Amino Acid Sequence of Rabbit Skeletal Muscle Myosin

50-kDa FRAGMENT OF THE HEAVY CHAIN*

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The amino acid sequence of the 50-kDa fragment that is released by limited tryptic digestion of the head portion of rabbit skeletal muscle myosin was determined by analysis and alignment of sets of peptides generated by digestion of the fragment at arginine or methionine residues. This fragment contains residues 205-636 of the myosin heavy chain; among the residues of particular interest in this fragment are N-trimethyllysine, one of four methyl-amino acids in myosin, and Ser-324, which is phosphorylated by an ATP analogue (Mahmood, R., Elzinga, M., and Yount, R. G. (1989) Biochemistry 28, 3989-3995). Combination of this sequence with those of the 23- and 20-kDa fragments yields an 809-residue sequence that constitutes most of the heavy chain of chymotryptic S-1 of this myosin.

Myosin is one of the key protein components of biological force-generating systems, and its structure and function are best understood in striated muscle. Myosin is a large molecule having two distinctly different halves which are tailored to specific functions. One part is a long threadlike structure that is assembled from two parallel α-helices wound about each other forming a highly stable coiled-coil. Each α-helix represents the COOH terminal about one half of the two heavy chains comprising each myosin molecule. Interactions (both parallel and antiparallel) between the coiled-coils are responsible for assembly of the myosin molecules into thick filaments. At the other end of the myosin molecule the two heavy chains separate from one another and fold into two roughly globular entities which, together with the light chains, constitute the two heads (each called subfragment-1, or S-1) of myosin. The heads are more complicated and less well understood than the helical section and are of particular interest because they contain the ATPase and actin-binding sites of myosin. Over the past several years, studies by many investigators have permitted the identification of certain parts of the molecule, and is some cases specific amino acid side chains, that are associated with structural or functional aspects of myosin. Such studies are most meaningful when viewed in the context of the amino acid sequence of the protein, and in this paper we present the sequence of the S-1 portion of the heavy chain of rabbit skeletal muscle myosin. This part of the adult rabbit skeletal muscle myosin sequence has not previously been described, and since it is a common myosin for biochemical studies of structure and function, the sequence as well as the methods that have been used to characterize individual peptides are of some interest. The data presented here document the sequence of a 50-kDa fragment; combination of this sequence with sequences of two other fragments (of 23 and 20 kDa size) yields the 809-residue sequence of the heavy chain of the rabbit S-1.

MATERIALS AND METHODS

Most of the methods and materials employed in this study have been described (Tong and Elzinga, 1983). The 50-kDa fragment of myosin was purified by gel filtration of a limited digest of tryptic heavy meromyosin or chymotryptic S-1 as shown in Fig. 1 of Tong and Elzinga (1983). Sodium dodecyl sulfate was removed from the protein by passage over a column of Extracti-Gel (Pierce Chemical Co.), as described by Elzinga and Phelan (1984). V6 protease (Drapier, 1975) was used for digestion of peptide fractions in 100 mM sodium phosphate, pH 7.5, containing 2 mM EDTA, at a ratio of 1 part enzyme:50 parts peptide. Chymotrypsin was used at the same ratio, but digestions were done in 0.5% NH4HCO3, pH 8.0.

RESULTS

DISCUSSION

Strategy—The overall strategy used in determining the sequence of the 50-kDa fragment of the myosin heavy chain included fragmenting of the parent polypeptide chain into successively smaller peptides and isolating these fragments for further study. The intact myosin was digested first with either trypsin, which yields heavy meromyosin (containing the S-1 moiety and part of the helical portion) and the helical light meromyosin (Szent-Gyorgyi et al., 1960), or chymotrypsin which yields chymotryptic S-1 as well as the entire helical portion (Weeds and Pope, 1977). Heavy meromyosin or chymotryptic S-1 was then digested with trypsin under conditions that lead to selective digestion at two sites (Balint et al., 1975, 1978), and upon denaturation of the trypsin-treated S-1 three fragments representing most of the head were released. These were separated by gel filtration (Mornet et al., 1980), and sequenced by conventional digestion, peptide separation, and automated sequencing. The connections between the large tryptic fragments (which have masses of 23, 50, and 20 kDa) were identified by analysis of cyanogen bromide peptides isolated from the intact heavy chain of chymotryptic S-1. The sequence as shown in Fig. 10 represents residues 1-809 of the heavy chain. It is most of the heavy chain portion of chymo-

