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Interaction between Stretch of Residues 633-642 (Actin Binding Site) and Nucleotide Binding Site on Skeletal Myosin Subfragment 1 Heavy Chain†

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ABSTRACT: Using a complementary sequence or antipeptide to selectively neutralize the stretch of residues 633-642 of skeletal myosin heavy chain, we recently demonstrated that this segment is an actin binding site operating in the absence as in the presence of nucleotide and that this stretch 633-642 is not part of the nucleotide binding site [Chaussepied & Morales (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 7471-7475]. In the present study, we determined that the covalent cross-linking of the antipeptide to the stretch 633-642 [induced by 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide] does not alter the overall polypeptide conformation since no changes were observed on the far-ultraviolet CD spectra and thiol reactivity measurements. The presence of the antipeptide did not influence significantly the enhancement of tryptophan fluorescence induced by ATP·Mg^{2+} or ADP·Mg^{2+} binding to the myosin head (S1) nor did it on the ATP·Mg^{2+}-induced tryptic proteolysis of S1 heavy chain. Moreover, fluorescence quenching studies, using acrylamide and the analogue, 1,N^6-ethenoadenosine 5′-triphosphate, indicated that the nucleotide bound to antipeptide–S1 complex has an accessibility to the solute quencher close to that observed when it is bound to native S1. Additionally, neutralization of the stretch 633-642 of the S1 heavy chain by the antipeptide did not influence the stabilization of the Mg^{2+}·ADP·sodium vanadate–S1 complex. On the other hand, experiments using antipeptide-induced protection against the cleavage of the S1 heavy chain by Arg-C protease demonstrated that the presence of Mg^{2+}·ADP·sodium vanadate in the S1 nucleotide site did not affect the interaction of the antipeptide with the stretch of residues 633-642. Together, these results show that the occupancy of the stretch of residues 633-642 by the antipeptide does not affect the overall structure or the ATP binding and hydrolysis properties of skeletal myosin head and that the reactivity of the stretch 633-642 is not significantly dependent on the presence of nucleotide in the active site. Because the stretch 633-642 seems available to actin interaction in the presence of nucleotide and because blocking it strongly decreases actin interaction with S1–nucleotide complex, it is proposed that the stretch of residues 633-642 represents an essential constituent of the so-called "weak" actin–S1 interface.

Muscle contraction, as well as cell movement processes based on the actomyosin system, take place through a cyclic interaction of actin and ATP with the myosin globular head (S1)[1] (Huxley, 1963). Although actin and ATP binding sites are structurally separated in the myosin molecule (Barany & Barany, 1959), these two ligand binding sites are known to be strongly interdependent. Thus, the binding of the polyphosphate moiety of ATP to S1 weakens the interaction between S1 and actin (Szent Gyorgyi, 1947), and the binding of actin to S1 alters the interaction between S1 and nucleotide, resulting in an activation of the S1 ATPase activity (Eisenberg & Moos, 1968; Highsmith, 1976).

Together, these experimental data led Morales and Botts (1979) to postulate that energy transduction in the myosin molecule is based on structural communication between the actin and the ATP binding sites. This hypothesis has recently been strengthened by kinetic evidence in which the ATP binding and hydrolysis described as a several-step process (Lymn & Taylor, 1971; Trentham et al, 1976) are associated with a two-step actin binding process (Eisenberg & Hill, 1985; Geeves et al., 1984; Coates et al., 1985). In the first step, actin is bound weakly to S1 (possibly bound to ATP·S1 or ADP·P_i·S1 intermediates), and during the second step, the acto–S1 complex isomerizes to reach finally the "rigor" acto–S1 complex (containing either bound ADP or no bound nucleotide).

Unfortunately, none of these observations have yet been interpreted in terms of structure, since neither actin nor ATP binding sites are definitively located in the myosin head. With the exception that the N-terminal part of the alkali light chain A1 can, under certain conditions, interact with actin (Henry et al., 1985), both binding sites have been located on the S1 heavy chain (Wagner & Giniger, 1981; Sivaranakrishnan &

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[1] Abbreviations: S1, subfragment 1; S1(A2), isoenzyme of S1 with alkali light chain 2; Vi, sodium vanadate; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; DTE, dithioerythritol; ε-ATP, 1,N^6-ethenoadenosine 5′-triphosphate; $NaDodSO_4$, sodium dodecyl sulfate; M_r, molecular weight; kDa, kilodalton; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); TNB, 5-thio-2-nitrobenzoic acid.

