Interaction of the Heavy Chain of Gizzard Myosin Heads with Skeletal F-Actin†

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ABSTRACT: To probe the molecular properties of the actin recognition site on the smooth muscle myosin heavy chain, the rigor complexes between skeletal F-actin and chicken gizzard myosin subfragments 1 (S1) were investigated by limited proteolysis and by chemical cross-linking with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide. Earlier, these approaches were used to analyze the actin site on the skeletal muscle myosin heads [Mornet, D., Bertrand, R., Pantel, P., Audemard, E., & Kassab, R. (1981) *Biochemistry 20*, 2110–2120; Labbé, J. P., Mornet, D., Roseau, G., & Kassab, R. (1982) *Biochemistry 21*, 6897–6902]. In contrast to the case of the skeletal S1, the cleavage with trypsin or papain of the sensitive COOH-terminal 50K–26K junction of the head heavy chain had no effect on the actin-stimulated Mg^{2+}-ATPase activity of the smooth S1. Moreover, actin binding had no significant influence on the proteolysis at this site whereas it abolished the scission of the skeletal S1 heavy chain. The COOH-terminal 26K segment of the smooth papain S1 heavy chain was converted by trypsin into a 25K peptide derivative, but it remained intact in the actin–S1 complex. A single actin monomer was cross-linked with the carbodiimide reagent to the intact 97K heavy chain of the smooth papain S1. Experiments performed on the complexes between F-actin and the fragmented S1 indicated that the site of cross-linking resides within the COOH-terminal 25K fragment of the S1 heavy chain. Thus, for both the striated and smooth muscle myosins, this region appears to be in contact with F-actin. But, in contrast to the skeletal actomyosin–S1 (acto–S1), no cross-linking between actin and the 50K fragment of the smooth S1 heavy chain was observed with the zero-length carbodiimide agent. The overall data suggest changes in the actin binding to the smooth head heavy chain near the 50K–25K area as compared to the skeletal acto–S1 complex. The carbodiimide-catalyzed covalent attachment of F-actin to the smooth S1 induced a significant increase of the Mg^{2+}-ATPase activity. Furthermore, the turnover rate of the isolated covalent F-actin–S1 complex was much lower than that for the skeletal acto–S1 species, as expected for a slow muscle type. This finding indicates that the chemical cross-linking reaction does not alter the specific enzymatic potential of a myosin head.

The molecular mechanisms involved in F-actin–myosin recognition are of prime importance for our understanding of muscle contraction. Vertebrate muscles exhibit a remarkable structural and functional diversity. The wide range of functions related to the diverse muscle types could be mediated in part by different specific modes of actin–myosin interaction. While the actin molecules from different sources have highly conserved structures and properties (Korn, 1982), myosins differ both chemically and in functional aspects, particularly, in the extent to which their Mg^{2+}-ATPase[1] is activated by actin and in the way this ATPase is regulated. In this regard, the comparison of the kinetics of the actomyosin ATPase of smooth and skeletal muscle S1 is of interest. It indicates that the maximum ATPase rate, the rate of dissociation of acto–S1 by ATP, and the rate of ADP release from the acto–S1 complex are slower with smooth muscle S1 (Marston & Taylor, 1980); also, the smooth actomyosin complex seems to be little dissociated by Mg^{2+}-PP_i (Takeuchi, 1982). Together, these features suggest possible changes in the mechanism of communication between the actin and nucleotide binding sites within the smooth head as recently proposed by Krisanda & Murphy (1980). Finally, while increasing ionic strength greatly diminishes the binding of skeletal muscle S1 to actin, it has almost no influence on the binding of smooth muscle S1, both in the absence and in the presence of nucleotide (Green et al., 1983). This observation suggests differences in the ionic properties of the interface between actin and the heads of the two myosins.

In this work, we have explored the interaction between skeletal F-actin and the head heavy chain of gizzard myosin, employing protein–protein protection effects against proteolysis and chemical cross-linking experiments on rigor acto–S1. Earlier, use was made of the same approaches for the study of the skeletal acto–S1 complex (Mornet et al., 1981a). The results indicate possible changes in the binding of the smooth heavy chain to actin close to the 50K–25K connector region; they also illustrate the involvement of the COOH-terminal 25K heavy chain segment in the interaction between F-actin and the smooth myosin head.

Materials and Methods

Chemicals. Trypsin [treated with L-1-(tosylamido)-2-phenylethyl chloromethyl ketone] was purchased from Worthington Biochemical Corp. Mercuripapain was from Sigma Chemical Co. (St Louis, MO). The hydrochlorides of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide and dimethyl suberimidate were from Fluka AG, Switzerland, and Pierce Chemical Co., respectively. All other chemicals were of analytical grade.