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tryptic S-1, but a short piece of the swivel (which connects S-1 to S-2) was presumably released by a tryptic split at Arg-809, since chymotrypsin would not be expected to hydrolyze an Arg-X bond. This fragment was not recovered in the tryptic fragments that we used for sequencing. However, we have found that a 22-kDa precursor of the 20-kDa fragment has the same NH2-terminal sequence, indicating that chymotryptic S-1 extends at least to residue 829.

The amino acid sequence of the heavy chain portion of rabbit skeletal muscle myosin S-1, as presented here, is the synthesis of studies on several fragments; the 50-kDa fragment (this work is described here); the sequence of residues 1–204, published earlier (Tong and Elzinga, 1983); a 92-residue fragment (residues 687–778) that contains SH-1, SH-2, and the single residue of N’-MeHis (Elzinga and Collins, 1977); the 20-kDa fragment that represents residues 641–809 (Gallagher and Elzinga, 1980); tryptic fragments that contain the methylated sidechains (Huszar, 1972a, 1972b); and a chymotryptic fragment from the amino terminus of the chain (Starr and Offer, 1973). Evidence for the alignment of the fragments is also presented here.

Microheterogeneity—Strictly speaking, we were not able to write a single sequence for the rabbit myosin because the myosin we studied contains at least two different polypeptide chains. The evidence for multiple chains is the fact that many positions are occupied by about equal amounts of two different amino acids. This situation is often called microheterogeneity, and was previously reported in myosin by Starr and Offer (1973) for residue 8. In Table 6 we list the positions at which microheterogeneity (both in 50 kDa and in the rest of S-1) is observed. The results are consistent with the presence of two different polypeptides, but since each instance could theoretically represent a point at which a different chain contains a substitution these results as presented are compatible with the presence of more than two gene products. The only direct evidence we have that substitutions are paired is from studies of the sequence near the COOH terminus of light meromyosin; evidence we have that substitutions are paired is from studies on several fragments; the 50-kDa fragment (this work is described here); the sequence of residues 1–204, published earlier (Tong and Elzinga, 1983); a 92-residue fragment (residues 687–778) that contains SH-1, SH-2, and the single residue of N’-MeHis (Elzinga and Collins, 1977); the 20-kDa fragment that represents residues 641–809 (Gallagher and Elzinga, 1980); tryptic fragments that contain the methylated sidechains (Huszar, 1972a, 1972b); and a chymotryptic fragment from the amino terminus of the chain (Starr and Offer, 1973). Evidence for the alignment of the fragments is also presented here.

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Features—The most unusual feature of the sequence is in residues 632–643 (Gly-Gly-Gly-Lys-Lys-Gly-Gly-Lys-Lys-Gly). This is preceded by a stretch of 9 residues in which the three sequences illustrated in Fig. 10 vary greatly. The lysines in the former sequences are rapidly digested by trypsin, the region is protected by actin binding, and actin can be cross-linked to residues in this vicinity. It seems likely that these sequences are flexible surface structures that may join two domains near an actin binding site.

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S. A. Davidoff and M. Elzinga, unpublished data.
Davidoff, 1987), it has been suggested that these fragments
describe independently folded domains that are linked by
easily digested connector peptides.

