isoenzymes, one containing the alkali-1 light chain, S-1(A1), and the other the alkali-2 light chain, S-1(A2) (2, 13). While the ATPase activities of these two isoenzymes in the absence of actin are indistinguishable, when assayed at low ionic strength their actin-activated ATPase are significantly different (2). S-1(A2) has both a higher maximal rate of ATP hydrolysis, V,,, and a higher K,,, the actin concentration required to achieve one-half of V,,, increases. However, when the KCl concentration is increased from 6 to 26 mM, the V,,, of the actin-activated ATPase of S-1(A1) increases to about that of S-1(A2) (14). The actin-activated ATPase of the heavy meromyosin isoenzyme which contains alkali-1 shows the same salt dependence as S-1(A1) (14, 15).

While it has not been possible to regenerate active myosin by recombining isolated heavy chains with purified light chains, several groups have developed methods for exchanging the essential light chains (15, 16, 17). Exchanges of alkali-1 into S-1(A2) and alkali-2 into S-1(A1) have been done using 4.7 M NH&l as a dissociant (16). These exchanges showed that the differences in the actin-activated ATPases of these isoenzymes are caused by the alkali light chains and not by some undetected differences in the heavy chains.

Myosins isolated from various muscle types have different ATPase activities; however, they all have similar subunit compositions, being composed of two heavy chains and two pairs of light chains (18, 19). These light chains appear to be analogous to the DTNB and alkali light chains of fast muscle myosin (3, 16, 20, 21). Hybrid S-1 molecules which contain heavy chains and essential light chains from different myosins have been made by exchange in NH&l (16). These hybridizations showed that in the absence of actin the ATPase activity is the same whether the essential light chain comes from fast, cardiac, slow, or smooth muscle myosin. However, in the presence of actin, the ATPase activity of the hybrid reflected the source of both the heavy chain and the essential light chain. In 6 mM KCl, the actin-activated ATPase of the hybrid between fast S-1 heavy chain and cardiac essential light chain (cardiac LCl) had a V,,, about one-half that of S-1(A2) and a K,,, close to that of cardiac S-1. At the time that these hybridizations were first done, the effect of increasing ionic strength on the actin-activated ATPase of S-1(A1) was not known and consequently the actin-activated ATPase of this hybrid was not examined at higher ionic strengths. In this paper, the actin-activated ATPase of the S-1 hybrid between fast S-1 heavy chain and cardiac LCl is examined as a function of ionic strength to see if cardiac LCl is simply mimicking the effect of alkali-1.

At a given ionic strength, the K,,, and V,,, of the actin-
activated ATPase of myosin are much lower than those of S-1 or heavy meromyosin. While the precise causes of these differences are not known, they are generally thought to result from the filamentous structure of myosin. The lower $K_{app}$ values for myosin allow its actin-activated ATPase to be examined at ionic strengths where the $V_{max}$ of the actin-activated ATPase of S-1 is prohibitively weak. Light chain exchange has been used previously to enrich fast myosin with either alkali-1 or alkali-2.2 This paper describes the formation of hybrid myosins between fast heavy chains and cardiac essential light chains and between cardiac heavy chains and fast essential light chains. The actin-activated ATPases of these hybrids are examined under a variety of conditions to see if the source of the essential light influences the actomyosin ATPase.

MATERIALS AND METHODS

Proteins—Fast twitch muscle myosin was isolated from rabbit back and white hind leg muscles, cardiac myosin from ox ventricles, and actin from rabbit skeletal muscle (18). Fast muscle S-1 was made by an α-chymotryptic digestion and fractionated by ion exchange chromatography into S-1(A1) and S-1(A2) (2). Cardiac S-1 was also prepared by an α-chymotryptic digestion and purified by ion exchange chromatography (16). This cardiac S-1 contains only the 21,000-dalton essential light chain, cardiac LC1. The DTNB light chain and the analogous cardiac LC2, molecular weight of 18,000 (22), were either lost during the digestions or cleaved to very small peptides.

Light chains were dissociated from fast and cardiac myosins in 5 mM guanidine HCl and fractionated by ion exchange chromatography (1, 23, 24). Prior to fractionation of the fast muscle light chains on DEAE-cellulose, most of the DTNB light chain was removed by ethanol fractionation (25). Purified alkali-1, alkali-2, and cardiac LC1 were freeze dried and stored at −20°C.