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[1] Abbreviations: ATPase, adenosine-5′-triphosphatase (EC 3.6.1.3); S1, subfragment 1; acto–S1, actomyosin–S1; $NaDodSO_4$, sodium dodecyl sulfate; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

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Preparation of Proteins. Chicken gizzard myosin and the corresponding native papain S1 were obtained as reported by Marianne-Pepin et al. (1983). The proteolytic derivative (75K–26K)–S1 was prepared by overnight digestion of gizzard myosin with papain and was isolated similarly to native papain S1 (Marianne-Pepin et al., 1983). Skeletal chymotryptic S1 was prepared according to Weeds & Taylor (1975). The skeletal tryptic derivative (27K–50K–20K)–S1 was obtained according to Mornet et al. (1981c). Rabbit skeletal muscle F-actin was isolated by the procedure of Spudich & Watt (1971) as modified by Eisenberg & Kielly (1974). F-Actin was labeled with the fluorescent dye *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine (1,5-IAEDANS) as reported by Mornet et al. (1981a).

Proteins concentrations were determinated by measuring the absorbance at 280 nm with an extinction coefficient of $E^{1\%}_{280nm}$ = 4.5 cm^{-1} for gizzard myosin (Okamoto & Sekine, 1978) and 11.0 cm^{-1} for skeletal actin (West et al., 1967). The concentration of S1 and all other proteolytic derivatives was determinated according to Bradford (1976) with bovine serum albumin as the standard. Calculations were based on molecular weights of 42000 for actin (Collins & Elzinga, 1975) and 115000 for S1 (Strzelecka-Golaszewska & Sobieszek, 1981).

Digestion of the Acto–S1 Complex. The tryptic digestion of gizzard papain S1 in the absence and in the presence of skeletal F-actin (molar ratio actin:S1 = 2) was conducted at a weight ratio of protease to S1 of 1:100 in 40 mM imidazole hydrochloride, 40 mM KCl, and 1 mM dithioerythritol (pH 7.0), 25 °C. The fragmentation process was monitored by $NaDodSO_4$ gel electrophoresis carried out in 5%–18% polyacrylamide slab gels (Mornet et al., 1981a). The fragmented derivative (29K–50K–25K)–S1 was purified according to Mornet et al. (1981c).

To follow the time course of changes in the acto–S1 ATPase activity during the proteolytic reactions, 100-μL aliquots were withdrawn at various times and were assayed for this ATPase activity. The actin-activated Mg^{2+}-ATPase was measured in a medium (1 mL) containing 35 mM KCl, 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.1 mM EGTA, and 5 mM ATP; actin was added at 1 mg/mL. For reproducible results, P_i was measured colorimetrically by an automated phosphate system as previously described (Mornet et al., 1981a).

Cross-Linking of F-Actin–S1 Complexes. Skeletal F-actin (4 mg/mL) was activated by a 2-min reaction with carbodiimide essentially as described by Mornet et al. (1981b). A sample was then mixed with S1 in 40 mM KCl and 40 mM imidazole hydrochloride (pH 7.0) so that the actin was diluted 5-fold and the molar ratio of F-actin to head was equal to 2. The cross-linking between F-actin and fragmented S1 with dimethyl suberimidate was performed as reported by Labbé et al. (1982). At appropriate times (0–15 min), protein aliquots (0.100 mg of S1) were removed from the reaction mixtures and assayed for Mg^{2+}-ATPase activity. The cross-linked species were separated by gel electrophoresis. Fluorescent bands were located in the gels by illumination with long-wave ultraviolet light before staining with Coomassie brilliant blue R-250 (Weber & Osborn, 1969).

The isolation of the covalent F-actin–S1 complex from cross-linking reaction mixtures was carried out by ultracentrifugation as reported by Mornet et al. (1981b) except that the dissociation of the unreacted S1 was conducted in 50 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid, 100 mM KCl, 25 mM $MgCl_2$, and 10 mM ATP (pH 8.0). The amount of S1 remaining in the supernatant was determined with the microassay of Bradford (1976) by using native S1 as the standard. The amount of residual nondissociated S1 still present in the pellet was estimated by densitometry of electrophoretic gels calibrated with native S1.

