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Location of an Essential Carboxyl Group along the Heavy Chain of Cardiac and Skeletal Myosin Subfragments 1†

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ABSTRACT: Cardiac and skeletal myosin subfragments 1 cleaved into three fragments were modified by 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate in the presence of the nucleophile nitrotyrosine ethyl ester. The effects observed (first-order kinetics of ATPase inactivation, incorporation of 1 mol of nitrotyrosine/mol of subfragment 1) were similar to those previously observed for the nondigested subfragments 1 [Lacombe, G., Van Thiem, N., & Swynghedauw, B. (1981) *Biochemistry 20*, 3648–3653; Körner, M., Van Thiem, N., Lacombe, G., &Swynghedauw, B. (1982) *Biochem. Biophys. Res. Commun. 105*, 1198–1207]. For both native and digested subfragments 1, which were inactivated to the extent of about 70%, the location of the label nitrotyrosine was performed by immunological blotting with ^{125}I-labeled anti-nitrotyrosine immunoglobulins. It was found that the modified residue was essentially located on the heavy chain for the native subfragments 1 and on the 50K peptide for the digested subfragments 1.

In recent years, a number of studies have been devoted to the structure–function relationship of myosin, an oligomeric enzyme consisting of two heavy chains and four light chains. The latter have been shown by several workers to play a role in the regulation of the myosin–actin interaction [see reviews by Taylor (1979) and Adelstein & Eisenberg (1980)]. For the heavy chains, the two important functions of myosin, adenosine 5′-triphosphate (ATP)[1] hydrolysis and actin activation, have recently been attributed to the isolated heavy chain (Maruta et al., 1978; Wagner & Giniger, 1981; Sivaramakrishnan & Burke, 1982). However, the chemical structure of the catalytic site of myosin is unknown, although a number of amino acid residues have long been suggested as being essential (cysteine, lysine, arginine, histidine, tyrosine). Some functional groups have been located, due to the fact that controlled fragmentation of the myosin head which contains the ATPase and the actin-binding sites produced three positioned peptides: 27K–50K–20K (Balint et al., 1978; Mornet et al., 1979; Yamamoto & Sekine, 1979a; Cardinaud, 1979). This is the case for the reactive lysyl residue located in the 27K domain (Mornet et al., 1980; Hozumi & Mühlrad, 1981; Miyanishi & Tonomura, 1981), the SH_1 and SH_2 cysteinyl residues in the 20K domain (Cardinaud, 1979; Walser et al., 1981; Sutoh, 1981), and the actin-binding sites in the 50K and 20K peptides (Mornet et al., 1981a; Yamamoto & Sekine, 1979b; Sutoh, 1982).

In previous studies (Lacombe et al., 1981; Körner et al., 1982), we showed that, through carbodimide modification, skeletal and cardiac myosin subfragments 1 incorporated 1 mol of nucleophile (nitrotyrosine) per mol of S1, resulting in a complete loss of ATPase activity. These results suggested the involvement of one carboxyl residue. As preliminary experiments using the ^{14}C-labeled nucleophile were not satisfactory, the location of this residue was carried out by an immunodetection procedure. For this, antibodies specific to the marker (nitrotyrosine) were prepared and used as probes in immunoblots of S1 fragments obtained with digestion by trypsin. These studies were carried out in parallel on cardiac and skeletal myosins. In both myosins, the modified residue was found essentially on the heavy chain in the case of native S1 and on the 50K peptide in the case of fragmented S1.

