Comparative Structure of the Protease-sensitive Regions of the Subfragment-1 Heavy Chain from Smooth and Skeletal Myosins*

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The heavy chain fragments generated by restricted proteolysis of the smooth chicken gizzard myosin subfragment-1 (S-1) with trypsin, Staphylococcus aureus V8 protease, and chymotrypsin were isolated and submitted to partial amino acid sequencing. The comparison between the smooth and striated muscle myosin sequences permitted the unambiguous structural characterization of the two protease-vulnerable segments joining the three putative domain-like regions of the smooth head heavy chain. The smooth carboxyl-terminal connector is a serine-rich region located around positions 632-640 of the rabbit skeletal sequence and would represent the "A" site that is conformationally sensitive to the myosin 10 S-6 S transition and to its interaction with actin (Ikebe, M., and Hartshorne, D. J. (1986) Biochemistry 25, 6177-6185). A third site which undergoes a nucleotide-dependent chymotryptic cleavage which inactivates the Mg\(^{2+}\)-ATPase (Okamoto, Y., and Sekine, T. (1981) J. Biochem. (Tokyo) 90, 833-842, 843-849) was identified at Trp-31/Ser-32. It is vicinal to Lys-34 that is monomethylated in the skeletal heavy chain but not at all in the smooth sequence. However, the two trimethyl lysine residues present in the skeletal sequence are conserved in the same regions of the smooth S-1 and may play a general functional role in myosin. The smooth central 50-kDa segment could be selectively destroyed by a mild tryptic digestion in the absence of any unfolding agent, with a concomitant inhibition of the ATPase activities. This feature is in line with the proposed domain structure of the S-1 heavy chain and also suggests a relationship between the specific biochemical properties of the smooth S-1 and the particular conformation of its 50-kDa region.

The vertebrate smooth and skeletal muscles display functional differences which are at least in part reflected by the different biochemical properties observed for the corresponding actomyosin-ATP complexes (1-3). To understand in detail the molecular mechanism of energy transduction in the smooth muscle cell, it is essential to elucidate the major structural features residing on the myosin heavy chain and that are the bases for the unique functional behavior of the smooth myosin.

Previous reports describing the proteolytic fragmentation of native gizzard smooth muscle myosin, heavy meromyosin, and S-1\(^*\) (4-6) have revealed similarities in the structural organization of the heavy chains of the smooth and the skeletal myosin heads. The two proteins are composed of three domain-like fragments, the 29-kDa, 27-kDa NH\(_2\)-terminal peptides, the 50-kDa central peptides, and the 25-kDa, 20-kDa COOH-terminal peptides, respectively, joined by two protease-sensitive loops. The growing information on the primary structure of sarcomeric and nonmuscle myosin heavy chains (7-11) indicated the presence of strongly conserved amino acid sequences within these three regions of the head. These homologous specific sequences are thought to be important for myosin function (8-9). On the other hand, the two linker regions exhibit much less conserved sequences. However, their conformation is very sensitive to binding of actin and nucleotides especially in the S-1 from vertebrate (12-15) and invertebrate striated muscle myosins (16).

In contrast, the primary structure of the smooth S-1 heavy chain is as yet unknown, except for the papain-produced C-terminal 24-kDa fragment of chicken gizzard S-1 whose amino acid sequence has been recently reported (17). This heavy chain segment is thought to be involved in the interaction with actin and the light chains (5, 18).

As a further step in the knowledge of the structure-activity relationships in the chicken gizzard smooth myosin head, we have determined in the present study the amino acid sequences of three typical protease-vulnerable regions of the smooth S-1 heavy chain. These include in particular the peptide stretch in between the 50- and 25-kDa segments that is cut by several enzymes together with the N-terminal heavy chain portion in which a new specific chymotryptic-sensitive site is introduced upon nucleotide binding to S-1 (19). These two particular regions are of special interest. The 50 kDa-25 kDa connector segment undergoes conformational changes during the transition of the native smooth myosin between the 10 S and 6 S forms, and it is located at or near the actin-binding site (6). Similarly, structural changes take place within the N-terminal heavy chain segment upon phosphorylation of the gizzard regulatory light chain (20) and the structural integrity of this area is essential for the maintenance of the smooth Mg\(^{2+}\)-ATPase activity (21, 22). Finally, we illustrate the unique conformational properties of the central 50-kDa region of the smooth S-1 heavy chain by its selective tryptic degradation under nearly physiological conditions with abolition of all ATPase activities. The overall

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*The abbreviations used are: S-1, myosin subfragment-1; SDS, sodium dodecyl sulfate; HPLC, high performance liquid chromatography.
Structural features of the protease-labile regions in the smooth S-1 heavy chain were compared to those known for the same regions in the skeletal myosin head.