Results

Effect of Peptide Bond Cleavage at the 50K–26K Junction on the Actin-Activated Mg^{2+}-ATPase of S1. When the gizzard papain S1 heavy chain of M_r 97K was cleaved with trypsin at the two joints connecting the 50K fragment to the adjacent 29K and 26K peptide segments (Figure 1B) that we have recently isolated and analyzed (Marianne-Pepin et al., 1983), the acto–S1 Mg^{2+}-ATPase remained unchanged (Figure 1A). Also, the isolated papain derivative (75K–26K)–S1 exhibited an intact actin-activated ATPase; as shown in Figure 1B, this derivative makes up a significant portion of the current gizzard S1. It should be noted that the Mg^{2+}-ATPase of S1 alone was not affected by tryptic digestion (0.028 μmol of P_i min^{-1} mg^{-1}). Consequently, the scission of the gizzard S1 heavy chain at the vulnerable 75K–26K junction by trypsin or papain has no apparent influence on the activation of the Mg^{2+}-ATPase by actin. This behavior of the smooth S1 is unlike that of the skeletal myosin head whose actin-stimulated Mg^{2+}-ATPase is specifically impaired upon tryptic hydrolysis of the COOH-terminal 75K–20K joint of the heavy chain (Mornet et al., 1981a). On the other hand, as illustrated in Figure 1B, the association of skeletal F-actin to gizzard S1 did not alter the fragmentation pattern of the heavy chain, except that the COOH-terminal segment of the heavy chain was released as an intact 26K fragment and not as the 25K product that forms in the absence of actin. There is no question that the 25K peptide is a breakdown derivative of the 26K region since it appears only in the digest of S1 and its amino acid composition is close to that found for the 26K fragment (Marianne-Pepin et al., 1983). In the presence of F-actin, a progressive degradation of the 26K peptide into fragments with mass equal or less than 14K was observed, but it did not lead to the formation of the 25K fragment. These results are also in striking contrast with those known for the skeletal S1, whose heavy chain was cleaved by trypsin into only two stable fragments of M_r 27K and 70K in the presence of actin because F-actin binding prevents specifically the cut of the heavy chain between the 50K and 20K segments (Mornet et al., 1979; Yamamoto & Sekine, 1979). The present data indicate that while the binding of actin to the smooth S1 presumably alters at least part of the structure of the COOH-terminal 26K region of the heavy chain, it has no apparent impact on the proteolytic events occurring at the 50K–26K joint within the free S1 molecule.

Cross-Linking of F-Actin to the COOH-Terminal 26K Segment of S1 Heavy Chain. To identify the regions of the gizzard myosin head heavy chain in close contact with F-actin, cross-linking experiments on S1 subfragments combined to fluorescent skeletal F-actin were performed, with the EDC-catalyzed reaction (Mornet et al., 1981a). The results obtained for the F-actin–papain S1 complex following gel electrophoresis of the reaction mixtures are shown in Figure 2. There was production of actin dimers and trimers present also in the actin control (Figure 2, lane d); their amount was increased in the presence of S1 as observed previously (Mornet et al., 1981b). The reaction led also to the formation of two new fluorescent species of M_r 160K and 68K, respectively (Figure 2, lanes A and a); the former single band displayed a mobility slightly higher than the 180K protein doublet generated by the cross-linking of the skeletal acto–S1 complex used as control