Materials and Methods

Chemicals. CNBr-activated Sepharose 4B was purchased from Pharmacia. 1-Cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide, nitrotyrosine ethyl ester, and bovine γ-globulin were purchased from Fluka. [^{14}C]Nitrotyrosine ethyl ester was prepared by Le Service des Molécules Marquées, Commissariat ã l'Energie Atomique, Saclay, France. 3-Nitro-L-tyrosine was an ICN Pharmaceuticals product. Bovine serum albumin and soybean trypsin inhibitor were purchased from Calbiochem. TPCK–trypsin and α-

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[1] Abbreviations: S1, α-chymotryptic myosin subfragment 1; CMC, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate; NTEE, 3-nitro-L-tyrosine ethyl ester; EDTA, ethylenediaminetetraacetic acid; ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; Tris, tris(hydroxymethyl)aminomethane; $NaDodSO_4$, sodium dodecyl sulfate; TPCK, tosylphenylalanyl chloromethyl ketone.

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chymotrypsin were from Worthington. ^{125}I-Iodinated 3-(4-hydroxyphenyl)propionic acid *N*-hydroxysuccinimide ester (Bolton–Hunter reagent) came from New England Nuclear and from Amersham.

Proteins. Dog cardiac left ventricle chymotryptic S1 was prepared from myofibrils as described by Cardinaud et al. (1973) with the modification introduced in a previous report (Körner et al., 1982). Rabbit skeletal S1 was obtained by the same procedure, with the minor modification that washing of the acto–S1 complex was carried out with phosphate buffer, pH 7.0, containing 0.1 M instead of 0.5 M KCl. Digestion of S1 (2 mg/mL) was conducted according to Yamamoto & Sekine (1979c) with a 1:100 (w/w) ratio of trypsin to S1, pH 7.0, 25 °C, in 50 mM Tris–maleate buffer and in 4 mM EDTA and 100 mM KCl for cardiac S1, and in 5 mM EDTA and 40 mM NaCl for skeletal S1. The reaction was stopped at 30 min for cardiac S1 and at 60 min for skeletal S1 by adding soybean trypsin inhibitor in an amount which was twice that of trypsin (w/w). The preparations were stored at 0 °C in 0.5 M KCl and 4 mM EDTA, pH 6.5, for cardiac S1 and in 40 mM NaCl and 5 mM EDTA, pH 6.5, for skeletal S1. S1 concentration was measured by the absorbance at 280 nm ($E^{1\%}_{280nm}$ = 7.5 cm^{-1}) Wagner & Weeds, 1977). The concentration of labeled protein was estimated by the Bradford method (Bradford, 1976) using the unlabeled protein as a standard. A molecular weight of 115 000 was used for S1 calculations (Weeds & Pope, 1977).

Kinetic Studies of S1 Modification. ATPase assays, inactivation kinetics, and the stoichiometry of the labeling with CMC plus nucleophile were carried out as previously described (Lacombe et al., 1981). For the stoichiometry of the labeling experiments, [^{14}C]Nitrotyrosine ethyl ester was used (Körner et al., 1982). Preliminary experiments indicated that the presence of 2.5 mM ADP provided no protection against inactivation for any of the S1 species studied here but significantly slowed down the precipitation phenomenon following inactivation. This was in full agreement with previous observations (Körner et al., 1982). For these reasons, all experiments were performed in the presence of 2.5 mM ADP.

Immunogens. A conjugate of bovine serum albumin and nitrotyrosine was prepared according to Helman & Givol (1971) by coupling 3-nitrotyrosine to the albumin with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide. The nitrotyrosylalbumin obtained contained 19 mol of nitrotyrosine/mol of albumin. Bovine γ-globulin similarly conjugated to nitrotyrosine contained 13 mol of nitrotyrosine/mol of γ-globulin.

Antibodies. Antisera were raised by injecting a sheep with 2 mg of nitrotyrosylalbumin emulsified with complete Freund's adjuvant in multiple intradermal sites. Two injections were made at an interval of 3 weeks. The serum was tested for antibodies with the nitrotyrosyl-γ-globulin by immunodiffusion according to the method of Ouchterlony. The antibodies showed good response to nitrotyrosyl-γ-globulin; this response was maximal 1 month after the first injection. After bleeding, the antiserum obtained was stored at −20 °C with 0.1% sodium azide. Antibodies were purified by adsorption on Sepharose 4B coupled with nitrotyrosyl-γ-globulin (Wilchek et al., 1971). The adsorbed antibodies were eluted from the column with 0.1 M acetic acid, dialyzed against 0.1 M ammonium bicarbonate, pH 7.5, lyophilized, and stored at −40 °C. The average yield of column-purified antibodies was 0.5–1.0 mg/mL of serum. The lyophilized antibodies retained a good affinity for nitrotyrosyl-γ-globulin.

