Subunit Interactions of Skeletal Muscle Myosin and Myosin Subfragment 1. Formation and Properties of Thermal Hybrids†

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ABSTRACT: The formation of hybrid myosin and subfragment 1 species by incubation of these proteins with free alkali light chains at physiological ionic and temperature conditions is described. Exchange of bound alkali light chains on myosin by free alkali light chains under these conditions is readily demonstrated from the subunit composition of the isolated myosin. Therefore, the light chain exchange previously described for the one-headed subfragment 1 [Sivaramakrishnan, M., & Burke, M. (1981) *J. Biol. Chem. 256*, 2607–2610] also occurs in the two-headed myosin molecule. It is found that the isozyme to hybrid transformation is dependent on both the temperature and the ionic strength of the incubation mixture but is relatively independent of pH in the range 6.5–8.0. A comparison of the SF1(A1) → $SF1(A2)_h$ system with the SF1(A2) → $SF1(A1)_h$ system indicates that more hybrid is formed in the latter case. With the assumption that hybrid formation reflects the degree of reversible dissociation exhibited by the isozyme, under the particular experimental condition employed, the data signify that the subunit interactions in the two isozymes are not identical and that the heavy chain–A1 interactions are significantly more stable than the heavy chain–A2 ones. An examination of the ATPase properties of the thermal hybrids in the presence and absence of actin indicates close similarities to their corresponding "native" isozymic counterparts.

It is now well established that myosin isolated from vertebrate skeletal fast-twitch muscles is an oligomeric protein comprised of two heavy polypeptide chains and two pairs of light chains known as the 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB)[1] chains and alkali light chains (Gershman et al., 1969; Gazith et al., 1970; Weeds & Lowey, 1971). In the native molecule, the two heavy chains, for half their mass starting at their C termini, are wrapped around one another in the form of an α-helical coiled-coil cable (Lowey et al., 1969; Burke et al., 1973). The two heavy chains then separate, and their N-terminal halves are each separately folded into globular regions known as the myosin heads or subfragment 1 moieties. The light polypeptide chains are associated with the heavy chains in the globular head regions. The two DTNB light chains are thought to be identical (Weeds & Lowey, 1971; Collins, 1976) and to bind at or near the junction between the α-helical cable and the two heads (Weeds & Pope, 1977; Bagshaw, 1977). The alkali chains are associated with the heads and appear to exist as two chemically similar but distinct forms (Frank & Weeds, 1974) designated as A1 and A2. Stoichiometric analyses of the amounts of A1 and A2 associated with the myosin molecule give values of 1.3 and about 0.7 mol, respectively (Sarkar, 1972; Weeds et al., 1975). These nonintegral values for A1 and A2 are now known to be due to multiple isozymic forms of fast-twitch myosin (Holt & Lowey, 1977; Hoh et al., 1976) existing in different proportions depending on the species and muscles employed for the isolation of the myosin. Although these isozymes of myosin have been separated on the basis of their alkali light chain differences, there is clear evidence that the heavy chains are heterogeneous from both sequence analyses (Starr & Offer, 1973; Gallagher & Elzinga, 1980; Pope et al., 1977) and isoelectric focusing studies (John, 1980). The fact that there are two distinct alkali light chain subunits and apparently two distinct heavy chains in fast-twitch skeletal myosin (John, 1980) raises the question of whether there is a predilection for certain heavy chain–alkali light chain combinations in vivo. Pope et al. (1977) have found that the heavy chains of subfragment 1 isozymes are heterogeneous, based on amino acid compositions of the N-terminal tripeptide, and this would argue against specific preferential interactions between the alkali light chains and particular heavy chains. The next point that can be raised is what is the physiological function for the myosin isoenzymes. Examination of the ATP functions has shown that there is no significant difference in catalysis between the two isozymes in the absence of actin (Weeds & Taylor, 1975). However, these workers have found that in the presence of actin the V_{max} and K_m values for the actin-activated subfragment 1 MgATPase differ markedly for the two isozymes albeit at very low salt concentrations, significantly below that occurring in the muscle cell. Wagner et al. (1979) and Reisler (1980) have recently shown that this difference is ionic strength dependent and is practically abolished at ionic strengths comparable to physiological levels. Since it appears that the ATPase and actin binding functions require the specific interactions between the alkali light chains and the heavy chains to form the subfragment 1 complex, the nature of these interactions is crucial to our understanding of the function of subfragment 1 and myosin.