**EXPERIMENTAL PROCEDURES**

**Materials**—Trypsin, l-1-(tosylamido)-2-phenylethyl chloromethyl ketone-treated and a-chymotrypsin, dialyzed and salt-free, were purchased from Worthington. Mercuriapain was from Sigma, *Staphylococcus aureus* V8 protease was obtained from Miles. Diisopropylphosphofluoridate-treated carboxypeptidases A and B were purchased from Boehringer Mannheim. Sequencer reagents were obtained from Applied Biosystems. All other chemicals were of analytical grade.

**Preparation of Proteins**—Myosin was prepared from fresh chicken gizzards by a combination of methods as described (4, 5).

Gizzard papain subfragment-1 and its trypptic derivative (29 kDa-50 kDa-25 kDa)-S1 were obtained as reported previously (5), except that the digestion of myosin with papain was conducted at a protease to substrate weight ratio of 1:6000 for 15 min at 20 °C.

Myosin from rabbit skeletal muscle was prepared according to Offer et al. (23).

Skeletal chymotryptic S-1 and trypsin-split (27 kDa-50 kDa-20 kDa)-S1 were prepared as described by Weeds and Taylor (24) and Mornet et al. (25), respectively.

Protein concentrations were determined by measuring the absorbance at 280 nm with an extinction coefficient of E_{280 nm} = 4.5 cm for gizzard myosin (26), and 5.6 cm^{-1} for skeletal myosin (24). The concentration of S-1 and the other proteolytic derivatives were determined according to Bradford (27). Calculations were based on a molecular weight of 115,000 for gizzard S-1 preparations (28).

**Proteolytic Digestions**—The limited cleavage of gizzard S-1 with the various proteases was performed at a protein concentration of 1-2 mg/ml in 25 mM Tris-Cl, pH 8.0, at 25 °C for 20-90 min, using enzyme to substrate weight ratios of 1:20 (*Staphylococcus aureus* V8 protease), 1:50 (chymotrypsin), and 1:1000 (trypsin and papain). The chymotryptic digestion of (29 kDa-50 kDa-25 kDa)-S1 was carried out in the presence of 5 mM MgATP. The following procedures were used to quench proteolysis: for trypsin, the addition of soybean inhibitor (twice the weight of trypsin); for chymotrypsin, the addition of phenylmethylsulfonyl fluoride to 1 mM; for papain, the addition of iodoacetic acid to 5 mM; for *S. aureus* protease, heat denaturation at 100 °C as previously described (29). The fragmentation of the gizzard (29 kDa-50 kDa-25 kDa)-S1 with trypsin was conducted under the same experimental conditions as for native S-1 and the skeletal (27 kDa-50 kDa-20 kDa)-S1 as control; protein aliquots were removed at various times and submitted in parallel to gel electrophoresis and ATPase assays.

**Peptides Isolation and Amino Acid Sequence Analyses**—The proteolytic fragments of S-1 heavy chain were purified by gel filtration over Sephacryl S-300 under the dissociating conditions reported earlier (25). The column was equilibrated in 50 mM Tris-Cl buffer, pH 8.0, containing 0.5% SDS. When necessary, the peptide fractions were further purified by reverse-phase HPLC using a Beckman Model 332 B gradient liquid chromatograph and a 4.6 × 75-mm "Ultrapore" RPSC column. A linear gradient elution was performed in 60 min using solvent A: 0.1% trifluoroacetic acid in water, and solvent B: 0.1% trifluoroacetic acid in 60% acetonitrile.

Amino acid sequences were determined in a liquid phase sequencer (Socios) as described (29) or on an Applied Biosystems Model 470 A gas-phase protein sequencer as described by Hewick et al. (30) and PTH-derivatives were analyzed by reverse-phase HPLC using an Applied Biosystems Model 120 A analyzer.