Antibodies were ^{125}I labeled according to Bolton & Hunter (1973). Lyophilized antibodies were dissolved in 150 mM phosphate buffer, pH 8.5, containing 150 mM NaCl. The few denatured proteins were removed by centrifugation. Antibodies (80 μg) in 80 μL of solution were made to react with dried Bolton–Hunter reagent, 1 mCi (specific activity 2000 Ci/mmol), for 1 or 2 h at 0 °C. The excess of reagent was then reacted with 0.2 M glycine in 150 mM NaCl and 150 mM phosphate buffer, pH 8.5. The ^{125}I-labeled protein was purified on an Ultrogel AcA 202 column equilibrated and eluted with 10 mM phosphate buffer, pH 7.4, containing 140 mM NaCl, 0.05% sodium azide, and 0.25% gelatin. Recovery of ^{125}I-labeled protein was about 90%. The protein diluted in 50% glycerol was stored in −20 °C.

Electrophoresis. The S1 and tryptic S1 from heart and skeletal muscle were modified by 100 mM CMC in the presence of 15 mM NTEE and 2.5 mM ADP at 25 °C, pH 5.9. The reaction was stopped at 70% of enzyme inactivation by 40 volumes of 5% trichloroacetic acid. After centrifugation, the pellets were washed by 40 volumes of 50% ethanol. The proteins were then collected by centrifugation and dissolved in 5% NaDodSO$_4$, 5 mM EDTA, and 1% mercaptoethanol, at 100 °C. Control samples were made by the same procedure, except that CMC was omitted. The peptide separation was performed by using a NaDodSO$_4$–acrylamide slab gel (1 mm thick) according to Porzio & Pearson (1977).

Immunoblots. The transfer of proteins from the NaDodSO$_4$ gel to nitrocellulose sheets was performed essentially according to Bowen et al. (1980) as modified by Schwartz et al. (1982). One of the two nitrocellulose sheets was revealed for proteins by Amido Black staining according to Schaffner & Weissmann (1973). The other sheet was incubated with 2% gelatin for 1 h at 37 °C in buffer A (140 mM NaCl and 10 mM sodium phosphate buffer, pH 7.8) and rinsed 5-fold with buffer B (140 mM NaCl, 10 mM sodium phosphate buffer, 0.2% gelatin, 0.2% NaDodSO$_4$, and 0.2% Tween, pH 7.8). Then the sheet was incubated with antibodies diluted with buffer B at 0.1 μg/mL (45 × 10^5 cpm/μg) for 2 h at 37 °C and washed thoroughly with 10 changes of buffer B for 2 h. After washing, the blots were sealed in a plastic bag and exposed to Kodak X-OMAT AR film in a Dupont Cronex Quanta III cassette for 30 h.