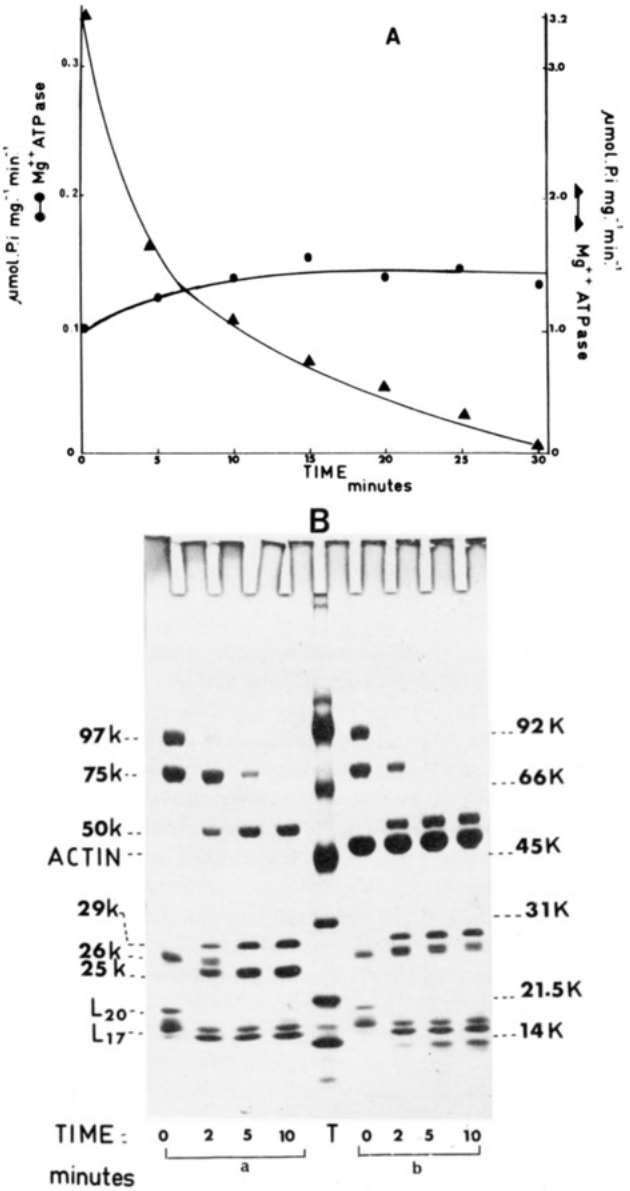

FIGURE 1: (A) Influence of the tryptic cleavage of gizzard S1 heavy chain on the actin-dependent Mg^{2+}-ATPase. Papain S1 (20 μM) in 40 mM KCl, 40 mM imidazole hydrochloride, and 1 mM dithioerythritol pH 7.0, was digested with a protease to enzyme weight ratio of 1:100 at 25 °C. At the times indicated, protein samples were withdrawn, and acto–S1 ATPase assays (●) were carried out as described under Materials and Methods. For comparison, the acto–S1 ATPase of rabbit skeletal S1, treated under similar conditions, was also measured (▲). (B) Comparison of the tryptic fragmentation patterns of gizzard S1 digested in the absence (a) and in the presence (b) of F-actin. Papain S1 was digested with trypsin as in (A); skeletal F-actin was added at a molar ratio of actin to S1 = 2. *T* = the following protein markers: phosphorylase B (92K); bovine serum albumin (66K); ovalbumin (45K); carbonic anhydrase (31K); soybean trypsin inhibitor (21.5K); lysozyme (14K). Note that in the native gizzard S1 preparations the heavy chain (M_r 97K) is always partially nicked by papain into a 75K–26K complex. Also, a noticeable amount of residual intact 20K light chain is consistently present in our preparations.

(Figure 2, lanes B and b); the velocity of the 68K entity was slightly higher than that of the 75 K band present in the native gizzard S1 preparation (Figure 2, lane C). Cross-linking of actin to the isolated papain derivative (75K–26K)–S1 produced only the 68K species (data not shown). Thus, the 68K band is likely to be a cross-linked product of an actin monomer with the 26K fragment, which represents the COOH-terminal counterpart of the 75K peptide within the 75K–26K complex.

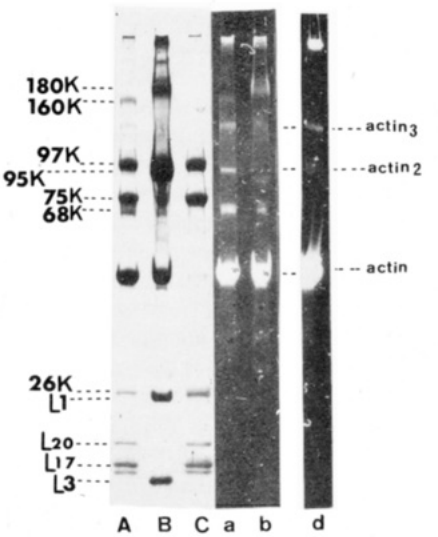

FIGURE 2: Carbodiimide-catalyzed covalent cross-linking between F-actin and gizzard S1. Native S1 (20 μM) (C) was reacted with EDC-activated fluorescent actin (40 μM) (A and a) as specified under Materials and Methods; after 15 min at 20 °C, the reaction mixture was analyzed by gel electrophoresis. The protein banding pattern (B) and the fluorescence profile (b) of the gel are shown for skeletal S1 and fluorescent actin used as control. A 20-min EDC-activated fluorescent F-actin containing dimers and trimers and higher oligomers at the top of the gel is also presented (d).