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Burke, 1982). Experiments using photoaffinity ATP analogues have partially located the adenosine moiety binding site near the residue Trp_{130} (Okamoto & Yount, 1985), while a sequence analogous to other nucleotide binding proteins, located at the stretches 149–159 and 178–184, may also interact with the base moiety (Walker et al., 1982; Fry, 1986). Additionally, by use of two different photoactivable analogues, the ribose part of ATP was found to interact either to Ser_{324} (Mahmood & Yount, 1984) or to Trp_{130} (Sutoh, 1987). Finally, the phosphoryl group subsite responsible for the actin dissociation was indirectly located within the stretch of residues 561–641 (Chaussepied et al., 1986a). More recently, by use of the ADP·Vi·S1 complex as an ADP·P_i·S1 analogue, the Vi-induced photocleavage at the residue Ser_{180} revealed another possible subsite for the polyphosphate chain (Grammer et al., 1988; Cremo et al., 1988).

The actin contact points, so far identified along the S1 heavy chain, have been separated into two different classes. The first class, revealed by direct binding studies between actin and peptides of S1 heavy chain, is located in the proximity of residues Cys_{697} and Cys_{707} (Katoh et al., 1985; Suzuki et al., 1987). However, this class of actin sites, identified in skeletal and cardiac myosin S1 (Suzuki & Morita, 1987), is apparently absent in scallop myosin S1 (Castellani et al., 1987; Labbé et al., 1988). The second class of actin binding sites, revealed by proteolytic cleavage and by chemical cross-linking experiments, involves two regions located at 18–22 and 37–45 kDa from the C-terminus of S1 heavy chain (Mornet et al., 1981; Sutoh, 1983; Chaussepied et al., 1983). Although this second class of actin sites is widely found among the different types of muscle (Marianne-Pépin et al., 1985; Atkinson et al., 1987; Labbé et al., 1988) and is revealed by cross-linkers of various reactivities (Bertrand et al., 1988), this class of sites is now believed to represent only a minor determinant of the acto–S1 interface in the rigor complex (Méjean et al., 1986; Miller et al., 1987). Moreover, while the first class of actin binding sites was postulated to be highly sensitive to the presence of nucleotide (Katoh & Morita, 1984), the second class of sites seems to exist in the absence as well as in the presence of nucleotide (Arata, 1984; Chen et al., 1985).

More recently, the presence of an actin binding site in the stretch of residues 633–642 (part of the subsite located at 18–22 kDa from the C-terminus of the S1 heavy chain) was directly confirmed by selectively blocking this sequence of S1 heavy chain with a complementary sequence or "antipeptide" (Chaussepied & Morales, 1988). Because the "antipeptide technology" leads to the specific modification (neutralization) of an actin binding site, we have further characterized the structural relationship existing between the stretch of residues 633–642 and the rest of the molecule and more particularly the nucleotide binding site. The results suggest that the stretch of residues 633–642 of S1 heavy chain is accessible to antipeptide, i.e., actin, even when ADP·P_i is present in the nucleotide binding site. Therefore, it is proposed that this actin binding site is an important determinant of the so-called "weak" acto–S1 interaction.

Materials and Methods

Chemicals. α-Chymotrypsin and trypsin treated with L-1-(tosylamino)-2-phenylethyl chloromethyl ketone were purchased from Worthington. Arginase C (Arg-C) protease was obtained from Boehringer Mannheim. EDC, ATP, and ADP were supplied by Sigma. ε-ATP was from Molecular Probes. Stock solutions of orthovanadate (VO_4^{3-}) were prepared according to the method of Goodno (1979). *N*-[7-(Dimethylamino)-4-methylcoumarinyl]maleimide was from Polyscience. All other chemicals were of the highest analytical grade.

Proteins. Myosin was prepared from rabbit skeletal muscle according to the method of Offer et al. (1973). Myosin subfragment1–alkali light chain 2 isoenzyme [S1(A2)] was purified as described by Chaussepied and Morales (1988).