Recent work from our laboratory (Sivaramakrishnan & Burke, 1981) has established that exchange between subunits of subfragment 1 isozymes readily occurs at ionic strengths and temperature conditions resembling those occurring in vivo. In the present paper, we examine the exchange of free alkali light chains for bound alkali light chains of subfragment 1 isozymes that occurs while the isozyme is active and hydrolyzing MgATP. We have studied the effects of ionic strength, pH, and temperature on this process. The data obtained indicate that the stabilities of the subunit interactions in SF1(A1) and SF1(A2) isozymes are not the same. From the degree of conversion to the hybrid species under identical

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[1] Abbreviations used: DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); Gdn·HCl, guanidine hydrochloride; SF1(A1) and SF1(A2), subfragment 1 isozymes containing the A1 and A2 alkali light chains, respectively; $SF1(A1)_h$ and $SF1(A2)_h$, thermal hybrids of subfragment 1 containing the A1 and A2 chains, respectively; HC, heavy chain subunit of subfragment 1; $NaDodSO_4$, sodium dodecyl sulfate.

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conditions, it appears that the A2 interaction with the heavy chain is intrinsically weaker than that occurring with the A1 light chain, with respect to both ionic strength and temperature. These studies raise the possibility that the off and on states of the alkali subunits on the myosin heads may have some physiological role. ATPase measurements of these isolated hybrids show that in the presence or absence of actin they are similar to their native isozymic counterparts.

Since it was of interest to see whether a physiologically more relevant molecule, containing two heads and a pair of DTNB light chains, would behave in a similar manner to the single-headed species, the possibility that light chain exchange can occur in myosin has also been studied. The results obtained by incubating a standard myosin preparation with either free A1 or A2 result in reciprocal changes in the alkali light chain composition of the isolated myosin, indicating that exchange of bound by free alkali light chains can occur on the heavy chains of the myosin molecule.

Materials and Methods

Deionized water prepurified in a Millipore QTM system was used throughout. Myosin was prepared by the procedure of Godfrey & Harrington (1972). Actin was prepared as described by Spudich & Watt (1971). The subfragment 1 isozymes were prepared by digestion of myosin with α-chymotrypsin and separated on DEAE-cellulose by the methods of Weeds & Taylor (1975). Alkali light chains were prepared by denaturing myosin in 6 M Gdn·HCl following the procedures described by Holt & Lowey (1975). These alkali light chains were separated on DEAE-cellulose, and only those fractions which were pure by $NaDodSO_4$ gel electrophoretic analysis employing Coomassie Brilliant Blue as the protein stain were pooled and concentrated for further studies. Protein concentrations were measured either by absorption, employing $A^{1\%}_{280nm}$ values of 5.5, 7.5, and 2.0 for myosin, subfragment 1, and alkali light chains, respectively, or by the Lowry procedure (Lowry et al., 1951) and the Bradford method (Bradford, 1976). Hybridization experiments were done by incubating a particular isozyme of subfragment 1 (1 mg/mL) with the required molar excess of the free alkali light chain of the alternate isozyme in a solution of the desired molarity of KCl buffered by 0.05 M imidazole, pH 7.0, and 5 mM dithiothreitol. When the studies were done at higher temperatures, the pHs of the buffer solutions were adjusted such that they were equal to 7.0 at the respective temperatures. In addition, the solvent contained 10^{-2} M MgATP. After incubation for the required period of time at the desired temperature, the solutions were cooled on ice. The incubated solution was then divided into two parts, and one part was dialyzed vs. 0.05 M imidazole, pH 7.0 (at 4 °C), for ion-exchange chromatography on DEAE-cellulose to separate the subfragment 1 species (Weeds & Taylor, 1975; Wagner & Weeds, 1977). The second portion was dialyzed against 0.01 M sodium pyrophosphate, 0.1 M glycine, and 0.01 M β-mercaptoethanol, pH 6.5, for subsequent electrophoretic separation on nondenaturing polyacrylamide gels. These gels were comprised of 5% acrylamide and 0.135% bis(acrylamide) polymerized in the same solvent but without 2-mercaptoethanol present. Electrophoresis was done on an LKB multiphor apparatus with the cooling water temperature set at 6 °C. The gels were stained with Coomassie Brilliant Blue and destained by the procedures of Weber & Osborn (1969). $NaDodSO_4$ gel electrophoresis was done on 10% acrylamide and 0.27% bis(acrylamide) by employing the procedures of Weber & Osborn (1969) except that 0.05 M imidazole, pH 7.0, and 0.1% $NaDodSO_4$ was used as the electrophoresis buffer. The amounts

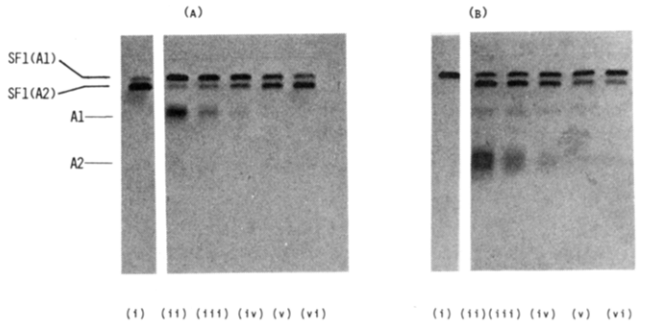

FIGURE 1: Gel electrophoretograms of subfragment 1 isozyme–free alkali light chain mixtures after incubation at 37 °C in 0.5 M KCl, 0.05 M imidazole, and 0.01 M MgATP, pH 7.0, for 30 min. (A) SF1(A2)–free A1 and (B) SF1(A1)–free A2. Columns ii–vi represent 8, 4, 2, 1, and 0.5 M excesses of free light chain to isozyme. Column i in (A) and (B) represents SF1(A2) and SF1(A1), respectively. For other details, see Materials and Methods.