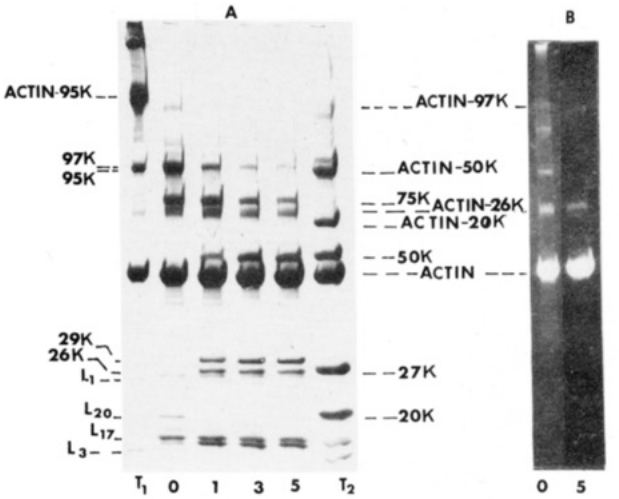

FIGURE 3: Tryptic sensitivity of the gizzard S1 heavy chain cross-linked to F-actin. (A) The reaction mixture containing gizzard S1 cross-linked to F-actin as described in Figure 2 was centrifuged, and the pellet was resuspended in the initial volume of 40 mM KCl, 40 mM imidazole hydrochloride, and 1 mM dithioerythritol, pH 7.0; trypsin was added at a protease to S1 weight ratio of 1:50. Protein samples were analyzed by gel electrophoresis at the times indicated. Controls were native skeletal S1 cross-linked to F-actin (T_1) and skeletal (27K–50K–20K)–S1 cross-linked to F-actin (T_2). (B) Same gel viewed under UV light at the indicated times with gizzard S1 cross-linked to fluorescent F-actin.

We attributed the 160K species to the cross-linking of the intact 97K S1 heavy chain to an actin monomer. As observed with the F-actin–S1 complex from skeletal myosin, the cross-linked adduct between one actin and one gizzard S1 heavy chain exhibits also in the gel system used a lower mobility than expected (Heaphy & Treager, 1983). No protein band accounting for the cross-linking of actin to the NH_2-terminal 75K portion of the smooth heavy chain was detected.

When the EDC-treated acto–S1 complex was submitted to limited proteolysis with trypsin (Figure 3A), the actin–97K entity was quickly digested and most of the 97K and 75K heavy chain fragments of the reversibly bound S1 were con-

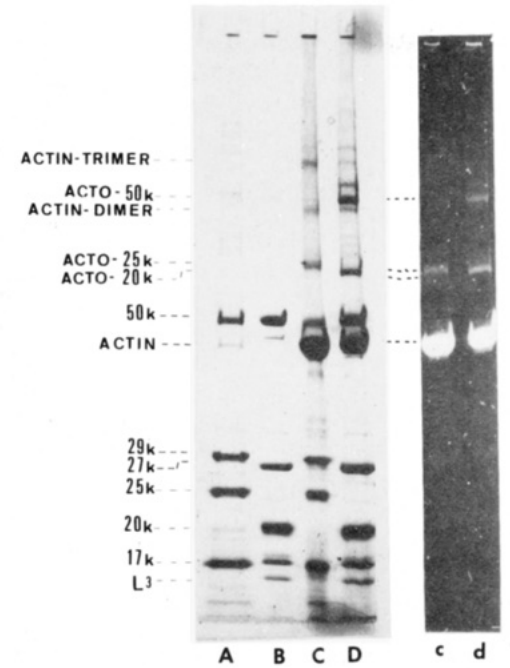

FIGURE 4: Cross-linking of F-actin to the COOH-terminal 25K segment of gizzard S1 heavy chain. EDC-activated fluorescent actin was reacted with gizzard (29K–50K–25K)-S1 (C and c) and with skeletal (27K–50K–20K)–S1 (D and d) under the conditions reported in Figure 2; the fluorescence pattern of the gels (c and d) is compared with the corresponding gel pattern after staining with Coomassie blue (C and D). (A and B) Native trypsin split gizzard and skeletal S1, respectively.