Purification of Antipeptide and Antipeptide–S1(A2) Complex. The antipeptide Cys-Gly-Gly-Asp-Asp-Gly-Gly-Asp-Asp-Asp-Gly was synthesized and HPLC-purified as recently described (Chaussepied & Morales, 1988). The antipeptide was covalently attached to S1(A2) by the EDC cross-linking procedure (Chaussepied & Morales, 1988); the ammonium sulfate precipitation was replaced by a concentration step on Amicon cells (using PM30 membrane). The antipeptide–S1(A2) complex eluted from the last ion-exchange column was extensively dialyzed against 30 mM Tris-HCl, 0.4 mM DTE, and 0.1 mM NaN_3, pH 7.8, clarified by centrifugation for 60 min at 150000*g*, filtered through Millipore membrane (pore size, 0.45 μm), and stored on ice for less than 5 days. Stoichiometry and specificity of the cross-linking reaction were evaluated by using DACM–antipeptide and by performing peptide mapping experiments (Chaussepied & Morales, 1988).

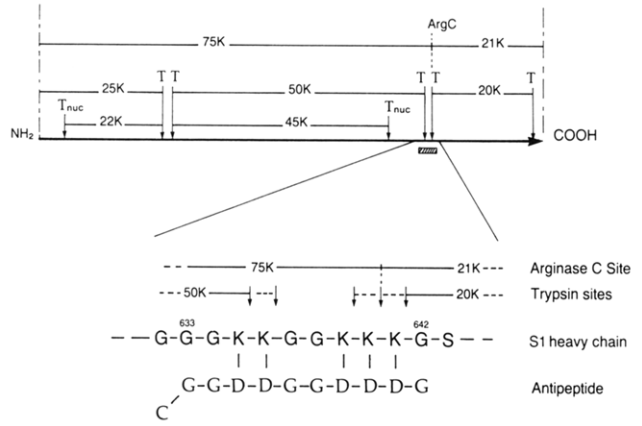
Protein Concentrations. Protein concentrations were optically measured by assuming $A^{1\%}_{280nm} = 7.5$ cm^{-1} for native S1(A2) (M_r = 115000), EDC-treated S1(A2) (M_r = 115000), and antipeptide–S1(A2) (M_r = 116000).

Preparation of ADP·Vi·S1(A2) Complex. ADP·Vi complex was trapped in the active site of S1(A2) derivatives as described by Goodno (1979) and Chaussepied et al. (1986b) with the following modifications. S1(A2), EDC-treated S1(A2), or antipeptide–S1(A2) (50 μM) was mixed with 2 mM ADP and 3 mM Vi in 50 mM Tris-HCl and 2.5 mM $MgCl_2$, pH 8.0, for 30 min at 20 °C in the dark. The excess of free nucleotide was then removed by G-100 chromatography (1.5 × 20 cm), equilibrated at 4 °C with 30 mM Tris-HCl and 0.4 mM DTE, pH 8.0. The yield of trapping was evaluated by measuring the S1(A2) K^+EDTA-ATPase activity and by assuming a direct correlation between the amount of S1(A2)-containing ADP·Vi and the extent of K^+EDTA-ATPase inhibition (Goodno & Taylor, 1982).

Proteolysis Experiments. Cleavage of EDC-treated S1(A2) or antipeptide–S1(A2) (1 mg/mL) by trypsin was performed in 30 mM Tris-HCl, 0.1 mM NaN_3, and 0.4 mM DTE, pH 8.0, for 45 min at 25 °C with a trypsin/S1 weight ratio of 1/100° and of 1/40° in the absence and in the presence of 4 mM ATP·Mg^{2+}, respectively. Proteolysis of S1 heavy chain (1 mg/mL) by Arg-C protease was achieved in 30 mM Tris-HCl, 0.1 mM NaN_3, and 0.4 mM DTE, pH 8.0 for 60 min at 25 °C by using 40 units of Arg-C protease/mg of protein. When Arg-C proteolysis was performed on S1(A2) or Mg^{2+}·ADP·Vi·S1(A2) complex in the presence of various amounts of antipeptide, S1(A2) (5 μM) was mixed with antipeptide in 5 mM Tris-HCl, 0.1 mM NaN_3, and 0.4 mM DTE, pH 8.0 (the other conditions were unchanged). All proteolytic reactions were terminated by incubating the samples for 5 min in 3 volumes of a boiling solution of 50 mM Tris-HCl, 2% (w/v) $NaDodSO_4$, 1% 2-mercaptoethanol, and 50% (v/v) glycerol, pH 8.0.