Amino acid compositions were determined on protein samples hydrolyzed at 110 °C under vacuum in 5.7 N HCl for 24 and 48 h using a Beckman analyzer (Model 119B). The analyses of methylated lysine and histidine were performed as previously described (25). For C-terminal analyses, digestions with carboxypeptidases A and B were carried out according to (31).

**Gel Electrophoresis**—The proteolytic reactions and peptide purification were followed by electrophoresis in SDS-polyacrylamide slab gels as described elsewhere (32, 33), using a 5-18% acrylamide gradient. The running buffer was 50 mM Tris/100 mM boric acid (pH 8.0). The following protein markers were used: skeletal subfragment-1 heavy chain (95 kDa), light chain 1 (25 kDa), light chain 3 (16.5 kDa), actin (42 kDa) and the three fragments of trypically split skeletal S-1 (50, 27, and 20 kDa).

**ATPase Measurements**—ATPase activities were determined at 25 °C in 50 mM Tris-Cl, pH 7.5, in the presence of 2.5 mM ATP. Conditions for K+ EDTA assay were: 1 M KCl, 5 mM EDTA; for calcium ATPase: 250 mM KCl, 5 mM CaCl2; for magnesium-dependent ATPase: 250 mM KCl, 5 mM MgCl2. P, liberated was measured colorimetrically as described previously (25).

**RESULTS**

**Primary Structures of the Connector I and II Regions of the Smooth S-1 Heavy Chain**—In the gizzard papain S-1, the connector I segment would represent a narrow part of the heavy chain joining the central 50-kDa domain to the C-terminal 24-kDa fragment, the N-terminal sequence of which is homologous to the trypptic skeletal 20-kDa peptide (17). In the parent intact myosin and heavy meromyosin, this region (also referred to as site A (6, 34)) is highly protease-sensitive. Consequently, to avoid loss of peptide material we first prepared papain S-1 under carefully controlled conditions using, in particular, a very low enzyme to substrate weight ratio. The yield of the S-1 so obtained did not exceed about 10%, but it contained at least 95% of intact 97-kDa heavy chain (Fig. 1), in contrast to the usual papain S-1 preparation which consistently included a significant amount of a nicked 75 kDa-26-kDa heavy chain species (5).

The isolated intact S-1 was then subjected to limited digests with *S. aureus* V8 protease, trypsin, and chymotrypsin. Three distinct gel patterns were obtained for the three proteolytic reactions; they are presented in Figs. 1, A-C, respectively. The bacterial protease cut only the heavy chain into two main fragments of M, 75,000 and 25,500 which correspond to the amino- and carboxyl-terminal portions of the heavy subunit, respectively. This assignment is in agreement with the mode of cleavage of myosin and heavy meromyosin by this protease, described previously (6, 34). In contrast, the trypptic pattern of the proteolysis showed the scission of the heavy chain at two sites with the formation of the expected three fragments corresponding to the NH2-terminal 29 kDa, the central 50-kDa and the COOH-terminal 25-kDa segments of the smooth S-1 heavy chain (5); however, the time course of this cleavage shows the rapid splitting of the heavy chain at the COOH-terminal 75 kDa-26 kDa junction followed by the breakdown of the NH2-terminal 75-kDa peptide into its 29- and 50-kDa components. Concomitantly the 26-kDa fragment is converted into the stable COOH-terminal 25-kDa entity.