of isozyme and hybrid present in the system were obtained by densitometric analyses of the stained gels by employing the linear transport attachment of the Gilford 250 spectrophotometer and measuring the absorbance at 550 nm. The absorbances were found to be proportional to the protein concentrations for subfragment 1 isozyme up to 1 mg/mL. In the case of the free alkali light chains, we observe that the relative absorbance at 550 nm for A1/A2 is about 4 for the native gels and about 1.5 for the $NaDodSO_4$ gels. Since A2 usually yields a more diffuse band than A1 on both types of gels, it photoreproduces as a very light band. CaATPase and EDTA-ATPase activities were measured at 37 °C by employing the methods of Kielley & Bradley (1956) and Kielley et al. (1956). Actin-activated MgATPase was measured by incubating 0.2 mg of subfragment 1 in a total volume of 1.0 mL containing actin varying from 0.5 to 2 mg at 25 °C for 30 min or at 37 °C for 10 min. The reaction was terminated by the addition of an equal volume of 1 M perchloric acid that was 5 mM in EDTA. The precipitated protein was removed by centrifugation, and the phosphate content of the supernatant was determined as described elsewhere (Kielley & Bradley, 1956; Kielley et al., 1956). The amount of MgATP hydrolyzed by subfragment 1 alone under the same conditions was subtracted from the actin-activated values to derive the actin-activated rates.

Results

(*i*) *Formation of Thermal Subfragment 1 Hybrids.* The formation of thermal hybrids due to exchange of free and bound alkali chains on the heavy chain of the subfragment 1 isozymes is shown in Figure 1. The gel electrophoretograms in Figure 1A show the species present after the subfragment 1 isozyme SF1(A2) is incubated at 37 °C in the presence of MgATP with the free alkali light chain A1. It is clear that, under these conditions where the protein is active and hydrolyzing MgATP, two new protein bands appear on these electrophoretograms having mobilities corresponding to the subfragment 1 isozyme SF1(A1) and the free alkali light chain A2.[2] These results indicate that there has been an exchange of the bound alkali light chain A2 by the free alkali light chain A1 to form $SF1(A1)_h$. This conclusion was verified by isolation of the two subfragment 1 species, the residual SF1(A2) and the new species $SF1(A1)_h$, by either ion-exchange chro-

[2] Although A2 is visible in the original gels, it photoreproduces poorly, and comparisons of the color yield for A2 on these native gels show that it is about one-fourth of that for A1. It is for this reason that it is not readily apparent in this photograph.

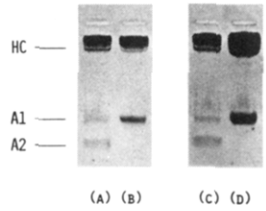

FIGURE 2: $NaDodSO_4$ gel electrophoretograms of thermal hybrids: (A) native SF1(A2) isozyme; (B) native SF1(A1) isozyme; (C) thermal hybrid $SF1(A2)_h$; (D) thermal hybrid $SF1(A1)_h$.

matography on DEAE-cellulose (Weeds & Taylor, 1975) or affinity chromatography on agarose–ATP columns (Winstanely et al., 1979; Burke, 1980). In both of these cases, the $SF1(A1)_h$ species was eluted at the same salt or ATP concentration, respectively, as the authentic SF1(A1) isozyme. The $SF1(A1)_h$ species when rerun on the native gels also exhibited the same mobility as the SF1(A1) isozyme, and its subunit composition determined by $NaDodSO_4$ gel electrophoresis shows that it has A1 as its sole light chain complement (Figure 2). Similar results where the free alkali light chain A2 replaces the bound A1 light chain from the isozyme SF1(A1) are presented in Figure 1B and Figure 2. In this case, there appears to be some contaminating SF1(A1) isozyme in this hybrid from the $NaDodSO_4$ gel electrophoretogram. It should be pointed out that the original isozymic separation in DEAE-cellulose showed, in agreement with the findings of Weeds & Taylor (1975), that the SF1(A1) isozyme was well fractionated whereas the SF1(A2) isozyme appeared to have a small amount of contaminating SF1(A1). Although the results shown here were done at 0.5 M KCl, significant isozyme to hybrid conversion also occurs, as will be shown below, at 0.12 M KCl, which is close to physiological ionic strength.