Circular Dichroism Spectrum. CD spectra of S1(A2) derivatives were measured with a Jasco J-500A spectropolarimeter under constant nitrogen flush at 20 °C. Spectra of S1(A2) derivatives (0.23 mg/mL) in 20 mM Tris-HCl, pH 7.8, prefiltered through Millipore membrane (pore size, 0.45 μM) were recorded from 185 to 250 nm. Percentage of α helicity was calculated from the spectra by the method of Chang et al. (1978).

Scheme I: Amino Acid Sequence and Localization of the Antipeptide on the Skeletal S1 Heavy Chain[a]



[a] The proteolytic sites and the size of the major peptides generated by Arg-C protease (ArgC) and trypsin in the absence (T) and in the presence of nucleotide (Tnuc) are also indicated.

Thiol Titration. The reactivity of the thiol group of S1(A2) derivatives was estimated by monitoring at 410 nm the time course of the reaction of the protein (7 μM) with DTNB (60-fold excess) in 100 mM $NaHCO_3$, pH 8.3 at 20 °C. The reactive SH groups/S1(A2) ratio was then calculated by assuming ϵ_{410nm} = 13 600 M^{-1} cm^{-1} for TNB (Ellman, 1959).

Spectral Procedures. Fluorescence measurements were carried out on an SLM Model 8000 fluorometer, thermostated at 20 °C. The interaction of ϵ-ATP with S1(A2) derivatives was studied by acrylamide quenching experiments (Ando et al., 1982). ϵ-ATP (8 μM) in 30 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.4 mM DTE, and 0.1 mM NaN_3, pH 7.8, was mixed at 20 °C with increasing amounts of filtered acrylamide in the absence or in the presence of S1(A2) derivatives (20 μM).

The Stern–Volmer quenching constant (K_{SV}) was determined from the slope of the plot $F_0/F = 1 + K_{SV}[Q]$, where F_0 and F are fluorescence intensities in the absence and in the presence of acrylamide, respectively, and [Q] is the concentration of acrylamide. This equation is valid in the range of acrylamide used (0–105 mM) since only one fluorescent component exists (Rosenfeld & Taylor, 1984).

$NaDodSO_4$/PAGE and the densitometric scanning of the gels (5–18% acrylamide gradient) were carried out as previously described (Chaussepied et al., 1986c).

ATPase Activities. K^+EDTA- and Ca^{2+}-dependent ATPase activities were measured at 25 °C as described by Chaussepied and Morales (1988).

Results

It has been shown that the interaction of the antipeptide with the stretch of residues 633–642 protects the S1 heavy chain against Arg-C proteolysis and that this property can be routinely used to determine the antipeptide specificity (Chaussepied & Morales, 1988; Scheme I). The gel electrophoretic pattern presented in Figure 1 revealed that, after EDC cross-linking of the antipeptide to the S1(A2) heavy chain, 82% of the S1 heavy chains (95-kDa band) were protected against Arg-C cleavage (lanes A and B), while 94% of the control EDC-treated S1(A2) heavy chains were cleaved (lanes a and b). These results are in good agreement with previous peptide mapping studies which showed that, under the same experimental conditions, 85% of the stretch of residues 633–642 were selectively modified and that 20–25% of the antipeptides interacted less specifically with the S1 heavy chains (Chaussepied & Morales, 1988). Although the peptide mapping studies used fluorescently labeled antipeptide, we found that the presence and the nature of the fluorescent probe attached to the residue Cys did not influence the interaction between antipeptide and the S1(A2) heavy chain (Kasprzak et al., 1989).