Localization of Residues within S-1—With the use of target-
gated photoaffinity analogs of ATP to probe the anatomy of the
myosin head, Winkelman and Lowey (1986). Miyashishi
et al. (1988), and Sutoh et al. (1989) have localized antibodies
made to the tryptic fragments or to synthetic peptides having
sequences selected from various regions of the heavy chain,
and these studies have shown that 23-kDa epitopes are local-
ized in the central part of the head, those for the 50-kDa
epitopes are generally in the distal portion (opposite from the
head-rod junction), and the 20-kDa epitopes seem to stretch
between the head-rod junction and a point about 10 mm
toward the distal end of S-1.

Chemical cross-linking has been used to identify pairs of
residues that are close to each other, and these studies have
given information primarily about side chains near the active
sulfhydryl group SH-1, which is Cys-707, and SH-2, which is
Cys-697. Wells and Yount (1979) showed that a disulfide
could be formed within S-1, resulting in "trapping" of MgATP
at the active site. This cross-link was shown (Huston et al.,
1988) to involve SH-1 and SH-2, and the fact that bifunctional
reagents of varying length could cross-link these groups
(Wells et al., 1980) suggested substantial flexibility in this
region of the molecule. Ue (1987) has shown that Cys-707 (in
the 20-kDa fragment) can be cross-linked by a short thiol
reagent. Sutoh et al. (1987) showed that Cys-707 could be cross-linked to
a residue(s) in the 50-kDa fragment. In the absence of MgATP
the binding is to the region near Cys 696 exclusively, while in
the presence of MgATP binding to the 50-kDa fragment also
occurs. Rajasekharan et al. (1987) showed that trapping of
MgADP also favors Cys-707-50-kDa cross-linking. Chauve-
pied et al. (1988) reported that Cys-697 can form a disulfide
with Cys-540. Hiratsuka (1988) used a trifunctional alkylation
agent, and found that when bound to Cys-707, the reagent
bound to side chains in both the 23- and 50-kDa fragments.
Taken together, these results clearly identify regions near the
reactive sulfhydryl groups and suggest that nucleotide-de-
dendent conformational changes occur in S-1. Attractive
models depicting the possible locations of various sites in S-1
have been published by Yanagisawa et al. (1987), Vibert and
Cohen (1988), and Sutoh et al. (1989).

ATPase Site and Actin Binding Sites—Side chains that
constitute the site responsible for myosin ATPase activity
have been sought experimentally, and by homology of the
amino acid sequence with that of other ATPases. Walker et al.
(1982) proposed that residues within the sequence 165-193
contributed to at least part of the binding or catalytic site,
and subsequent experiments have corroborated this proposal;
Atkinson et al. (1986) photoaffinity labeled Acanthamoeba
myosin II with UTP, and reported binding to a Glu in a
position corresponding to Val-186 of the rabbit myosin, and
Cremo et al. (1989) have implicated Ser-180 by photoxidation
of an S-1 MgATPase complex containing trapped vanadium.
Other reagents have targeted different regions. Okamoto and
Yount (1985) photoaffinity labeled myosin with the ADP
analog N-(4-azoido-2-nitrophenyl)-2-aminoethyl diphosphate,
and found that the major site of binding was Trp-130. Mah-
moor et al. (1989) used the photoaffinity label 3'(2')-O-(4-
benzoyl-benzoyl)ATP and reported that it bound to Ser-324,
which is in the 50-kDa fragment. In the first myosin photo-
affinity labeling study to be reported, Szilagyi et al. (1979)
found that an arylazido-β-alanine derivative of ATP bound
covalently to the 20-kDa fragment.