**FIG. 1. Comparative gel patterns of the gizzard papain subfragment-1 digested by various proteases.** The time course of the proteolysis of S-1 is presented for *S. aureus* V8 protease (A), trypsin (B), or chymotrypsin (C) using the conditions reported in the experimental section. The fragmentation of the heavy chain is initiated by the breakdown of the connector I segment into a NH2-terminal 75-kDa fragment and a COOH-terminal 26- to 25-kDa fragment. The NH2-terminal fragment gives rise to the NH2-terminal 29- and 50-kDa fragments only upon trypsin action on a putative "connector II"
Finally, the chymotryptic digestion led to a slow and incomplete splitting of the heavy chain into two fragments representing the NH2-terminal 75-kDa and COOH-terminal 25.5-kDa moieties of the S-1 heavy chain. In addition, both the 97- and 75-kDa bands were partially converted into two species of slightly higher electrophoretic mobility. The overall gel pattern is essentially consistent with the reported property of this protease to cut the head region of native smooth myosin at two clip sites; one is located at about 71 kDa from the NH2-terminal end and the other is within the NH2-terminal 5-kDa segment (19). As indicated in Table I, this region is cut only by trypsin for the smooth S-1, whereas it is severed by any COOH-terminal sequences analyzed. The results are summarized in Fig. 2. Sequerencer runs on the COOH-terminal \textit{S. aureus} protease 25.5-kDa fragment permitted the unambiguous identification of up to 21 amino acid residues which provided most of the information on the structure of the connector I region. Similarly, the structure of the connector II segment was approached by sequencing the NH2-terminal 35 residues of the trypsin 50-kDa fragment. In Fig. 2, the sites of proteolytic attacks on the smooth and rabbit skeletal S-1 heavy chain sequences are compared, and a more general comparison of the smooth sequences with those established for various other striated muscle (7, 8, 11) and nonmuscle myosins (9, 10) is presented in Table II. The 25.5-kDa peptide sequence overlaps the papain 24-kDa fragment which starts with the sequence (Lys-Gly-Met) KGM (17), whereas we found the trypsin 25-kDa fragment to start one amino acid further with the sequence (Gly-Met-Arg) OM. Thus the papain and trypsin sites are adjacent, and three proteolytic sites are located within a 12-residue peptide stretch (Fig. 2).

The smooth sequence we have determined corresponds to residues 629-653 in the rabbit skeletal sequence (Table II). In both cases the \textit{S. aureus} protease cuts the S-1 heavy chain at the same site (Glu-629). The \textit{S. aureus} protease cleavage site on the rabbit skeletal S-1 was identified during the present work by microsequencing of the corresponding 22-kDa peptide (29) up to 24 amino acid residues. Similarly, trypsin severs the two connector I segments at a (Lys-Gly) K-G bond and releases the COOH-terminal 25- and 20-kDa domains, respectively. Homology between the smooth and skeletal sequences is apparent only from position 643, whereas most of the connector I region itself displays a different primary structure, the Gly residues in the skeletal structure being replaced by serines in the smooth sequence (Table II and Fig. 2).

The NH2-terminal sequence we have determined for the smooth 50-kDa peptide starts from positions 203 to 238 (Table II). Previously, we have suggested that the rabbit skeletal 27 kDa-50 kDa connector II region could span residues 205-213 (29). This 9-residue segment includes three proteolytic sites one of which (Glu-208) corresponds to the \textit{S. aureus} protease...
Table II

Sequence homologies of myosin in rabbit skeletal (a), chicken skeletal (b), nematode (c), rat embryo skeletal (d),
dictyostelium (e), acanthamoeba II (f), acanthamoeba 1B (g), chicken gizzard (h)

A) NH2-terminal sequence of the NH2-terminal 22-kDa K fragment (obtained by nucleotide-induced chymo-
tryptic cleavage); B) NH2-terminal sequence of the central 50-kDa fragment (obtained by trypsin cleavage); C) 
NH2-terminal sequence of the COOH-terminal 25.5-kDa fragment (obtained by S. aureus V8 protease cleavage).

Structures are identical in at least two myosin sequences are capitalized. Sequences composed of at least three
adjacent amino acids and that are homologous in at least two myosin sequences are capitalized and red colored. X
represents gaps inserted to maximize homology. Numbers refer to the amino acid positions where proteolysis takes
place as detailed in Fig. 3. Dashed line corresponds to unknown sequences. Sequence homologies were calculated
according to Staden (40).

|        | Me                           |             |
|--------|------------------------------|-------------|
|        | dMAVFGEAPA**K**EKRK**E**RIEAQQKPA**D**AKKnSVFVaxDPKESYVAATvQSR**G**GKV**x**TVKT | (a)         |
|        | EMAaFGEAPA**K**EKRK**E**RIEAQQKPA**D**AKKnSFVVhxPKESFVKTQGK**G**GKV**x**TVKT | (b)         |
|        | HehekDpgwq**Y**LrteqvlEdQsKPyDsKKnWViFxEgY1AAeltat**K**GDqVxxTivT | (c)         |
| A)     | EMeVFGiAAPfL**R**EKRRI**E**AQnPFDAtYcFVvxDsKeYaK**G**Kl**S**qGK**V**xxTVvT | (d)         |
|        | HdrtsDyhkk**Y**Lkvk$q$dsdlfktvxDxKrYiWyntDPKErdsyegeivsetsdsxsstf | (e)         |
|        | sAKKlVWVPsexKhGF**eA**asIKeeK**G**DeVxxTV | (h)         |