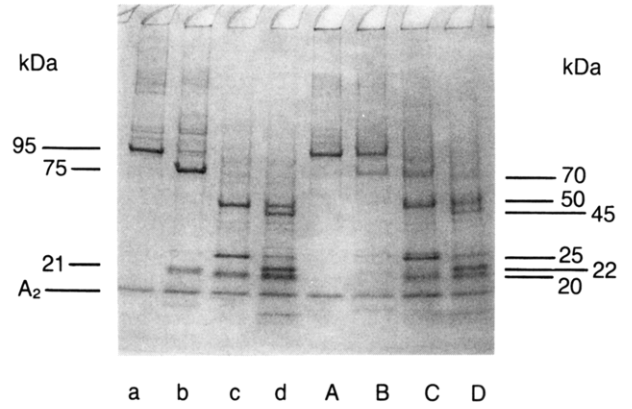


FIGURE 1: $NaDodSO_4$/polyacrylamide gel comparing the proteolytic degradation of EDC-treated S1(A2) and of antipeptide:S1(A2) complex. EDC-treated S1(A2) (lane a) or antipeptide–S1(A2) complex (lane A) were digested by Arg-C protease (lanes b, B) and by trypsin in the absence (lanes c, C) or in the presence of ATP (lanes d, D) as described under Materials and Methods.

Conformational Properties of the Antipeptide–S1(A2) Complex. To assess whether the overall conformation of S1(A2) is affected by antipeptide attachment to its target sequence, we first compared its far-ultraviolet CD spectrum with those obtained with native and EDC-treated S1(A2). The α-helix contents determined by the method of Chang et al. (1978) were 38.0, 33.4, and 34.0% for native S1(A2), EDC-treated S1(A2), and antipeptide–S1(A2), respectively. The slight difference between the value of helicity for native S1(A2) (38.0%) and the value reported for the mixed population S1(A1),(A2) (40.8%; Chaussepied et al., 1986b) can be explained either by the use of different methods of calculation or by structural changes induced by the extra 41 residues present in the alkali light chain A1 subunit. Although the slight decrease of α-helical structure induced by EDC treatment is not yet explained, it is important to note that no significant difference in the S1(A2) secondary structure was observed before and after covalent attachment of the antipeptide.

To complement these spectroscopic data, we compared the chemical thiol reactivity of antipeptide–S1(A2) with that of control S1(A2) derivatives in the DTNB reaction. Figure 2 shows the amount of modified SH group per S1(A2) derivative versus time of DTNB reaction. Data obtained for EDC-treated S1(A2) and antipeptide–S1(A2) were not distinguishable in the range of time used, although the SH groups of native S1(A2) seemed slightly more reactive (with a plateau value of 0.4 SH group/S1 higher). The difference in SH group reactivity between EDC-treated and native S1(A2) derivatives may explain the change in ATPase activities also observed after EDC treatment of S1(A2) (Chaussepied & Morales, 1988) since the modification of a reactive cysteinyl (Cys_{707} of the S1 heavy chain) is also known to affect in a similar way the S1 ATPase activities (Sekine & Kielly, 1964).

Interaction of Nucleotide with the Antipeptide–S1(A2) Complex. To characterize further our previous finding that antipeptide binding to S1 heavy chain does not alter the Ca^{2+}-, K^+-, and Mg^{2+}-dependent ATPase activities of S1(A2) (Chaussepied & Morales, 1988), we examined in more detail the effect of blocking the stretch of residues 633–642 on the critical nucleotide binding to the S1 heavy chain.

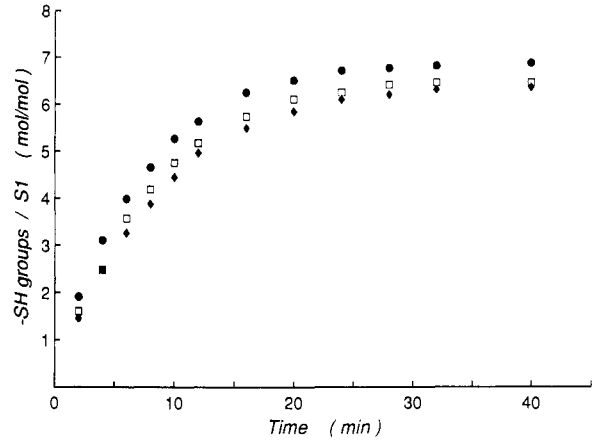


FIGURE 2: Comparative time course of the SH groups titration with DTNB. Native S1(A2) (●), EDC-treated S1(A2) (□), and antipeptide–S1(A2) complex (◆) were reacted with DTNB at 20 °C as described under Materials and Methods. The number of reactive SH groups was determined by monitoring at 410 nm the release of TNB groups and by assuming that 1 mol of TNB was released/mol of reactive SH group.