Results

Cardiac and Skeletal Preparations. Digestion by trypsin of dog cardiac S1 provided three peptides, 50K, 27K, and 20K, issued from the heavy chain and a degradation peptide from the alkali light chain (LC1) (Figure 1), as previously observed for skeletal S1 (Balint et al., 1978; Mornet et al., 1979; Yamamoto & Sekine, 1979a; Cardinaud, 1980) and beef cardiac S1 (Flink & Morkin, 1982). Furthermore, a 47K peptide, a degradation product of the 50K peptide, was also systematically observed for skeletal S1 and cardiac S1. This 47K peptide has been noted by several authors (Yamamoto & Sekine, 1979a; Cardinaud, 1979; Mocz et al., 1982). Such a digestion pattern suggested that the sequential arrangement of the fragments within the heavy chain of cardiac S1 is identical with that of skeletal S1 (Flink & Morkin, 1982). Although the digestion pattern obtained with trypsin was very similar for cardiac and skeletal S1, the time dependence was very different. For skeletal S1, the formation of the 50K peptide was complete after 60 min, in agreement with previous observations (Yamamoto & Sekine, 1979c; Cardinaud, 1979). Cardiac S1 was cleaved more rapidly, and a full conversion to the 50K peptide required less than 10 min.

After digestion with trypsin at pH 7.0 (see Table I), the Ca^{2+}-ATPase activities of skeletal and cardiac S1 were identical with those of native S1. But when digestion was

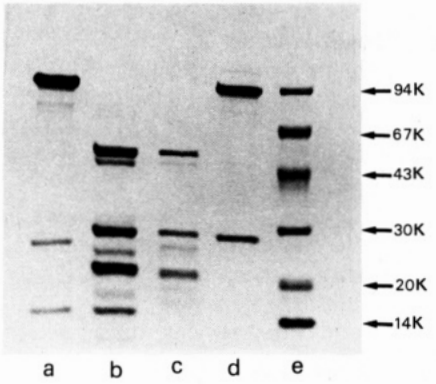

FIGURE 1: $NaDodSO_4$ gel electrophoresis of native and trypsin-digested cardiac and skeletal S1. (a) Skeletal S1; (b) skeletal S1 digested with trypsin for 60 min; (c) cardiac S1 digested with trypsin for 30 min; (d) cardiac S1; (e) mixture of protein markers: phosphorylase *a*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and α-lactalbumin (94K, 67K, 43K, 30K, 20K, and 14.4K, respectively).

Table I: Ca^{2+}-ATPase Activity and Half-Time of Inactivation of S1 Species[a]

enzyme preparation	Ca^{2+}-ATPase (nmol of $P_i \cdot mg^{-1} \cdot min^{-1}$)	half-time of inactivation	
		min[b]	s[c]
native cardiac S1	1200	5.0	22
trypsinized cardiac S1	1180	4.5	20
native skeletal S1	4300	5.0	28
trypsinized skeletal S1	4500	5.0	25

[a] For Ca^{2+}-ATPase activity and inactivation by carbodiimide, see Materials and Methods. Inactivation was carried out at pH 5.9, 25 °C, in the presence of 2.5 mM ADP. The concentrations of reagents were as indicated. The half-time of inactivation was calculated from the semilogarithmic plots of Ca^{2+}-ATPase activity. [b] Inactivation in the presence of 50 mM CMC + 15 mM NTEE. [c] Inactivation in the presence of 100 mM CMC + 15 mM NTEE.

performed at pH 8.0 (not shown), cardiac S1 completely lost its ATPase activity, whereas skeletal S1 retained its full activity. For this reason, digestion with trypsin was usually carried out at pH 7.0, as suggested by Yamamoto & Sekine (1979c).

Carbodiimide Modification of Cardiac and Skeletal S1. The modification by CMC was performed on native cardiac and skeletal S1 as well as on cardiac and skeletal S1 after digestion by trypsin. The kinetics of inactivation of Ca^{2+}-ATPase by CMC in the presence of 15 mM NTEE were first order for the four species, and the observed half-times of inactivation were nearly the same (Table I). The ratio between the rates of inactivation with 50 mM CMC and 100 mM CMC was identical for skeletal and cardiac S1 (Lacombe et al., 1981; Körner et al., 1982) and for native and trypsin-digested S1. The stoichiometry of labeling was 1 mol of nitrotyrosine/mol of S1 for the four species (not shown). These results suggested that digestion of S1 by trypsin did not change the reactivity of carboxyl groups toward carbodiimide.