The following extinction coefficients, $e_{280}$, and molecular weights were used: actin 11.0 cm$^{-1}$ (26) and 42,000 (27); myosin 5.6 cm$^{-1}$ and 470,000; S-1 7.5 cm$^{-1}$ (16) and 115,000 (2). An $e_{280}$ of 2.2 cm$^{-1}$ was used for alkali-1, alkali-2, and cardiac LC1.

Exchange Reactions—The conditions for light chain exchange with S-1 were 200 μM cardiac LC1, 20 μM S-1(A2), 0.10 mM imidazole (pH 7.0), 2 mM dithiothreitol, 2 mM EDTA, and 4.7 mM NH4Cl at 4°C. After stirring for 30 min, the NH4Cl was removed by dialyzing overnight against 50 mM imidazole and 10−4 M dithiothreitol, pH 7.0 at 4°C. The S-1 hybrid was purified by ion exchange chromatography (16).

The conditions for light chain exchange with myosin were 300 μM light chain, 0.25 M KCl, 0.10 mM imidazole (pH 7.0), 2 mM dithiothreitol, 2 mM EDTA, and 4.7 mM NH4Cl at 4°C. After stirring for 30 to 60 min (see “Results”), the NH4Cl was removed by dialyzing overnight against 0.3 M KCl, 10 mM imidazole, and 10−4 M dithiothreitol, pH 7.0 at 4°C. The samples (2 or 3 ml) were diluted to 35 ml with cold water and after 20 min centrifuged at 35,000 × g for 20 min. The myosin pellets which were less than 1 ml in volume were dissolved in 1 ml of 0.80 M KCl, 10 mM imidazole, and 2 × 10−4 M dithiothreitol, pH 7.0 at 4°C. The myosin was repurified by dialuting with cold water to 35 ml and centrifuging as before. The resulting pellets were dissolved in 1 ml of 0.80 M KCl, 10 mM imidazole, and 2 × 10−4 M dithiothreitol, pH 7.5 at 4°C, diluted to 2 ml with cold water, and centrifuged at 35,000 × g for 30 min. Approximately one-half of the myosin was lost during these precipitations. Two types of control myosin were used, myosin treated with 4.7 mM NH4Cl but with no added light chain and myosin which was not exposed to NH4Cl. Both control myosins went through the same precipitation procedure as the hybridized myosins.

All ATPase assays were performed at pH 8.0 and 25°C using a pH stat to measure the rate of H+ release. All velocities are reported as turnover rates, in terms of moles of ATP hydrolyzed per s per mol of myosin heads.

The conditions for the EDTA ATPase were 2 mM EDTA, 5 mM ATP, and 0.60 mM KCl and for the Ca$^{2+}$ ATPase 10 mM CaCl2, 5 mM ATP, and 0.30 mM KCl. The actin-activated ATPases of S-1 were measured in 3.5 mM MgCl2, 2 mM ATP, and 6 and 26 mM KCl. The actin was varied from 3 to 300 μM. The actin-activated ATPases of myosin were measured in 3.5 mM MgCl2, 2 mM ATP, and 60 and 100 mM KCl. The actin concentration was varied from 6.0 to 45 μM. The myosin concentrations were approximately 0.15 mg/ml for fast myosin and 0.40 mg/ml for cardiac myosin. To obtain reproducible actomyosin ATPases, the following protocol was used. Myosin in 0.4 mM KC1 was diluted to 100 mM KCl. After 1/2 min at 25°C, actin was added and after an additional 1/2 min, the MgATP was added. Frequently the actomyosin ATPase did not become linear until after 1 or 2 min had elapsed. Rates used were those observed between 2 and 8 min after adding the ATP.

The actin-activated ATPase of S-1 has a hyperbolic dependence on actin concentration (28) and can be described by the equation

\[ v = V_{	ext{max}}[	ext{Actin}]/(K_{	ext{app}} + [	ext{Actin}]) \]

where $v$ is the observed rate of ATP hydrolysis by S-1 in the presence of actin less that of S-1 alone, $V_{	ext{max}}$ the extrapolated rate of ATP hydrolysis at infinite actin, and $K_{	ext{app}}$ the concentration of actin required to achieve $1/2 V_{	ext{max}}$ (the apparent dissociation constant of actin from S-1 in the presence of ATP).