|        | 204 213             |             |
|        | I**T**GDxx**K**KKEatSGKMQGTL**E**DQIItANPLLEAFGNAKqVRN | (a)         |
|        | IAASGe**K**KKEeaxSGKMQGTL**E**DQIItANPLLEAFGNAKqVRN | (b)         |
|        | vgaSqqeG**a**eVdPknKv**T**LEDQIVqNTNP**L**EAFGNAKqVRN | (c)         |
| B)     | ATGD1A**K**KxxSxMK**G**TLEDQIItANPLLEAFGNAKqVRN | (d)         |
|        | AxxGxnqanGSxxxxxxxGvLEQQILQANPLLEAFGNAKTTTRN | (e)         |
|        | tAiag**e**eGxxxxxxxlLEQQ**L**e**f**NPL**E**AFGNAKTT**N** | (f)         |
|        | AAvS**G**eatGdvxxxxxxxxxLLEAFGNAK**T**fR | (g)         |
|        | --kDxxxtiSqqPtf$$t$$yGeL**e**kQLQ**A**NP**L**EAFGNAKTV | (h)         |

|        | 202                   |             |

|        | 269 363 640          |             |
|        | A**f**L**F**AgxxxxxxxAg**A**cEGGGGKKGGKKK**G**SSF**Q**TVSA | (a)         |
|        | AllFATYxxxxxxx**x**geAEGGGKKGGKKK**G**SSF**Q**TVSA | (b)         |
|        | vei**q**wqytteAAaA**K**e**G**GGG**G**KKK**G**SxGxF**V**sm | (c)         |
| C)     | AhlyAt**f**xxxxxAxxttxdadG**k**K**v**a**K**K**G**SSF**Q**TVSA | (d)         |
|        | xxxxxv**K**lfnpiA**s**rxxxxxxxKKGAnF**i**T**V**a**A | (e)         |
|        | --**e**AKEvaxtS**K**KxпttAgFkixxx | (f)         |
|        | --**e**AGmEs**s**s**s**StK**K**GxxmFrT**V**gq | (h)         |

The site identified earlier (29). On the basis of the strong homology of the smooth and rabbit skeletal 50-kDa sequences from
positions 216 to 238 (Table II), we have positioned, for comparison in Fig. 2, the putative smooth connector II region
which would encompass the NH2-terminal 10-residue portion of the smooth tryptic 50-kDa sequence. The skeletal and
smooth connector II sequences are quite different.

Structure of the NH2-terminal Smooth S-1 Heavy Chain Region—Nucleotide binding to skeletal myosin S-1 is known
to induce a new proteolytic cleavage at the heavy chain NH2
terminus that is reflected by the rapid degradation of the NH$_2$-terminal tryptic 27-kDa fragment into a stable 22-kDa derivative (15, 36, 37). Fig. 3 illustrates the ATP-dependent proteolytic production of a homologous 22-kDa peptide from the smooth tryptic (29 kDa-50 kDa-25 kDa)-S-1. However, the proteolytic processes on the smooth and skeletal S-1 exhibit striking differences. To hydrolyze the Mg$^{2+}$-ATP-gizzard S-1 complex, we used several proteases including trypsin, clostripain, subtilisin, and chymotrypsin; only the latter protease was effective giving rise only to the breakdown of the NH$_2$-terminal 29-kDa fragment into a 22-kDa product. In contrast, the skeletal 25 kDa-22 kDa reaction is catalyzed by various proteases such as trypsin, subtilisin, and elastase (14) but not at all by chymotrypsin as assessed in separate experiments. Furthermore with the skeletal S-1, there is an additional cleavage occurring concomitantly at the COOH terminus of the 50-kDa fragment which is converted into a 45-kDa species (38). No such reaction was observed with the smooth S-1.