Table I: Enhancement of Tryptophan Fluorescence Induced by Nucleotide Binding to S1(A2) Derivatives or to the Antipeptide–S1(A2) Complex

protein	fluorescence enhancement[a] (%)	
	+ATP·Mg^{2+}	+ADP·Mg^{2+}
native S1(A2)	29.2 (332)[b]	4.3 (333)
EDC-treated S1(A2)	16.7 (333)	2.7 (333)
antipeptide–S1(A2)	17.3 (332)	3.4 (333)

[a] Percent of fluorescence enhancement at the maximum emission wavelength was calculated from the emission spectra of 1.5 μM S1(A2) derivatives measured in the absence and in the presence of 150 μM of ATP or ADP in 30 mM Tris, 2.5 mM $MgCl_2$, and 0.4 mM DTE, pH 7.8, 20 °C, (excitation = 295 nm). [b] Maximum emission wavelength is given in parentheses.

In a first approach, we investigated the effect of nucleotide binding on the tryptophan fluorescence properties of the different S1(A2) derivatives. The fluorescence spectrum of antipeptide–S1(A2) exhibited features very similar to those exhibited by native or EDC-treated S1(A2) (data not shown; Werber et al., 1972). Moreover, the binding of Mg^{2+}·ATP and of Mg^{2+}·ADP to the S1(A2) derivatives induced an enhancement of the tryptophan fluorescence emission spectra without shifting of the maximum emission wavelength (Table I). Although the relative fluorescence enhancement was of lower extent with antipeptide–S1(A2) than with native S1(A2), regardless of the nature of bound nucleotide, this apparent change in the internal response to nucleotide binding seemed due to EDC treatment itself since values obtained for EDC-treated S1(A2) and for antipeptide–S1(A2) were within experimental error.

A proteolytic approach was also used as a probe of nucleotide binding to S1 (Scheme I; Hozumi, 1983; Mornet et al., 1985). As shown in Figure 1, degradation of antipeptide–S1(A2) by trypsin in the absence of nucleotide produced 70-, 50-, 25-, and 20-kDa fragments (lane C). While the three last fragments were normally found with native and EDC-treated S1(A2) (lane c), the 70-kDa fragment results from the partial protection of the 50- and 20-kDa fragment junction with which the antipeptide is known to interact. When the digestion was performed in the presence of 4 mM Mg^{2+}·ATP, the nucleotide-induced degradation of 50- into 45-kDa fragments and of 25- into 22-kDa fragments were identical with those of EDC-treated S1(A2) and with antipeptide–S1(A2) (lanes d and D).

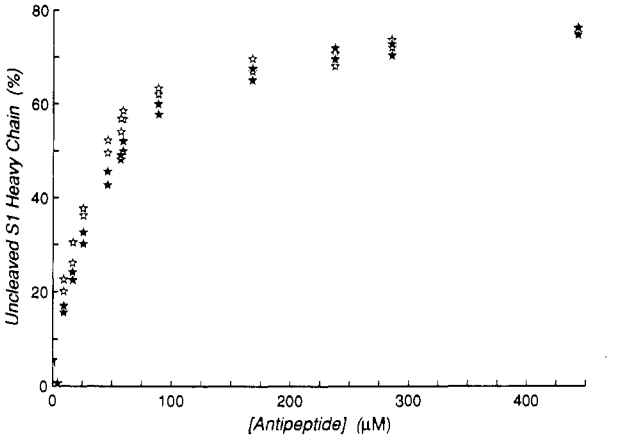


FIGURE 3: Effect of Mg^{2+}·ADP·P_i trapping on the antipeptide-induced protection of S1 heavy chain against Arg-C proteolysis. Arg-C proteolysis of S1(A2) (☆) and Mg^{2+}·ADP·Vi–S1(A2) complex (★) was performed in the presence of various amounts of antipeptide in 5 mM Tris-HCl, 1 mM NaN_3, and 0.4 mM DTE, pH 8.0 at 20 °C, as described under Materials and Methods. The amount of uncleaved S1 heavy chain was determined by densitometry of the 95-kDa band on $NaDodSO_4$ gel electrophoresis.