Location of the Reactive Carboxyl Group. During carbodiimide modification, nitrotyrosine binds covalently to the reactive carboxyl groups (Hoare & Koshland, 1967). We were thus able to locate the reactive carboxyl residues by detection of nitrotyrosine with anti-nitrotyrosine antibodies. After electrophoresis under denaturing conditions, modified cardiac and skeletal species were transferred simultaneously to two nitrocellulose sheets. A control of the transfer was made by staining one of the two nitrocellulose sheets with Amido Black. Figure 2, lanes a–f, shows that the heavy and light chains, as

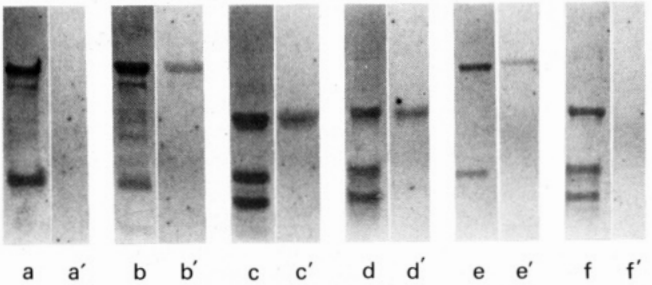

FIGURE 2: Immunoblots of native and digested S1. The modification was carried out at pH 5.9, 25 °C, in the presence of 2.5 mM ADP with 100 mM CMC + 15 mM NTEE. Amido Black staining is shown in lanes a–f, and autoradiography with ^{125}I-labeled antibodies is shown in lanes a′–f′. (Lanes a and a′) native cardiac S1 used as control (15 mM NTEE alone), (lanes b and b′) modified native skeletal S1; (lanes c and c′) modified digested skeletal S1; (lanes d and d′) modified digested cardiac S1; (lanes e and e′) modified native cardiac S1; (lanes f and f′) digested cardiac S1 used as control (15 mM NTEE alone).

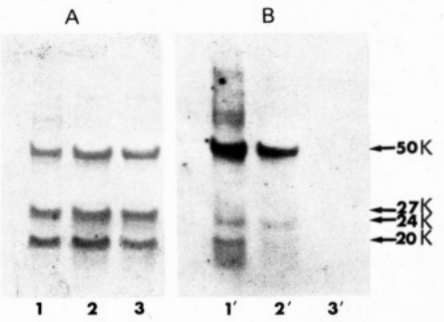

FIGURE 3: Immunoblots of digested cardiac S1. Detection of the label on peptides other than the 50K peptide. The modification experiments were carried out as described in Figure 2 for 32 and 90 s. (A) Amido Black staining; (B) autoradiography; (lanes 1 and 1′) S1 modified for 90 s; (lanes 2 and 2′) S1 modified for 32 s; (lanes 3 and 3′) S1 used as the control (15 mM NTEE alone). 24K is the degraded LC1.

well as the peptide fragments, were transferred. While the transfer was complete for the light chains, for the 20K peptide, and for the 27K peptide, it was not complete for the heavy chain and for the 50K peptide. The 47K peptide was visible, but diffusion of the protein bands during transfer rendered the 47K and 50K bands less clearly separated than in the polyacrylamide gel stained by Coomassie Brilliant Blue (see Figure 1). The second nitrocellulose sheet was used for the antigen–^{125}I-labeled antibody reaction. It contained all the protein bands shown by Amido Black staining, but autoradiography revealed only four protein bands (Figure 2, lanes a′–f′). As seen in Figure 2, lanes a′ and f′, the controls (native cardiac S1 and trypsin-digested cardiac S1, treated with 15 mM NTEE alone) showed no radioactivity. Thus, not only was nonspecific adsorption of antibodies to protein bands inexistent but also the noncovalently bound reagent nitrotyrosine did not remain on the protein bands.