Previous amino acid sequence studies performed on the skeletal tryptic 22-kDa peptide (15, 37) showed the clip site to reside at Arg-23/Ile-24. We have isolated the smooth 22-kDa fragment by a combination of gel filtration and reverse-phase HPLC. In the heavy chains from rabbit and chicken myosins. However, according to Maita et al. (54) the position was recently described as a tryptophan (Trp). It is therefore located 8 residues apart from the skeletal trypsin site, and it is consistent with the protease specificity. On the other hand, it is adjacent to Lys 34. In the heavy chains from rabbit and chicken skeletal S-1 this residue is monomethylated (11, 39); however, no PTH-monomethyl lysine was identified in the chicken gizzard myosin (b) during the additional trypsic cleavage. The data are expressed as semilogarithmic plots of the activities remaining after incubation with trypsin during the times indicated.

Table III

| Amino acid               | Peptides from trypsin split gizzard S-1 |
|--------------------------|----------------------------------------|
|                          | Central 50 kDa | NH$_2$-terminal 29 kDa | COOH-terminal 25 kDa |
| 3-Methyl-histidine       | 0             | 0                       | 0                     |
| N-Trimethyl-lysine       | 1.0-1.1       | 1.0-1.1                 | 0                     |
| Monomethyl lysine        | 0             | 0                       | 0                     |

Values in parenthesis are for the corresponding central (50 kDa), NH$_2$-terminal (27 kDa), and COOH-terminal (20 kDa) fragments from trypsin split skeletal S-1 (25).

The ATP-dependent chymotryptic cleavage of gizzard (29 kDa-50 kDa-25 kDa)-S1. SDS-polyacrylamide gel electrophoresis pattern at different times of degradation for the gizzard trypsin cut (29 kDa-50 kDa-25 kDa)-S1 during further treatment with trypsin under the conditions described under "Experimental Procedures." The 22-kDa peptide produced was further isolated by reverse-phase HPLC.

FIG. 3. The ATP-dependent chymotryptic cleavage of gizzard (29 kDa-50 kDa-25 kDa)-S1. SDS-polyacrylamide gel electrophoresis pattern of the trypsin-cut (29 kDa-50 kDa-25 kDa)-S1 before (A) and after (B) chymotryptic digestion in the presence of Mg$^{2+}$-ATP under the conditions outlined under "Experimental Procedures." The 22-kDa peptide produced was further isolated by reverse-phase HPLC.

FIG. 4. Correlation between the selective tryptic fragmentation of the 50-kDa fragment and the inactivation of the gizzard S-1 ATPase. A, SDS-polyacrylamide gel electrophoresis pattern of the 50-kDa fragment and the inactivation of the gizzard S-1 ATPase. A, comparative K$^+$-ATPase activities of the tryptic subfragment-1 from rabbit skeletal myosin (a) and chicken gizzard myosin (b) during the additional trypsic cleavage. The data are expressed as semilogarithmic plots of the activities remaining after incubation with trypsin during the times indicated.

Structural Homologies between Smooth and Skeletal Myosin Heads
residues are present and are distributed over the 29- and 50-kDa fragments similarly to the skeletal S-1 (11, 25, 39) indicating that these particular residues are conserved in the smooth S-1 heavy chain sequence.

**Relationship between the Structural Integrity of the 50-kDa Heavy Chain Domain and the Expression of the Smooth S-1 Enzymatic Activity**—We have previously determined that the skeletal trypsin split S-1 could express full K$^{+}$EDTA- or Ca$^{2+}$-dependent ATPase activities while its actin-dependent Mg$^{2+}$-ATPase is abolished after the cleavage of the 50 kDa-20 kDa junction (12). The same properties were displayed by the smooth trypsin split S-1 except that its actin-activated ATPase remained unaffected (4-6). Herein we have investigated the stability of each of the smooth trypic fragments when the S-1 derivative is further digested with a moderate trypsin to S-1 weight ratio (1:100) at pH 8.0, 25°C. Starting with the trypsin split smooth (29 kDa-50 kDa-25 kDa)-S-1 as presented in Fig. 4A, we found that the two heavy chain fragments of M$\_2$29,000 and 25,000 were stable during the course of the proteolysis, but the 50-kDa fragment was progressively degraded, first into a 45-kDa fragment and then into small peptides.

Concomitantly to the proteolytic breakdown of the 50-kDa fragment, there is a progressive abolition of the S-1 K$^{+}$-ATPase (and also of the Ca$^{2+}$-ATPase) activity as illustrated in Fig. 4B (curve b). Comparatively in the same Fig. 4B (curve a) is presented the good stability of the rabbit skeletal trypsin split (27 kDa-50 kDa-20 kDa)-S-1 that we have used under the same conditions as a control.