comitantly converted, as expected, into the constituting peptides, 29K, 50K, and 26K. If the cross-linking of S1 to actin had promoted the protection of the 50K–26K joint against trypsin cleavage, only the NH_2-terminal 29K fragment would have been released from the actin–97K species together with the apparition of the COOH-terminal heavy chain segment cross-linked to actin; this latter entity is homologous to the actin–70K entity reported previously for skeletal acto–S1 (Mornet et al., 1981b). Such a species was not observed in the digestion pattern of the cross-linked acto–S1 from gizzard. As shown in Figure 3B, after 5 min of trypsin digestion of EDC-treated fluorescent F-actin–S1 complex only the fluorescent 68K species remained present on the gel. The lability of the actin–97K band indicates that gizzard S1 heavy chain retains its susceptibility to trypsin even when it is covalently bound to actin. The actin–26K band was the only clearly discernible cross-linked product remaining present in the digest; it displays a mobility slightly lower than that corresponding to the actin–20K product formed by cross-linking between actin and the skeletal (27K–50K–20K)–S1 used as control (Figure 3A, lane T2). The band with slight intensity present at the 97K position after 5-min digestion together with the 75K species disappears upon prolonged tryptic digestion; also, the 97K product is not fluorescent when fluorescent actin was employed. Therefore, the latter entity cannot be an eventual actin–50K cross-linked derivative but rather a residual intact S1 heavy chain. The 25K fragment includes the site of the chemical cross-linking between actin and the gizzard S1 heavy chain as indicated by the results obtained on cross-linking fluorescent F-actin to the tryptic (29K–50K–25K)–S1 (Figure 4); a single fluorescent new band was observed (Figure 4, lanes C and c) with mobility slightly lower than that of the actin–20K peptide band issued from the cross-linking between actin and the homologous skeletal derivative (27K–50K–20K)–S1 used as control (Figure 4, lanes D and d). This product was

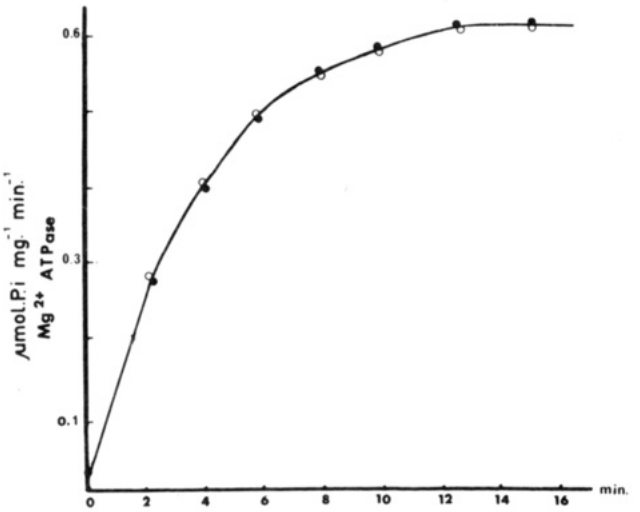

FIGURE 5: Stimulation of the Mg^{2+}-ATPase of gizzard S1 covalently cross-linked to F-actin. Papain S1 (O) and the tryptic (29K–50K–25K)–S1 (●) were incubated at pH 7.0 and 20 °C with EDC-activated actin as specified under Materials and Methods. At the time indicated, protein samples (0.100 μg of S1) were removed from the reaction mixtures and submitted to Mg^{2+}-ATPase assays.

assumed to be a cross-linked actin–25K peptide. In contrast to the case of the skeletal S1 derivative, no species accounting for the cross-linking of actin to the 50K fragment of gizzard S1 heavy chain was noticed. This finding is in agreement with the absence of cross-linking, already observed, between actin and the smooth 75K peptide.

Enhancement of the Mg^{2+}-ATPase Activity of Gizzard S1 Cross-Linked to F-Actin. The skeletal S1 covalently cross-linked to actin catalyzes Mg^{2+}-ATP hydrolysis at a rate as high as the maximum rate measured kinetically at infinite actin concentration for the reversible acto–S1 complex (Mornet et al., 1981b; Labbé et al., 1982). Figure 5 illustrates the stimulation of the Mg^{2+}-ATPase induced by the cross-linking of EDC-activated actin to either native gizzard papain S1 or the tryptic derivative (29K–50K–25K)–S1. Similar results were also obtained with the isolated papain-produced (75K–26K)–S1. After 15-min reaction, the covalent complex between F-actin and the S1 species was isolated by sedimentation in the presence of Mg^{2+}-ATP. Generally, about 10% of the initial amount of S1 remained irreversibly bound to actin. The efficiency of the EDC-catalyzed cross-linking reaction is, therefore, 3 times lower for the smooth S1 as compared to the skeletal S1 (Mornet et al., 1981b). The rate of the Mg^{2+}-ATPase activity of the freshly prepared complex was 3.0 s^{-1}; the value measured in the absence of actin was 0.05 s^{-1}, in agreement with published data (Marston & Taylor, 1980). The value of the covalent complex is within the range of values (2.3–3.7 s^{-1}) reported for the V_{max} of the ATPase of gizzard S1 reversibly activated by skeletal muscle actin (Strzelecka-Golaszewska & Sobieszek, 1981).