The light chain compositions of the various myosin and S-1 hybrids were analyzed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (29). The light chain ratios were determined using a scanning gel densitometer. All of the light chains were assumed to stain equally.

RESULTS

A hybrid S-1 containing fast S-1 heavy chain and cardiac LC1 was made by exchanging the alkali-2 light chain of S-1(A2) with cardiac LC1. S-1(A2) rather than S-1(A1) was used as a starting material as the hybrid elutes from the ion exchange column close to S-1(A1) making its purification very difficult. Since S-1(A1) and S-1(A2) have the same heavy chain content (16), exchange of cardiac LC1 with either iso-enzyme will give the same S-1 hybrid.

As reported previously (16), this purified hybrid had an EDTA ATPase activity equal to that of S-1(A2) and very different from that of cardiac S-1 (Table I). The actin-activated ATPase of this S-1 hybrid was determined in both 6 and 26 mM KC1 and compared to control S-1(A2). As shown in Fig. 1, in 6 mM KC1 the actin-activated ATPase of this hybrid was significantly different from that of S-1(A2), having both a lower $V_{	ext{max}}$ and $K_{	ext{app}}$. In 26 mM KC1, the $V_{	ext{max}}$ of the hybrid increased, approaching that of S-1(A2). The proportional difference in their $K_{	ext{app}}$ values also decreased (Table I). Other preparations of the S-1 hybrid between fast S-1 heavy chain and cardiac LC1 gave similar results and in one instance had a $V_{	ext{max}}$ in 26 mM KC1 greater than that of S-1(A2) (52 and 50 s−1, respectively). As shown in Table I, increasing the ionic strength also increased the $V_{	ext{max}}$ of S-1(A1) to that of S-1(A2). The $V_{	ext{max}}$ of the actin-activated ATPase of cardiac S-1 was much less than that of the hybrid and did not show any increase upon raising the ionic strength. The actin-activated ATPases of S-1(A2) and the S-1 hybrid between fast S-1 heavy chain and cardiac LC1 were also compared in 46 mM

<table>
<thead>
<tr>
<th>Table I</th>
<th>S-1 ATPase activities</th>
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</tr>
<tr>
<td>EDTA 6 mM KC1</td>
<td>26 mM KC1</td>
</tr>
<tr>
<td>S-1(A2)</td>
<td>9.4</td>
</tr>
<tr>
<td>S-1(A1)</td>
<td>29 (1)</td>
</tr>
<tr>
<td>Hybrid S-1</td>
<td>9.4</td>
</tr>
<tr>
<td>Cardiac S-1</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Myosin Hybrids

FIG. 1. Eadie-Hofstee plots of the actin-activated ATPase of S-1(A2) (A) and hybrid S-1 containing cardiac LC1 and fast S1 heavy chain (B). Assays were performed at pH 8.0 and 25°C in 3.5 mM MgCl₂, 2.0 mM ATP, and 6 mM KCl (A) or 26 mM KCl (B) as described under “Materials and Methods.”

KCl. While only one set of assays was performed above \( K_{\text{app}} \), there were no apparent differences in their activities. S-1(A2) had an extrapolated \( V_{\text{max}} \) of around 48 s⁻¹ with a \( K_{\text{app}} \) of 300 \( \mu \)M, while the hybrid gave 46 s⁻¹ and 270 \( \mu \)M.