The results suggest that the smooth central 50-kDa segment possesses particular conformational features that are revealed by its greater susceptibility to proteolysis under nondenaturing conditions as compared to the skeletal 50-kDa domain.

**CONCLUSION**

Our sequence studies show that the two linker regions on either side of the central 50-kDa segment of the smooth S-1 heavy chain contain the major sites attacked by specific proteases. This was also observed in the case of the rabbit skeletal S-1 (41). On the other hand the production of the three heavy chain fragments upon digestion of the gizzard S-1 with several proteases of different specificities was recently briefly reported (42). Thus, the smooth myosin S-1 heavy chain seems also to comprise the three spatially distinct domains encountered in the skeletal counterpart, suggesting an evolutionary conserved domain organization of the myosin head structure. The proposed domain architecture of the smooth S-1 heavy chain is further supported by the proteolytic behavior of its 50-kDa fragment. In the skeletal myosin head, this region is known to bear an intrinsic structural instability which contrasts with the stability of the two other heavy chain segments (15); this property is revealed by the slow and limited degradation of the 50-kDa peptide into a stable 45-kDa product without an apparent influence on the enzymatic activities. However, the 50-kDa region is also capable of an independent proteolytic fragmentation which leads to a concomitant loss of the ATPase function, but which occurs only under unfolding conditions, such as mild thermal denaturation (43, 44), treatment with an organic solvent (45), or chemical modification of some thiol groups (46). From this property it has been inferred that the expression of the skeletal S-1 ATPase is intimately related to the structural integrity of the 50-kDa segment. In the present work, we found that the smooth 50-kDa domain exhibits a naturally enhanced vulnerability to digestion by trypsin and its selective degradation is accompanied by ATPase inhibition. The latter event was expected since the 50-kDa segment was recently shown to be involved in nucleotide binding to the smooth S-1 (47). Protection by Mg$^{2+}$-ATP against the selective trypic cleavage of the 50-kDa fragment was also briefly outlined in the recent report for the gizzard myosin S-1 (42).

Most importantly, the natural protease sensitivity of the smooth 50-kDa domain is also displayed by the homologous region of the heads from two other slow type myosins, the cardiac V3 isoenzyme (48) and the skeletal slow muscle myosin (49). Because the 50-kDa heavy chain segment seems to be involved in the intercommunication between the myosin ATPase and actin sites (15, 50, 51), some unique variable structural features in this domain would determine at least part of the differences observed in the kinetic properties of the actomyosin ATPases from the fast and slowly contracting muscles (1) and would contribute, in general, to the functional diversity of the myosins.

The protease-sensitive NH$_2$-terminal portion of the smooth S-1 heavy chain represents another part of this subunit whose structure could be related to the functional specificity of this myosin. In the skeletal S-1, monoclonal antibodies have located this particular region in the middle of the head close to the adenine binding site, and their interaction alters the S-1 ATPase activities (52). In the absence of nucleotide, the chymotryptic proteolysis at the smooth NH$_2$-terminal segment still occurs but at a much slower rate. This natural cleavage is abolished by light chain phosphorylation and further enhanced by nucleotide binding (20). Both opposite effects reflect different conformational changes taking place in the NH$_2$-terminal heavy chain region. Because light chain phosphorylation in the smooth myosin is now known to affect kinetic steps of the actin-S-1 ATPase reaction, rather than the actin-S-1 interaction (53), the influence of the regulatory light chain could be transmitted to the ATPase site through a specific conformational change within the vicinal NH$_2$-terminal segment. The communication between the latter region and the ATPase site is illustrated not only by the nucleotide-induced acceleration of the heavy chain cleavage but also by the immediate and parallel Mg$^{2+}$-ATPase inhibition which was reported to result from an increased binding of Mg$^{2+}$-ADP to the ATPase site (22). In the skeletal S-1, the tryptic cleavage at Arg-24 was found to alter ATP binding with the suppression of its ability to protect the S-1 against thermal denaturation (37); also it was recently observed that the truncated skeletal S-1 looses progressively its enzymatic activity upon storage. Table III shows a remarkable diversity of the NH$_2$-terminal sequences of the various myosin heavy chains; these structural changes could provide additional molecular features necessary for the specific expression and modulation of the enzymatic properties of the myosins.

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