Discussion

The tryptic digestion of the smooth S1, presented here, confirms and extends our recent study describing the fragmentation of gizzard myosin and HMM with trypsin (Marianne-Pépin et al., 1983). The results show that the structural organization of the heavy chain is apparently similar for the smooth and skeletal myosin heads. Like its skeletal counterpart, the smooth S1 heavy chain is built up of three tryptic fragments (29K, 50K, 25K), which are presumably linked by two protease-vulnerable connector segments. As observed earlier for the skeletal S1 (Mornet et al., 1979), the COOH-

terminal junction between the 50K and 25K segments of the smooth heavy chain exhibits the highest susceptibility to proteolysis; this explains the presence in the native S1 preparation of a significant amount of the 75K–26K heavy chain complex consistently produced by papain hydrolysis of this particular region. Also, chymotryptic cleavage at this place, is likely to be responsible for the reported nicking of the gizzard HMM heavy chain into the 70K–65K complex (Okamoto et al., 1980; Seidel, 1980).

However, in contrast to the case of the skeletal S1, the binding of F-actin to the smooth S1 does not appreciably protect the 50K–25K cut under the mild proteolytic conditions employed. Also, no significant protection was noticed with the parent myosin or in the presence of added gizzard or skeletal tropomyosin. The protection by F-actin of the 50K–20K region within the skeletal S1 heavy chain was shown to occur not only during the tryptic digestion of the acto–S1 complex (Mornet et al., 1979; Yamamoto & Sekine, 1979) but also during its treatment with staphylococcal protease, which splits the connector segment at a different site (Chaussepied et al., 1983). In the course of the digestion of the complex between F-actin and gizzard myosin with papain, only a slight protection of the 75K–26K link was noticed (results not shown), but the effect was far from that currently obtained upon tryptic digestion of the skeletal actomyosin complex both in vitro (Mornet et al., 1981a) and in vivo (Lovell & Harrington, 1981). The protective effect observed with the skeletal S1 was thought to be related to the interaction of actin with this S1 molecule because the cleavage specifically abolished the activation of the Mg^{2+}ATPase by actin (Mornet et al., 1979). The loss of the activation was the result of the reduction of actin affinity for the S1–ADP–P_i complex (Botts et al., 1982). Interestingly, not only the binding of actin to smooth S1 failed to prevent the 50K–25K cut of the heavy chain, but also this cleavage had no influence on the measured actin-stimulated Mg^{2+}-ATPase. Since the kinetics of this activity are similar with smooth and skeletal muscle S1 (Marston & Taylor, 1980; Greene et al., 1983), the present results raise the possibility of changes in the conformation of the 50K–25K region of the smooth S1 and/or in the geometry of the actin site on the smooth S1 heavy chain.

The interaction between F-actin and the COOH-terminal 26K fragment of the smooth myosin head is suggested by both the proteolytic data and the chemical cross-linking experiments carried out with the carbodiimide reagent. Also, recently, the observed production by trypsin of a S1 species with a degraded 26K heavy chain segment and displaying a weak affinity for actin has led to the proposal that the structural integrity of this region is required for the effective binding of the smooth head to F-actin (Marianne-Pepin et al., 1983). Thus, the COOH-terminal segment of the S1 heavy chain appears to be involved in the association of F-actin to both the striated and smooth muscle myosins. However, the previous cross-linking studies conducted on the skeletal acto–S1 complex and employing the zero-length cross-linker EDC, as well as the longer bis(imido ester) agents (Mornet et al., 1981a, b; Labbé et al., 1982; Yamamoto & Sekine, 1979), have shown that the 50K fragment contains potent sites for the interaction with actin. Although the proximity of the smooth 50K region to F-actin was demonstrated by cross-linking with dimethyl suberimidate (data not shown), no such contacts could be revealed by the reaction of EDC with the complexes between F-actin and the intact smooth S1 heavy chain or its various proteolytic fragments. Two causes seem plausible for the lack of EDC-induced cross-linking between F-actin and the smooth 50K fragment. The lysine residues required for the cross-linking were replaced by arginine side chains. The amino acid composition of the isolated smooth 50K peptide indicated that its content in arginine was twice that found in the skeletal peptide, but their contents in lysine were quite similar (Marianne-Pépin et al., 1983). The other cause would be a definite alteration in the positive charge distribution within the smooth 50K peptide, generating a specific change in the ionic structure of the interface between this region and actin. The latter possibility is more attractive for two reasons. First, it is consistent with the marked differences recently observed in the effect of ionic strength on the interaction of F-actin with the skeletal muscle S1 and the smooth muscle S1; increasing ionic strength affected only slightly the binding of the smooth S1 whereas it considerably weakened the binding of the skeletal S1 (Greene et al., 1983). Second, it is in line with the above conclusions drawn from the proteolytic probe and which suggest changes in the actin binding near the 50K–25K region of the smooth heavy chain.