Additionally, the antipeptide–S1(A2) derivative was able to trap the Mg^{2+}·ADP·Vi complex in the ATPase site with a yield of 76%, compared to 82 and 84% of nucleotide trapped in EDC-treated S1(A2) and native S1(A2), respectively.

Finally, we used the method of fluorescence quenching by acrylamide to analyze the accessibility of the fluorescent ε-ATP analogue bound to antipeptide–S1(A2) (Ando et al., 1982). The quenching constants calculated from the slope of the Stern–Volmer plots were 63.0, 6.5, 6.4, and 5.3 M^{-1} for unbound ε-ATP, ε-ATP bound to antipeptide-S1(A2), EDC-treated S1(A2), and native S1(A2), respectively. These results showed that ε-ATP bound to antipeptide–S1(A2) or bound to EDC-treated S1(A2) is equally shielded from solvent, although slightly less than when it is bound to native S1(A2).

Interaction of the Antipeptide with the Mg^{2+}·ADP·Vi·S1(A2) Complex. Because the reversible interaction between antipeptide and S1(A2) is highly ionic strength dependent (Chaussepied & Morales, 1988), the interaction between antipeptide and the nucleotide·S1(A2) complex could not be studied in the presence of free nucleotide but only by using purified Mg^{2+}·ADP·Vi·Si(A2) complex. Moreover, the low stability of this complex at zero ionic strength forced us to study the antipeptide S1(A2) interaction in 5 mM Tris-HCl, 0.1 mM NaN_3, and 0.4 mM DTE, pH 8.0, by evaluating the amount of S1(A2) heavy chain protected against Arg-C cleavage by various amounts of antipeptide. Determination of the amount of trapped nucleotide at the end of the proteolysis showed that under these experimental conditions more than 90% of the trapped nucleotide was still present in the S1(A2) active site, regardless of the amount of antipeptide added or attached to its target sequence. In Figure 3, the results of duplicate binding experiments showed that the antipeptide-induced protection of S1 heavy chain was roughly identical in the absence and in the presence of trapped nucleotide in the S1 active site, although the protection seemed slightly more efficient at low antipeptide concentration in the absence of trapped nucleotide.

Discussion

Assuming that the binding of antipeptide to the stretch 633–642 mimics the binding of actin to the same stretch of S1 heavy chain (Chaussepied & Morales, 1988), we have first

evaluated the degree of structural changes induced by blocking this particular actin binding site.

It was found that the covalent attachment of the antipeptide to the residues 633–642 does not affect the overall structure of S1 as judged by far-ultraviolet CD spectrum or by thiol reactivity. The absence of change in the α-helical content of S1(A2) upon blocking of this actin binding site by the antipeptide is consistent with a recent neutron scattering study that revealed that less than 20% of the S1 mass could be deformed by interaction with actin (Curmi et al., 1988). On the other hand, it is known that actin interaction affects the environment of at least the two most reactive thiols of the molecule (Duke et al., 1976; Kameyama, 1980; Cooke, 1986). So the fact that antipeptide binding does not modify the thiol reactivity of S1 suggests that the actin binding site which alters the thiol environment is not the stretch of residues 633–642 of the S1 heavy chain. Finally the absence of change in the overall structure of S1 demonstrates that the decrease of the acto–S1 interaction, observed when antipeptide is attached to S1 heavy chain (Chaussepied & Morales, 1988) is not consequent to secondary structural changes within S1 but is more likely due to the electrical neutralization of stretch 633–642 by the antipeptide. This conclusion can also explain the diminution in actin affinity previously observed after trypsin proteolysis of the S1 heavy chain (Botts et al., 1982). In that case, the decrease in actin affinity would result from the extensive degradation of stretch 633–642 and not from changes in structure or in flexibility of the S1 molecule that also appear after trypsinolysis (Cheung et al., 1985; Chaussepied et al., 1986; Highsmith & Eden, 1987; Rajasekharan & Burke, 1989).