Nitrotyrosine was found on the heavy chain of both native skeletal and cardiac S1 (Figure 2, lanes b′ and e′). Digested cardiac and skeletal S1 showed strong radioactivity in the 50K peptide and in the 47K peptide (Figure 2, lanes c′ and d′).

A trace of radioactivity was occasionally noticed on the degraded LC1 and 20K peptide. This can be seen in Figure 3, showing the results of a particular experiment in the modification of digested cardiac S1, which was carried out in order to detect the labeling of peptides other than the 50K peptide. For a reaction time of 32 s, resulting in 70% inactivation, the distribution of the radioactivity on the transferred protein bands obtained by scanning the photograph with a Vernon microdensitometer was 91%, 6.7%, and 2.2% on the

50K peptide, the degraded LC1, and the 20K peptide, respectively (Figure 3, lanes 2 and 2'). However, since the transfer was better for the degraded LC1 and the 20K peptide than for the 50K peptide (see above), labeling was strongly preferential on the 50K peptide. In contrast, when the modification was carried out for a more extended duration of 90 s, the distribution of radioactivity was 76%, 1.9%, and 4.4% on the 50K peptide, the degraded LC1, and the 20K peptide, respectively (Figure 3, lanes 1 and 1'). In addition, 16% of the radioactivity was on a doublet peptide band of a molecular mass higher than 50K daltons. The identity of this doublet peptide was not examined, as its proportion was insignificant.

Discussion

The location of the carboxyl residue involved in the ATPase activity of the cardiac and skeletal myosins led us to perform a chemical modification on the subfragment 1 digested by trypsin. It was not possible to carry out a controlled proteolysis of the modified S1 despite the presence of ADP, which stabilized the S1 structure, because the modified S1 was precipitated after only a few minutes. However, modification of the fragmented S1 was possible because digested S1 maintains its Ca^{2+}-ATPase activity (see Table I) as previously observed (Mornet et al., 1979; Yamamoto & Sekine, 1979c). In carbodimiide modification, fragmented S1 showed the same kinetics of inactivation and the same stoichiometry of labeling as those of native S1. It therefore appeared likely that the reactivity of the essential carboxyl group did not change and that it was the same residue which was reactive in both fragmented S1 and native S1.

To locate the residues modified by carbodiimide treatment, the covalently bound nucleophile, nitrotyrosine, was detected by the use of its antigenic properties (Helman & Givol, 1971). We tried to use the direct, classical method employing ^{14}C-labeled nitrotyrosine ethyl ester. In our hands, this approach was unsuccessful because we could not completely remove noncovalently bound radioactive NTEE from the polyacrylamide gel. A similar difficulty in desorbing [^{14}C]glycine ethyl ester used as a nucleophile was mentioned by Rachamandran & Colman (1977). It should be pointed out that the use of [^{14}C]NTEE did not cause the same problems in the calculation of the stoichiometry (Lacombe et al., 1981; Körner et al., 1982), since the noncovalently bound nucleophile was eliminated by extensive dialysis by the use of NaOH solution. This washing procedure could not be used here, as it brought about uncontrolled cleavages in the peptide chains which were not suitable for location studies (not shown). It is likely that the efficiency of desorption of the immunological procedure (see Figure 2) could be explained by the extensive washings which included the use of detergents, gelatin, and especially specific anti-nitrotyrosine immunoglobulins. It might be postulated that, due to the affinity of the antibody with its antigen, this contaminating nitrotyrosine was specifically removed from the nitrocellulose sheets.