As found earlier for the skeletal S1, the covalent attachment of actin to the smooth S1 or to its fragmented derivatives induced an acceleration of the Mg^{2+}ATPase activity. Furthermore, as expected for a slow muscle type, the enhancement of this enzymatic activity was much less than that observed with the skeletal muscle S1 (Mornet et al., 1981b), but the rate of the stimulated ATPase was quite similar to the V_{max} of the ATPase of the reversible smooth acto–S1 complex. Thus, the chemical cross-linking between actin and S1 does neither change the ATPase mechanism (Webb & Trentham, 1982) nor alter the intrinsic enzymatic properties of a myosin head. So far, and following the suggestion of Barany et al. (1966), one cannot convert the smooth acto–S1 ATPase into skeletal acto–S1 ATPase.

Registry No. ATPase, 9000-83-3.

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Biochemical Properties of 9-*cis*- and *all-trans*-Retinoylopsins†

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ABSTRACT: The stoichiometry of the reaction between [^{14}C]-9-*cis*-retinoyl fluoride, a close isostere of 9-*cis*-retinal, and bovine opsin and the biochemical and spectral properties of this new pigment were investigated. The stoichiometry of retinoid incorporation is approximately one in dodecyl maltoside, a detergent in which opsin is capable of regeneration with 11-*cis*-retinal. Interestingly, in Ammonyx LO, a detergent that does not permit rhodopsin regeneration, the stoichiometry of binding is still approximately one. By contrast, heat-denatured opsin does not irreversibly bind substantial [^{14}C]retinoyl fluoride. This result strongly suggests that the nucleophilicity of the active site lysine is retained in Ammonyx LO but that further conformational changes in the protein, required to form rhodopsin, are not possible. These results are all consistent with an active site directed mechanism for the irreversible reaction of 9-*cis*-retinoyl fluoride with opsin probably at the active site lysine residue. The ultraviolet spectra of 9-*cis*-retinoylopsin and its all-trans congener show λ_{max}'s at 373 and 380 nm, respectively, somewhat bathochromically shifted from their respective model *N*-butylretinamides which absorb at 347 and 351 nm. Photolysis of both 9-*cis*- and *all-trans*-retinoylopsins leads to the same photostationary state. This shows that, as expected, photoisomerization without bleaching occurs. The photolysis of either 9-*cis*- or *all-trans*-retinoylopsin in the presence of the G protein (transducin) does not lead to the activation of the latter. This is consistent with the notion that a protonated Schiff base is critical for the function of rhodopsin.

Vertebrate scotopic vision begins with absorption of light by rhodopsin, the major membrane-bound protein in rod outer segments (Hubbell & Bownds, 1979). Bovine rhodopsin is a 39-kDa protein comprised of a single polypeptide chain linked via a protonated Schiff base between lysine-296 and the chromophore 11-*cis*-retinal (Hargrave et al., 1983). Absorption of light by this holoprotein results in the isomerization of the chromophore to its all-trans congener (Hubbard & Kropf, 1958) followed by a series of conformational changes in the protein, which eventually results in the hydrolysis of the *all-trans*-retinal–opsin Schiff base (Wald, 1968). This process is called bleaching, because without the chromophore opsin does not absorb in the visible range. One of the conformers (probably metarhodopsin II) (Parkes et al., 1979; Calhoon et al., 1981) on the way to Schiff-base hydrolysis activates the G protein (transducin), a biochemical step proposed to be of importance in the cascade of events that eventually leads to closing of sodium channels with subsequent hyperpolarization of the rod outer segments (Shinozawa et al., 1979).

The state of protonation of the Schiff base of rhodopsin is thought to be of prime importance for its functioning. The ability of rhodopsin to efficiently transduce light energy into the chemical potential energy of the intermediates is almost certainly related to charge movement caused by light absorption (Honig et al., 1979). This necessitates that the Schiff base be protonated. Furthermore, in the critical metarhodopsin I → metarhodopsin II conversion, a proton is gained by the protein (Cooper & Converse, 1976) at the same time as the Schiff base is probably deprotonated (Doukas et al., 1978), suggesting that proton translocations are important in this step. Finally, the protonated Schiff base is also important in determining the λ_{max}'s for the various visual pigments (Honig et al., 1976).

On the basis of what has been stated concerning the importance of protonated Schiff base formation, it would be of some interest to study the function of an opsin containing a vitamin A analogue not capable of being protonated but, nevertheless, a close isostere of the chromophore of rhodopsin or isorhodopsin. To these ends we have developed 9-*cis*-retinoyl

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