Tokunaga et al. (1987) have used a biotin-containing photo-
affinity analog of ATP to localize the ATP-binding site within
the native S-1; the modified ATP was labeled with avidin,
which was then localized by electron microscopy, and it was
found to be approximately 5 mm from the tip (distal end) of
the myosin head, and about 4 mm from the actin-binding
region. Localization of the actin-binding site(s) (reviewed by
Vibert and Cohen, 1988) has given less definitive answers,
probably because the site(s) occupy relatively large surfaces.
It seems clear that the biologically important activities of
myosin, ATPase and actin binding, involve sites that are
physically separate within S-1; yet they must be functionally
coordinated in generating contractile force. It is anticipated
that further chemical studies, which should be facilitated by
knowledge of the exact sequence of the rabbit skeletal muscle
myosin, combined with analysis of the structure of S-1 (Ray-
ment and Winkelmann, 1984; Winkelmann et al. 1985) will
lead to an understanding of the key events underlying force
generation in the acto-myosin system.

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259, 2775-2781
Amino Acid Sequence of Myosin


SUPPLEMENTARY MATERIAL

The Amino Acid Sequence of Steady State Myosin


This section contains a description of the test employed in determination of the sequence of residues 259–630, as well as Figures 1 through 12 and Tables 1 through 7.

Desalination of the sequence

The purified 55,014 M, fragment obtained by G-50 gel filtration was <98% homogeneous as judged by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The pooled homogenous fractions were dialyzed against 0.1 M NaHCO₃ and lyophilized.

The amino acid composition of the purified myosin chain was determined by the procedures of Bowers and Doty (1961) (Fig. 1). The amino acid composition of the purified myosin chain was determined by the procedures of Bowers and Doty (1961) (Fig. 1). The amino acid composition of the purified myosin chain was determined by the procedures of Bowers and Doty (1961) (Fig. 1).

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Table 1. Amino Acid Sequence of Myosin

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Table 2. Sequencer run on 105 mol of peptide N-5. Steps 1 and 16 were identified as amino butyric acid after N1 hydrolysis.

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Table 3. Residues found in the sequence of rabbit skeletal muscle myosin 5-1 at which microheterogeneity was observed.

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<thead>
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<th>Position</th>
<th>Residues found</th>
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<td>40</td>
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<td>870</td>
<td>Met, Val</td>
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</table>

Table 4. Locations of low abundance amino acids in the myosin head.

| Tryptophan | 522,422,522,567,577,697,707,794 |
| Cysteine   | 122,422,522,535,674,697,707,794 |
| e-tetrahydro-pyridine | 129,651 |
| e-monosulfone-pyridine | 129,651 |
Amino Acid Sequence of Myosin

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Figure 1. Diagram of the peptides that were characterized for determination of the sequence of 50 kDa. Upper case letters denote positions identified by sequencing peptides. Lower case letters (b-d) designate positions that were not unambiguously identified. Solid lines together with the letters indicate the exact locations within 50 kDa of the individual peptides, the arrowed line indicates, in each case, the COOH-terminal residue. 50 kDa begins at both 205 and 214, and terminates at 656. Numbers above the sequence denote the locations of residues within the intact myosin molecule.

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Figure 2. Gel filtration of peptides generated by pepsin digestion of subproteolytically 50 kDa, after reversibly blocking all amine groups with diisopropylethylene. 2 amounts were digested for 10 hr with 20% glycerol. Buffer equilibrated with 20% glycerol was added to the digestion, glycerol and ethanol (1:1) to 25%, and the solution was left overnight at 20°C for defreezing of the amine groups. The solution was then as denatured and concentrated 2 to 5-fold. NADH were added and the solution was adjusted to 60% for the following filtration analysis. Filtered fractions were collected by a fraction collector to which fractions were collected and pooled. The above figure shows, fractions were pooled as indicated by the solid bars, and peptides (designated by "A" and "B") were eluted in the same order as shown.
Amino Acid Sequence of Myosin

Fig. 11. Overlay of the amide nitrocellulose of a rabbit skeletal muscle myosin (broken line) and chicken gizzard muscle myosin (open line). The overlap between the two lines is shown in black. The identity of the residue is shown above or below the residue number. The shaded area indicates the regions which are conserved between the two myosins.
Amino acid sequence of rabbit skeletal muscle myosin. 50-kDa fragment of the heavy chain.
S W Tong and M Elzinga


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