The same exchange procedure in \( \text{NH}_4\text{Cl} \) used to form S-1 hybrids can be used to exchange the essential light chains of whole myosin. However, while the S-1 hybrids can be purified by ion exchange chromatography, there is no easy method available to separate the myosin hybrid from the native myosin. Therefore, ATPase assays must be performed on mixed myosins. The greater the extent of exchange the easier it is to determine the properties of the myosin hybrid. The amount of exchange depends on light chain concentration, temperature, and length of time in \( \text{NH}_4\text{Cl} \). The light chain concentration used in these hybridizations was around 300 \( \mu \)M. Higher concentrations gave slightly better levels of exchange; however, the increase was too small to warrant the amount of light chain required. Temperature and length of time are limited by the irreversible inactivation of myosin by \( \text{NH}_4\text{Cl} \). Since the rate of inactivation increases more rapidly with increasing temperature than does the rate of exchange, low temperatures were used. Fast myosin can be left in 4.7 M \( \text{NH}_4\text{Cl} \) at 4°C for 1½ h with only a small loss of activity. In the experiments reported here with fast myosin, the length of exchange was 1 h which resulted in less than a 10% loss of EDTA ATPase activity. Cardiac myosin is more susceptible to inactivation by \( \text{NH}_4\text{Cl} \) than is fast myosin. After 30 min at 4°C in 4.7 M \( \text{NH}_4\text{Cl} \), the EDTA ATPase of cardiac myosin is reduced by approximately 10% and by 20% after 1 h. The exchange experiments reported here with cardiac myosin used 30-min incubations at 4°C which gave reasonable amounts of hybridization with little inactivation. In those instances where there was greater than a 10% reduction in the EDTA ATPase of the \( \text{NH}_4\text{Cl} \)-treated control the results were not used.

The extent of hybridization was determined using polyacrylamide gel electrophoresis in sodium dodecyl sulfate. One set of hybridization experiments is shown in Fig. 2. The essential light chain content of the myosin from the exchange between fast myosin and cardiac LC1 was 55% cardiac LC1 and 45% alkali light chains. Myosin from the exchange of fast alkali-1 into cardiac myosin contained about 60% alkali-1 and 40% cardiac LC1. Other exchanges gave from 50 to 75% hybridization. Exchanging alkali-2 into cardiac myosin proved difficult and only about one-third hybridization was obtained. For native and hybridized myosins, the ratio of the essential light chain (alkali-1, alkali-2, and cardiac LC1) to either the DTNB light chain or cardiac LC2 varied between 0.90 and 1.10.

A mock exchange was performed to ensure that the washing procedure removed free light chains. Cardiac LC1 was mixed with fast myosin under exchange conditions except no \( \text{NH}_4\text{Cl} \) was added. The mixture was dialyzed and the myosin washed as usual. The isolated myosin had less than a 5% contamination by cardiac LC1.

As shown in Table II, in the absence of actin, the ATPase activities of the myosin hybrid formed from cardiac LC1 and fast myosin heavy chain were the same as those of fast myosin and very different from those of cardiac myosin. Hybrid myosin from the reciprocal exchange, alkali-1 into cardiac myosin, had the same EDTA and Ca⁺ ATPase activities as cardiac myosin. These results, like those with S-1, show that in the absence of actin the heavy chains and not the essential light chains control the rate of ATP hydrolysis.

The actin-activated ATPase of fast myosin does not have a simple hyperbolic dependence on actin concentration but rather has a biphasic response. At low actin concentration, \( K_{\text{app}} \) for this hybrid

![Fig. 2. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate of control fast myosin (A), myosin from the hybridization of cardiac LC1 with fast myosin (B), myosin from the hybridization of alkali-1 with cardiac myosin (C), and control cardiac myosin (D).](image-url)
Myosin Hybrids

Table II
Myosin ATPase activities

The ATPase activities were determined as described under "Materials and Methods." Values in parentheses are standard deviations of V_max obtained from the linear regressions.

<table>
<thead>
<tr>
<th></th>
<th>EDTA</th>
<th>Ca**</th>
<th>60 mM KCl</th>
<th>100 mM KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>s⁻¹</td>
<td>s⁻¹</td>
<td>µM</td>
<td>s⁻¹</td>
</tr>
<tr>
<td>Fast myosin</td>
<td>11.0</td>
<td>1.4</td>
<td>3.3 (0.2)</td>
<td>6.2</td>
</tr>
<tr>
<td>NH₄Cl-treated fast myosin</td>
<td>10.7</td>
<td>1.3</td>
<td>3.6 (0.2)</td>
<td>3.0</td>
</tr>
<tr>
<td>Fast myosin and cardiac LC1</td>
<td>10.3</td>
<td>1.3</td>
<td>3.2 (0.2)</td>
<td>3.8</td>
</tr>
<tr>
<td>Cardiac myosin</td>
<td>2.2</td>
<td>0.63</td>
<td>0.22 (0.05)</td>
<td></td>
</tr>
<tr>
<td>NH₄Cl-treated cardiac myosin</td>
<td>2.0</td>
<td>0.63</td>
<td>0.20 (0.03)</td>
<td></td>
</tr>
<tr>
<td>Cardiac myosin and alkali-1</td>
<td>2.1</td>
<td>0.57</td>
<td>0.19 (0.05)</td>
<td></td>
</tr>
</tbody>
</table>