Formation of the hybrid subfragment 1 species suggests that the extent of subunit dissociation of the subfragment 1 isozyme is time dependent and that with increasing incubation times progressively higher degrees of subunit dissociation are occurring. This possibility was examined in the following way. The subfragment 1 isozyme was incubated alone for different periods of time at 37 °C in the presence of MgATP, and, just prior to being cooled, excess free alkali light chain of the alternate isozyme was added to give an 8 M excess over that of the isozyme. Under these conditions, reassociation of the subunits would be expected to favor the formation of hybrid species if dissociation of the isozyme increases with the time of preincubation at 37 °C. We have, however, consistently observed that little, if any, hybrid is formed under these conditions for periods of subfragment 1 preincubations of up to 30 min. This suggests that extensive dissociation of the subunits of subfragment 1 does not occur at least for these time periods. It should be pointed out in this connection that Dreizen & Richards (1972) also found no evidence for subunit dissociation in myosin when the protein was incubated at 37 °C under similar conditions. The fact that hybrid subfragment 1 species are formed under conditions where the enzyme is apparently active and hydrolyzing MgATP (Sekine & Kielley, 1964; Dreizen & Richards, 1972) suggests that the subunits of the protein exist in a dynamic equilibrium between their associated and dissociated states with the equilibrium favoring the associated complex.

Previous studies by other workers have established that the subunit interactions between the heavy and alkali light chains in myosin and subfragment 1 have a significant ionic component, since titration to pH 11 or incubation in high concentrations of neutral salts such as LiCl or NH_4Cl causes

Table I: Effect of the Ionic Strength of the Incubation Solution on the Degree of Hybrid Formation at 37 °C at pH 7.0

	% hybrid formed[a]	
KCl concn (M)	$SF1(A1)_h$	$SF1(A2)_h$
0.05	40.1	21.5
0.12	51.8	45.0
0.50	72.9	64.4

[a] Subfragment 1 isozymes were at 1 mg/mL, and free alkali light chains were present at an 8 M excess. Incubations were done for 30 min in the presence of 10 mM MgATP.

Table II: Effect of the pH of the Incubation Solution on the Extent of Hybrid Formation at 37 °C in 0.5 M KCl and 0.05 M Imidazole

	amount of hybrid formed relative to that at pH 7.0	
pH	$SF1(A1)_h$	$SF1(A2)_h$
6.5	1.0	0.93
7.5	0.96	1.02
8.0	1.0	0.84

subunit dissociation (Kominz et al., 1959; Gershman & Dreizen, 1970; Dreizen & Richards, 1972). Since increasing ionic strength of the solution should weaken the ionic interactions between the subunits, it would therefore be anticipated that the stability of the subfragment 1 isozymes should decrease as the ionic strength is raised, and this should favor the dissociated state of the equilibrium. This should result in an enhancement of the amount of hybrid formed as the ionic strength is increased. The results presented in Table I are in agreement with this expectation. It is clear from the densitometric analyses of the separate bands, corresponding to isozyme and hybrid on the native gels, that there is more conversion to hybrid as the ionic strength of the incubation solution increases. It is pertinent to note that significant conversion occurs at ionic strengths corresponding to those in vivo.

The effect of pH on the isozyme to hybrid transformation for the range 6.5–8 has also been examined, and the results obtained are presented in Table II. It is clear that there is little variation in the degree of hybrid formation over the pH range studied. This finding suggests that the p*K*s of the ionizable groups that are involved in the subunit interactions lie at least 1 pH unit to either side of this range.

Since the formation of subfragment 1 hybrids occurs readily at 37 °C, it was of interest to examine the temperature dependence of this process. This has been done for the two isozymes SF1(A1) and SF1(A2) by observing the relative amounts of isozyme and hybrid present after a constant incubation time of the respective isozyme–free alkali light chain systems at temperatures ranging from 25 to 46 °C. As mentioned under Materials and Methods, care was taken to ensure that the pH of these solutions was constant over this temperature range. The results obtained are plotted in Figure 3, from which it is clear that the temperature dependence of the isozyme to hybrid conversion is not identical for the two isozymes. Apparently there is a sharp transition where the interactions between the subunits in the isozymes change from a state favoring the associated complex to one that favors the dissociated subunits, over a relatively narrow temperature range. This type of transition is reminiscent of cooperative interactions. The temperatures corresponding to the midpoints of these transitions for SF(A1) to $SF1(A2)_h$ and for SF1(A2) to $SF1(A1)_h$ systems show that it is higher for the former case. This suggests that the subunit interactions in the SF1(A1)