A critical feature of actin binding to S1 is its weakening of the nucleotide–S1 interaction. None of the approaches tried here, however, revealed that the covalent attachment of antipeptide to the stretch of residues 633–642 significantly affects the interaction between ATP and S1.[2] Thus, blocking the stretch 633–642 neither modifies the k_{cat} of the different ATPase activities of S1 (Chaussepied & Morales, 1988) nor seems to alter the critical formation of the Mg^{2+}·ADP·P_i intermediate that follows the ATP binding and cleavage steps. For instance, processes such as Mg^{2+}·ADP·P_i-induced enhancement of tryptophan fluorescence or trypsin proteolysis of the S1 heavy chain (Bagshaw & Trentham, 1974; Mornet et al., 1985) and trapping of the Mg^{2+}·ADP·Vi complex in the active site (Goody et al., 1980; Wells & Bagshaw, 1984) are not affected by the presence of antipeptide. In addition to the binding of the polyphosphate chain, the binding of the adenosine moiety to the active site of S1 is not altered by the presence of antipeptide, as revealed by fluorescence acrylamide quenching studies.

Another crucial characteristic of S1 is that the effect of the nucleotide binding to the active site is transmitted to the actin binding sites. However, the presence of Mg^{2+}·ADP·Vi trapped in the active site, as Mg^{2+}·ADP·P_i intermediate, does not reduce significantly antipeptide binding to the stretch of residues 633–642. One should note that the presence of antipeptide slightly diminishes the yield of trapping (with 8% decreasing) and that the antipeptide-induced protection of the S1 heavy chain against Arg-C proteolysis is slightly less efficient when Mg^{2+}·ADP·Vi is trapped in the active site (Figure 3). These small effects can be explained either by a weak structural dependence between stretch 633–642 and ATP binding site [also described by Applegate and Reisler (1984)] or by an aspecific effect due to the orthovanadate (Vi) interaction with S1. However, since these effects are of a small scale, the absence of large mutual influence between the ATP binding site and the residues 633–642 of S1 heavy chain is here interpreted as a real structural independence between these two loci. This interpretation is further strengthened by the recent measurement of the distance separating these two sites (Kasprzak et al., 1989); this distance of >4.4 nm appears definitely too large to allow a direct contact between these two loci of the S1 heavy chain.

The straightforward implication of a structural independence between stretch 633–642 and the nucleoside binding site of the S1 heavy chain is that actin binding to stretch 633–642 is very likely when the ATP or ADP·P_i intermediate is present in the active site, as was suggested by EDC-induced cross-linking experiments performed on the acto–S1–nucleotide complex (Arata, 1984; Chen et al., 1985; Yamamoto, 1989). Furthermore, this actin contact point may represent the predominant acto–S1 interface in the presence of the ATP or ADP·P_i intermediate, since blocking this site with the antipeptide strongly inhibits the interaction between actin and S1·nucleotide and since the interactions between antipeptide and stretch 633–642 and between actin and S1·nucleotide are similarly dependent on the ionic strength (Chalovich et al., 1983; Arata, 1984; Chaussepied et al., 1988).

Finally, it was previously demonstrated that the interaction between actin and S1 in the rigor state (absence of nucleotide) is also affected, but to a smaller extent, by the presence of antipeptide on the stretch 633–642 (Chaussepied & Morales, 1988). Together these data tend to demonstrate that the interaction of actin with the stretch 633–642 of skeletal S1 heavy chain exists during the entire cross-bridge cycle, evolving from being a predominant interface at the beginning of the power stroke (while ADP·P_i is present in the active site) to being a less predominant interface at the end of the power stroke.

[2] To avoid cross-linking between S1 heavy chain and alkali light chain 1(A1) or aspecific binding of the antipeptide to the N-terminal part of A1 light chain, this work was performed with the S1(A2) isoenzyme only. However, the lack of experimental evidence for the involvement of the alkali light chains in ATP binding or hydrolysis properties of skeletal S1 allows the generalization of these conclusions to both S1(A1) and S1(A2) molecule.

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