* The actin-activated ATPases of myosins containing fast heavy chains were analyzed on Lineweaver-Burk plots and values for V_max and K_m obtained by linear regression.
* V_max and K_m values for the high affinity actin-activated ATPases.
* V_max and K_m values for the low affinity actin-activated ATPases.
* The values in parentheses are the standard deviations of V_max obtained from the linear regressions.
* The average value for the actin-activated ATPase of myosins containing cardiac heavy chains obtained between 7.5 and 30 µM actin.
* Values in parentheses are standard deviations of these turnover rates.
* This myosin hybrid was formed by the exchange of alkali-1 into cardiac myosin.

Hydrolysis with increasing actin concentration, 7.5 to 30 µM. Therefore, only average turnover rates are given in Table II. The actin-activated ATPase activities of this myosin hybrid were about the same as those of cardiac myosin and much less than those of fast myosin. If anything, the hybrid had less activity than cardiac myosin. This probably results from a small amount of inactivation by NH₄Cl, as the NH₄Cl-treated control cardiac myosin also had an actin-activated ATPase slightly lower than that of cardiac myosin. The exchange of alkali-1 into cardiac myosin was performed three times and always gave results comparable to those in Table II.

While the myosins resulting from the exchanges are referred to as hybrids, they were really mixtures of hybrid and native myosins. To see if the presence of a hybrid with altered ATPase could be detected, equal molar amounts of fast and cardiac myosins were mixed and assayed. The EDTA ATPase of the mixture was within 5% of the average of the separate cardiac and fast myosin and the actin-activated ATPase within 15% of the calculated average.

Discussion

Previous hybridization experiments have shown that in the absence of actin the source of the essential light chains does not influence the rate of ATP hydrolysis (16). However, the actin-activated ATPase of S-1 is affected by both the heavy chain and the essential light chain. The K_m values for the actin-activated ATPase of the various S-1 hybrids were closer to those of control S-1 with the same essential light chain than to those of control S-1 with the same heavy chain. The results for V_max were more variable. While the S-1 hybrid between cardiac heavy chain and alkali-2 had the same V_max as did cardiac-S-1, the reciprocal S-1 hybrid, fast heavy chain, and cardiac LC1 had a V_max about one-half way between those of cardiac-S-1 and S-1(A2). Similarly, the S-1 hybrid between fast heavy chain and slow muscle myosin essential light chain had a V_max about one-half way between those of slow S-1 and S-1(A2) (16). The meaning of these results was unclear since under the assay conditions used for these hybrids, the V_max of S-1(A1) is also about one-half that of S-1(A2).
Myosin Hybrids

The actin-activated ATPases were originally assayed in 6 mM KC1 where the affinity of S-1 for actin is high and, therefore, the assays relatively easy to perform. It was subsequently found that when the ionic strength was increased, the $V_{max}$ of the actin-activated ATPase of S-1(A1) increased to about the same level as that of S-1(A2) (14). The percentage difference in their $K_{app}$ values also decreased at higher ionic strengths. This suggested a means to test whether cardiac LC1 when hybridized to fast S-1 heavy chain inhibited the actin-activated ATPase by mimicking alkali-1 or if it caused this hybrid to have some characteristics of cardiac S-1.

The effect of increasing ionic strength on the actin-activated ATPase of the S-1 hybrid containing cardiac LC1 and fast heavy chain was determined. As with S-1(A1), increasing the KC1 concentration from 6 to 26 mM caused the $V_{max}$ of this hybrid to increase almost to that of S-1(A2) and caused the percentage difference in their $K_{app}$ values to decrease. In the one set of assays in 46 mM KC1 where data could be obtained above the $K_{app}$, the actin-activated ATPases of S-1(A2) and this S-1 hybrid were almost identical. Cardiac LC1 appears to interact with fast S-1 heavy chain in the same manner as alkali-1.