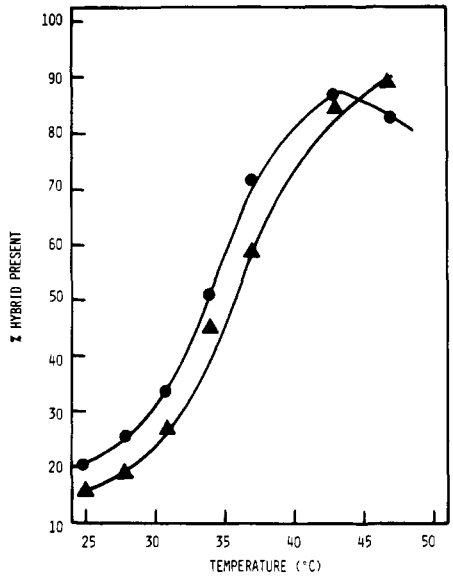

FIGURE 3: Temperature dependence of the isozyme–hybrid transformation: (●) SF1(A2) → SF1(A1)$_h$; (▲) SF1(A1) → SF1(A2)$_h$. Samples were incubated for 30 min in 0.5 M KCl, 0.05 M imidazole, and 0.01 M MgATP, pH 7.0, at the temperatures indicated. The extent of hybrid formation was obtained by densitometric analysis of gel electrophoretograms obtained under nondenaturing conditions.

Table III: ATPase Activities of Subfragment 1 Isozymes and Thermal Hybrids

	activity, k (s^{-1})		actin activation [b]		
	Ca-ATPase [a]	EDTA-ATPase [a]	Mg-ATPase [b]	V_{max} (s^{-1})	K_m (μM)
SF1-(A1)	8.53	15.0	0.032	2.1	89
SF1-(A2)	7.92	14.1	0.033	5.8	343
SF1-$(A1)_h$	9.89	15.9	0.034	2.5	54
SF1-$(A2)_h$	9.95	15.3	0.037	3.6	136

[a] Activities were measured at 37 °C. [b] Activities were measured at 25 °C by incubating 0.2 mg of subfragment 1 isozyme with 2 mM ATP and 3 mM $MgCl_2$ in 0.01 M Tris-histidine and 0.05 M KCl, pH 7.5, in a total volume of 1.0 mL containing actin varying from 0.5 to 2 mg for 30 min.

isozyme are somewhat more stable than those of the SF1(A2) isozyme to temperature perturbation.

(*ii*) *ATPase Activities of the Thermal Hybrids.* Although the ion-exchange and electrophoretic analyses indicate that subfragment 1 hybrids can be formed under physiological conditions, it was of interest to examine the ATPase properties of these thermal hybrids to see whether the exchange of free for bound Alkali light chain has impaired the active site of the reconstituted protein. These results are shown in Table III. In the absence of actin, it is clear that the Ca^{2+}- and EDTA-activated ATPase activities of the thermal subfragment 1 species are very similar to those of the native isozymes, and no clear distinction between these species is evident. In the case of the actin-activated MgATPase activities measured at 25 °C in 0.05 M KCl, the SF1(A1) and SF1(A2) hybrids exhibit the same trends in V_{max} and K_m as their native isozymic counterparts (Table III) even though there are some differences in the absolute values for these parameters. Since the subunit interactions in SF1(A1) and SF1(A2) are destabilized to slightly different extents by raising the temperature from 25 to 46 °C (Figure 3), it was of interest to examine whether this difference would also be reflected in their actin-activated

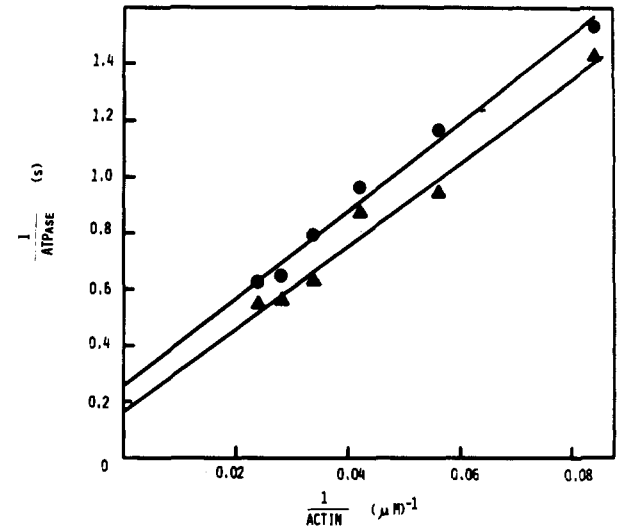

FIGURE 4: Double-reciprocal plot of actin activation of subfragment 1 MgATPase at 37 °C in 0.12 M KCl, 0.01 M Tris–histidine, pH 7.35, 2 mM ATP, and 3 mM $MgCl_2$. Subfragment 1 isozymes (0.2 mg/mL) were incubated with F-actin ranging in concentration from 0.5 to 1.75 mg/mL. The reaction was started by addition of subfragment 1 and was terminated after 10 min by the addition of an equal volume of 1 M perchloric acid containing 0.005 M EDTA. The amount of MgATP hydrolyzed by the subfragment 1 in the absence of actin was subtracted from the actin-activated values to obtain the actin-activated rates. (●) SF1(A1); (▲) SF1(A2).