By the use of fragmented S1 and immunoblot detection, it was found (Figure 2) that the dicarboxylic acid which reacts with carbodiimide was located in the 50K peptide, and more precisely in the 47K peptide, which probably resulted from cleavage of a 2–3K peptide in one of the extremities of the 50K peptide. Only a very poor immunological reaction was observed on the degraded LC1 and the 20K peptide, in spite of the better transfer of these peptides onto the nitrocellulose sheets and the high sensitivity of this detection technique. Indeed, the sensitivity of this technique is (10–20) $\times 10^3$-fold higher than the sensitivity of the technique using [^{14}C]nitrotyrosine (specific activities 2×10^6 mCi/nmol of anti-nitrotyrosine immunoglobulins vs. 1×10^2 mCi/nmol of nitrotyrosine ethyl ester). Labeling of a single peptide is consistent with results of kinetic studies which suggested that only one amino acid residue was blocked by the reagent (Lacombe et al., 1981; Körner et al., 1982). However, in spite of first-order kinetic inactivation and the stoichiometry of labeling results (Lacombe et al., 1981; Körner et al., 1982), we cannot exclude the possibility of labeling of more than one residue of the 50K peptide. In that case, several residues would be partially labeled simultaneously with a global stoichiometry of one. The answer to this question will only be provided by identifying the modified residues and fragmentation of the 50K peptide.

The striking identity of both reactivity and localization of the modified residue in cardiac and skeletal S1 species suggests that it is part of a conserved structure.

This is the first demonstration that an essential amino acid residue is located in the 50K peptide. In addition, this 50K domain contains an actin-binding site (Mornet et al., 1981a; Yamamoto & Sekine, 1979b) and a natural marker, trimethyllysine (Mornet et al., 1981b; Flink & Morkin, 1982). Sukla et al. (1982) suggested that natural markers could regulate the catalytic functions by changing the rates of intermediate reactions; this seemed to be the case with SH_1 and the reactive lysine modifications, which caused marked inhibition of "intermediate oxygen exchange". We might therefore postulate that the 50K domain, which contains an active carboxyl residue, a natural marker, and a binding site for the potent natural modifier actin, plays an important role in such physiological activity of myosin.

At the present time, it appears that the three domains 27K, 50K, and 20K participate in the active site by spatial configuration. The 27K domain contains a reactive lysine residue (Mornet et al., 1980; Hozumi & Mühlrad, 1981; Miyanishi & Tonomura, 1981), a monomethyllysine, and a trimethyllysine (Mornet et al., 1981b; Flink & Morkin, 1982). The 20K domain of skeletal S1 contains a methylhistidine (Huszar & Elzinga, 1969; Mornet et al., 1981b) and the two so-called essential SH_1 and SH_2 groups (Elzinga & Collins, 1977; Balint et al., 1978; Cardinaud, 1979; Walser et al., 1981; Sutoh, 1981). Trapping of metal nucleotide by cross-linking these two thiols (Wells & Yount, 1980) suggests that this 20K domain is in some way related to the active site. The fact that the 27K domain (which is separated from the 20K domain by a stretch of 50K) is labeled by an azido derivative of ATP suggests the involvement of two spatially separated regions in the active site (Szilagyi et al., 1979). By chemical cross-linking, Mornet et al. (1981b) showed that actin binds to two domains, 50K and 20K, so that one S1 heavy chain contacts two actin monomers. By electron micrograph analysis, Amos et al. (1982) found two or more independent sites of contact between an individual S1 and F-actin. The fact that an essential carboxyl group is located on the 50K fragment indicates that this fragment also participated in the catalytic activity. The role of this residue has not been precisely determined at this time. This residue is not involved in the ATP binding site, both because ADP does not protect S1 against carbodiimide modification and because it slows down precipitation of the protein following modification (Körner et al., 1982). Blocking this residue brought about the loss of both phosphate burst and steady-state ATPase activity (Lacombe et al., 1981; Körner et al., 1982). These results suggest that this residue might be a participant in some ATP hydrolysis step (protein isomerization or ATP cleavage).

The results presented here are relevant to a structural study since they introduce a specific marker in the 50K fragment

which is stable and antigenic. Investigation is now in progress to define the nature of the modified amino acid residue and to more precisely locate its position.

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Registry No. CMC, 2491-17-0; NTEE, 53497-45-3; ATPase, 9000-83-3.

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