The primary difference between the alkali-1 and the alkali-2 light chains is 41 additional amino acids at the NH$_2$-terminus of alkali-1. This peptide has an unusual sequence being very rich in lysine, proline, and alanine (8). The NH$_2$-terminal regions of cardiac LC1 and the essential light chain of slow muscle myosin appear to be similar to that of alkali-1 (20, 21). It seems likely that the similarity of the effects of cardiac LC1, slow muscle myosin essential light chain, and alkali-1, when bound to fast S-1 heavy chain, result from their similar NH$_2$-terminal amino acid sequences. Consistent with this is the observation that occasionally during storage the alkali-1 light chain of S-1(A1) is cleaved to a fragment which runs just above the DTNB light chain when electrophoresed on polyacrylamide gels in sodium dodecyl sulfate. (The source of the proteolysis is not known.) This proteolysis causes S-1(A1) to have the same actin-activated ATPase as S-1(A2) even in 6 mM KC1. Since a similar proteolysis of alkali-2 has never been observed, the cleavage is thought to occur in the NH$_2$-terminal region of alkali-1.

The actin-activated ATPase of myosin has very different properties than those of S-1 or heavy meromyosin. For instance, in 60 mM KC1 at 20°C, the actin-activated ATPase of fast myosin has a $V_{max}$ of around 5.0 s$^{-1}$ with a $K_{app}$ of 20 $\mu$M, while under the same conditions, the actin-activated S-1 has a $V_{max}$ of 50 s$^{-1}$ and a $K_{app}$ greater than 300 $\mu$M. While the essential light chains had no modulating effects on the actin-activated ATPases at intermediate ionic strengths, it is not correct to assume that they will have none at higher ionic strengths. The low $K_{app}$ values of the actomyosin ATPases allow for assays to be performed at ionic strengths higher than can be done with S-1 and closer to those in living muscle. Also, the limiting step in the actomyosin ATPase may be different from that in S-1 and it may be influenced by the essential light chain.

As shown in Fig. 3, the actin-activated ATPase of fast myosin does not show a simple hyperbolic dependence on actin concentration but rather has a distinct biphasic response. It is thought that even though the precise reasons for the behavior are not known, it is still possible to make valid comparisons of fast myosin with myosin hybrids. However, this biphasic response does complicate the comparisons. A more thorough discussion of this phenomenon is presented elsewhere.7

Exchange in NH$_4$Cl has been used previously to make fast myosin enriched either in alkali-1 or in alkali-2. Over a wide range of ionic strengths, 30 to 130 mM KC1, and at all actin concentrations, there were no large differences in the ATPase activities of these two myosin isoenzymes.8 Similar results have been obtained using native myosin isoenzymes isolated from immunoabsorbent columns (90) but thus far their actin-activated ATPases have only been determined at intermediate ionic strengths.

Analysis of the ATPase activities of myosin hybrids formed by light chain exchange is complicated by the inability to separate the hybrid from the native myosin. Fortunately, at the ionic strengths used, 60 and 100 mM KC1, there are 20- and 10-fold differences in the $V_{max}$ values of actin-activated ATPases of fast and cardiac myosins. These large differences in rates, especially when there is greater than 50% light chain exchange, make it easy to show that the actin-activated ATPases of the hybrids are dominated by the heavy chains and not by the essential light chains.

It can be calculated from the data in Table II that an equal mixture of fast and cardiac myosins should have an actin-activated ATPase of 1.8 s$^{-1}$ or 3.0 s$^{-1}$ depending on whether the high or low affinity $V_{max}$ is used for fast myosin. These average values are well outside the experimental deviation of either the exchange of cardiac LC1 into fast myosin or alkali-1 into cardiac myosin. In 100 mM KC1, the calculated average of 0.54 s$^{-1}$ is also clearly different from the observed values.

Taking into consideration the level of exchange achieved in these experiments and the precision of the ATPase assays, these hybridizations show that the actin-activated ATPases of the hybrid myosins differ by less than one-third from those of the control myosins with the same heavy chains and, therefore, that the heavy chains dominate the actin-activated ATPase of myosin. However, it is not possible with impure hybrids to eliminate small modulations of the actomyosin ATPase by the essential light chains.

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REFERENCES