MgATPase activities at 37 °C in 0.12 M KCl. Typical results are shown in Figure 4. Although some variation in the kinetic parameters was observed with different protein preparations, we have consistently found that the V_{max} for SF1(A2) is somewhat higher than that for SF1(A1). It should be noted that the difference in actin activation for the two isozymes observed under these conditions is far smaller than that observed at 25 °C under much lower salt conditions (Weeds & Taylor, 1975). Whether the difference at 37 °C in the actin activation of the SF1(A1) and SF1(A2) MgATPase is physiologically significant and is related to the differential destabilization of the subunit interactions in these two isozymes must await more work.

(*iii*) *Studies of Thermal Hybrid Formation with Mysoin.* The preceding studies with the subfragment 1 isozymes show that the alkali light chain–heavy chain interactions are extremely labile in the isolated myosin heads. However, it was also of interest to examine whether a similar exchange could be detected in myosin, since it contains two heads and intact DTNB chains. Because there is no convenient way of isolating the pure myosin isozyme by conventional procedures, we have used a standard myosin preparation containing presumably homodimers and heterodimers with respect to the alkali light chains. This myosin was incubated with a 16 M excess of free A1 or A2 under similar conditions described for the experiments with the subfragment 1 isozymes. The myosin was subsequently separated from the free light chains by precipitation with 20 volumes of cold distilled water and collected by centrifugation. The pellet was gently resuspended in 20 volumes of cold distilled water and collected once more by centrifugation. After one more wash cycle, the myosin was subsequently denatured by boiling for 3 min in 1% $NaDodSO_4$ and 1% 2-mercaptoethanol and then examined by $NaDodSO_4$ gel electrophoresis on 10% polyacrylamide gels. After being stained with Coomassie Brilliant Blue, the gels were scanned at 550 nm on a linear transport attachment on a Gilford 250 spectrophotometer. These scans are presented in Figure 5. An examination of the light chain patterns of these myosins shows

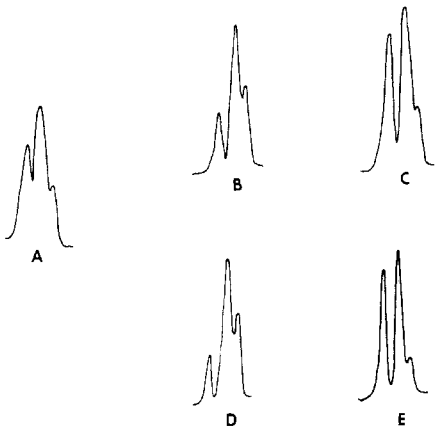

FIGURE 5: Densitometric scans of the light chains of myosin and myosin hybrids separated by $NaDodSO_4$ gel electrophoresis. The three peaks from left to right in each scan correspond to A1, DTNB, and A2 light chains, respectively. (A) Native myosin; (B and C) myosins incubated with A2 and A1, respectively, in 0.12 M KCl and 10 mM MgATP at 37 °C for 60 min; (D and E) myosins incubated with A2 and A1, respectively, in 0.5 M KCl and 10 mM MgATP at 37 °C for 60 min.

that incubation of the myosin with excess free light chain in either 0.5 M KCl or 0.12 M KCl, in the presence of 10 mM MgATP at 37 °C, resulted in reciprocal changes in the A1 and A2 compositions of the myosin. For example, incubation of myosin with excess free A2 resulted in an enhancement in the A2 content of the isolated myosin and, importantly, produced a concomitant decrease in A1 in comparison to that of the control myosin (Figure 5A,B,D). Qualitatively, it appears that in these myosins the A1 plus A2 content is close to the DTNB content, making it unlikely that the altered A1 or A2 compositions of these myosins are caused by fortuitous coprecipitation of free light chains with myosin during the isolation of the latter. Similar results involving myosin hybridization in 4.7 M NH_4Cl and 2 mM EDTA (pH 7.0) at 4 °C have been reported recently by Wagner (1981). Therefore, it appears that exchange of bound by free alkali light chains can also occur in native myosin under physiological conditions of temperature and ionic strength and that the presence of the DTNB light chains does not affect this process. Measurements of the Ca^{2+}- and EDTA-activated ATPase activities of these "hybrid" myosins showed a slight elevation of about 10% for both activities. Because pure isozymes of myosins were not obtained, the actin activations were not studied.

Discussion

The results presented in this paper indicate that the subunit interactions in the subfragment 1 isozymes are sufficiently labile at 37 °C and that incubation with MgATP in the presence of free alkali light chains of the alternate isozyme results in an exchange of free and bound alkali light chains to form hybrid species. Previous studies done on myosin by Dreizen & Richards (1972) have indicated, by criteria such as ATPase activity or denaturation, that there is no subunit dissociation occurring when the protein is incubated under similar conditions. The fact that no hybrid is formed when the isozyme is preincubated alone and excess free alkali light chain is added just prior to cooling to 0 °C indicates that progressive irreversible dissociation of the subunits is not occurring. Therefore, this result would be in agreement with the conclusions of Dreizen & Richards (1972). On the other hand, since hybrids are formed when free alkali light chains and the isozymes are incubated together, it appears that subunit dissociation must be occurring. These apparently contradicting pieces of evidence can be reconciled if it is assumed that at 37 °C subfragment 1 is in equilibrium with its dissociated subunits with the equilibrium very much in the direction of the associated complex. Such an equilibrium would be consistent both with the earlier observation of Dreizen & Richards (1972) and with our observations. Since it is assumed that an equilibrium of the type

$$HC{\cdot}A1 \rightleftharpoons HC + A1 \qquad (1)$$

exists with a very small K_d, addition of excess A2 just prior to cooling to 0 °C would result in very little hybrid being formed. On the other hand, in the situation where the isozyme HC·A1 is incubated at 37 °C in the presence of an excess of free A2 light chain, significant amounts of hybrid would form since, by the law of mass action, each time an isozyme dissociated it would have higher probability of reassociating to form the hybrid species than to form the original isozyme. A mechanism consistent with the data can be formulated as follows:

$$HC{\cdot}A1 + A2 \rightleftharpoons HC + A1 + A2 \rightleftharpoons HC{\cdot}A2 + A1 \qquad (2)$$

It should be stressed that this is just one of many possible mechanisms that would be consistent with the available data. Clearly, more complex situations would arise if, as it is likely, the heavy chain-alkali light chain interaction is a multibond type. Elucidation of the actual mechanism of the isozyme to hybrid conversion must await more work.

Irrespective of the precise mechanism, the formation of the hybrid species must reflect the stability of the subunit interactions in the particular isozyme from which the bound alkali light chain is being replaced. Clearly, the greater the stability of the subunit interaction in the isozyme, the lower will be the extent of its conversion into the hybrid species during incubation with the excess free alkali light chain. An examination of the two exchange systems, SF1(A2)–free A1 chain and SF1(A1)–free A2 chain, under exactly the same protein concentrations and incubation conditions, suggests that the subunit interactions between A1 and the heavy chain are intrinsically more stable than those between A2 and the heavy chain. This is readily apparent from the fact that more hybrid is formed from the SF1(A2) than the SF1(A1) isozyme as shown in Figure 1A,B. This difference in all likelihood represents the differences in the light chains, since the heavy chains in both the SF1(A1) and SF1(A2) isozymes are heterogeneous (Pope et al., 1977), existing in at least two distinct types and in similar proportions in the two isozymes. Another indication of the differences between A1 and A2 in their interactions with the heavy chains comes from both the ionic strength and temperature-dependence studies of hybrid formation shown in Table I and Figure 3, respectively. At each ionic strength investigated, hybrid formation from the SF1(A2) isozyme always exceeds that formed from SF1(A1). This indicates that the ionic component of the heavy chain–alkali light chain interaction is more stable with the A1 light chain. This finding is not completely unexpected since, although A1 and A2 have extensive sequence homology, A1 has an additional 41-residue N-terminal fragment which is highly positively charged (lysine rich), and this segment could, therefore, play a role in the ionic interaction with the heavy chain, thereby conferring additional stability to the subunit interaction of the SF1(A1) isozyme. It should also be noted that differential stability of the subunit interactions between myosin isozyme was noted by Dreizen & Richards (1972), who observed that incubation of myosin at 37 °C in the absence of MgATP or at 0 °C with concentrated NH_4Cl released the A1 subunit preferentially. Sarkar (1972), on the other hand, has

observed that the A2 chain is preferentially released by the 4.7 M NH_4Cl incubation. More recently, Leger & Marotte (1975) and Wagner & Weeds (1977) have shown that both A1 and A2 are released from subfragment 1 by the NH_4Cl procedure. We have found by measuring the amount of hybrids formed from both SF1(A1) and SF1(A2) by incubation with free alkali light chain and NH_4Cl that the subunit interactions are weaker in the SF1(A2) isozyme (M. Burke, M. Sivaramakrishnan, and A. Hess, unpublished experiments).

The fact that the formation of hybrid species is extremely temperature dependent indicates that there is also a hydrophobic component in the interactions between the heavy and alkali light chains in the subfragment 1 isozymes. The data presented in Figure 3 demonstrate that the subunit interactions in the SF1(A1) isozyme are more stable to temperature than is the case for the SF1(A2) species.

The data of the ATPase activities of the thermal subfragment 1 hybrids in the presence and absence of actin show that the hybrids behave catalytically in a similar way to their "authentic" isozymic counterparts although there are some variations in the absolute V_{max} and K_m values. In the presence of actin, $SF1(A1)_h$ behaves like SF1(A1), and $SF1(A2)_h$ acts like SF1(A2). Therefore, it appears that the ATPase and actin binding sites are regenerated during the exchange of the alkali light chains on the heavy chains under these conditions.

We have recently shown that incubation of a mixture of two isozymes under physiological conditions of ionic strength and temperature results in an exchange of subunit between the isozymes (Sivaramakrishnan & Burke, 1981). The data presented in Figure 5 demonstrate that exchange of free for bound alkali light chains on the myosin molecule can also occur under physiological conditions to form thermal hybrids. Therefore, the presence of the intact DTNB chains or the two-headed nature of the myosin molecule does not prevent this process from occurring. Whether or not subunit scrambling can occur between myosin heads in a single molecule or between two molecules must await studies using preparations of pure myosin isozymes. The data obtained with myosin, however, raise the possibility that interconversion between the isozymes may conceivably occur under resting conditions in vivo. Whether the exchange process has any relationship to the ATPase mechanism must wait for kinetic studies of the on and off rates of the heavy chain–alkali light chain equilibrium done for subfragment 1 and myosin in the M**MgADP·P_i and M*·MgADP conformations.

References

Bagshaw, C. R. (1977) *Biochemistry 16*, 59–67.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Burke, M. (1980) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 39*, 1936.

Burke, M., Himmelfarb, S., & Harrington, W. F. (1973) *Biochemistry 12*, 701–710.

Collins, J. H. (1976) *Nature (London) 259*, 699–702.

Dreizen, P., & Richards, D. H. (1972) *Cold Spring Harbor Symp. Quant. Biol. 37*, 145–152.

Frank, G., & Weeds, A. G. (1974) *Eur. J. Biochem. 44*, 317–334.

Gallagher, M., & Elzinga, M. (1980) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 39*, 2168.

Gazith, J., Himmelfarb, S., & Harrington, W. F. (1970) *J. Biol. Chem. 245*, 15–22.

Gershman, L. C., & Dreizen, P. (1970) *Biochemistry 9*, 1677–1687.

Gershman, L. C., Stracher, A., & Dreizen, P. (1969) *J. Biol. Chem. 244*, 2726–2736.

Godfrey, J. E., & Harrington, W. F. (1970) *Biochemistry 9*, 886–893.

Hoh, J. F. Y., McGrath, P. A., & White, R. I. (1976) *Biochem. J. 157*, 87–95.

Holt, J. C., & Lowey, S. (1975) *Biochemistry 14*, 4609–4620.

Holt, J. C., & Lowey, S. (1977) *Biochemistry 16*, 4398–4402.

John, H. A. (1980) *Biochem. Biophys. Res. Commun. 92*, 1223–1230.

Kielley, W. W., & Bradley, L. B. (1956) *J. Biol. Chem. 218*, 653–659.

Kielley, W. W., Kalckar, H. M., & Bradley, L. B. (1956) *J. Biol. Chem. 219*, 95–101.

Kominz, D. R., Carroll, W. R., Smith, E. N., & Mitchell, E. R. (1959) *Arch. Biochem. Biophys. 79*, 191–199.

Leger, J. J., & Marotte, F. (1975) *FEBS Lett. 52*, 17–21.

Lowey, S., Slayter, H. S., Weeds, A. G., & Baker, H. (1969) *J. Mol. Biol. 42*, 1–29.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Pope, B. J., Wagner, P. D., & Weeds, A. G. (1977) *J. Mol. Biol. 109*, 470–473.

Reisler, E. (1980) *J. Mol. Biol. 138*, 93–107.

Sarkar, S. (1972) *Cold Spring Harbor Symp. Quant. Biol. 37*, 14–17.

Sekine, T., & Kielley, W. W. (1965) *Biochim. Biophys. Acta 81*, 336–345.

Sivaramakrishnan, M., & Burke, M. (1981) *J. Biol. Chem. 256*, 2607–2610.

Spudich, J. A., & Watt, S. (1971) *J. Biol. Chem. 246*, 4866–4871.

Starr, R., & Offer, G. W. (1973) *J. Mol. Biol. 81*, 17–31.

Wagner, P. D. (1981) *J. Biol. Chem. 256*, 2993–2998.

Wagner, P. D., & Weeds, A. G. (1977) *J. Mol. Biol. 109*, 455–470.

Wagner, P. D., Slater, C. S., Pope, B., & Weeds, A. G. (1979) *Eur. J. Biochem. 99*, 385–394.

Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406–4412.

Weeds, A. G., & Lowey, S. (1971) *J. Mol. Biol. 61*, 701–725.

Weeds, A. G., & Taylor, R. S. (1975) *Nature (London) 257*, 54–56.

Weeds, A. G., & Pope, B. (1977) *J. Mol. Biol. 111*, 129–157.

Weeds, A. G., Hall, R., & Spurway, N. C. S. (1975) *FEBS Lett. 49*, 320–324.

Winstanley, M., Small, D. A., & Trayer, I. P. (1979) *Eur. J. Biochem. 98*, 441–446.