Probing Functional Regions in Cardiac Isomyosins with Monoclonal Antibodies†

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Received July 20, 1992; Revised Manuscript Received November 6, 1992

ABSTRACT: Seven Mabs prepared against subfragment 1 (S1) of either bovine cardiac β-specific or rabbit fast skeletal muscle myosin were used to identify functional regions in cardiac isomyosin heavy chains. This approach was designed to improve the understanding of structure–function relationships within the myosin molecule and between α and β myosin heavy chains (MHCs). We used bacterial expression of human β myosin fragments and determined that the seven antibodies were localized within four different MHC subdomains: amino acid residues 33–37 (one β-specific antibody), 67–84 (one α/β-specific antibody), 85–106 (four α/β-specific antibodies) and 215–248 (one α/β-specific antibody). All epitopes were accessible on myosin and actomyosin with the same affinities. Therefore, none of these MHC epitopes were located on the interfaces between the myosin head and actin. Three antibodies reacting at three out of the four investigated epitopes enhanced acto-S1 ATPase activities but not myosin, S1, or actomyosin activities. One antibody, which was strictly β-specific and bound to five amino acid residues near the most N-terminal MHC end, substantially inhibited all myosin or S1 ATPase activities measured with or without actin. The epitope of this antibody coincides with one difference cluster observed between both cardiac MHC isoforms [McNally et al. (1989) *J. Mol. Biol. 210*, 665–671], suggesting that this small variable MHC area could be one of the stuctural bases to explain observed functional differences in cardiac α and β myosin isoforms.

The hydrolysis of ATP by myosin in the presence of actin is the molecular basis of muscle contraction and plays an essential role in the overall cell motility process. Myosin is a hexameric protein molecule, comprising two heavy chains and two pairs of light chains. It has a long asymmetrical shape and is composed of two globular heads (at the N-terminus) with one rod segment (at the C-terminus). Myosins are encoded by a multigene family whose members exhibit tissue specificity and development regulation.

Some MHC[1] residues or larger MHC areas were found to be involved in the binding of actin, ATP, and LC1 within the myosin head [the so-called S1, residues 1–808 according to the amino acid numbering scheme of Mornet et al. (1989)], obtained by chymotryptic treatment of the whole myosin molecule. Using ATP analogs with cross-linking capacities, a few residues [i.e., Lys-83, Trp-130, Ser-180, Ser-243, and Ser-324; for review see Audemard et al. (1988)] were found to be close to the main ATP binding site. A wide variety of bifunctional chemical agents (Mornet et al., 1984; Hiratsuka, 1987; Lu & Wong, 1989; Maruta et al., 1990; Botts et al., 1989) have demonstrated a close proximity between the ATPase site and the two distal tryptic S1 fragments, the N-terminal 23-kDa (residues 1–206) and the C-terminal 20-kDa (residues 643–808) S1 fragments. Many lines of evidence obtained by various approaches (Mornet et al., 1981; Chaussepied et al., 1986; Morita et al., 1991; Trayer et al., 1991; Eldin et al., 1990, 1991), suggest that the acto–myosin interface may be composed of multiple contact sites, mainly occuring in the portion of the myosin head overlapping the central 50-kDa (residues 216–636) and 20-kDa S1 fragments. There could be another actin site in the 23-kDa fragment (Muhlrad et al., 1989, 1991). However, even if the S1/actin ratio is 1:1 in the rigor state, the S1 molecule would be able to bind two actin molecules (Andreev & Borejdo, 1991; Valentin-Ranc et al., 1991). On the other hand, the MHC region (residues 792–808) interacts with the MLC1, as revealed by limited proteolysis and mutagenesis experiments (McNally et al., 1991). Structural–functional analysis of myosin heads using specific anti-MLC and anti-MHC monoclonal antibodies (Winkelmann & Lowey, 1986; Dan-Goor et al., 1990; Dan-Goor & Muhlrad, 1991), has also provided some information. Antibody fixation experiments have established the functional role of certain MHC and MLC residues or areas. The use of antibodies to alter some enzymatic properties of myosin (Higashihara & Ikebe, 1990), or to influence contractile activities (Miyanishi et al., 1988; Lovell at al., 1988; Margossian et al., 1991), has also revealed the importance of certain conformational states within the myosin molecule (Reinach & Fischman, 1985; Van Eyk & Hodges, 1991; Cornillon et al., 1992; Alessi et al., 1992). These results indicate that myosin heads contain multiple sites where actin, ATP, and LC could bind in a cooperative or serial way.

The full primary structures of both α and β MHC isoforms expressed in rat (McNally et al., 1989) and human (Jaenicke et al., 1990; Matsuoka et al., 1991) hearts were recently determined. Most divergence sequences are grouped in four clusters within the myosin head (McNally et al., 1991). Interestingly, three divergence clusters correspond to presumed areas of MHC binding with ATP, actin, and MLC. One divergence cluster is located at the MHC N-terminal ends. These structural differences in cardiac isomyosins are probably the molecular basis of their ATPase activity differences and

† This work was supported by the Association Française contre les Myopathies (AFM).

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[1] Abbreviations: BCIP, bromo chloro indolyl phosphate; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; IPTG, isopropyl thiogalactoside; Mab, monoclonal antibody; MHC, myosin heavy chain; MLC, myosin light chain; NANDP, *N*-(4-azido-2-nitrophenyl)-2-aminoethyl diphosphate; NANTP, *N*-(4-azido-2-nitrophenyl)-2-aminoethyl triphosphate; NBT, nitro blue tetrazolium; S1, myosin subfragment 1; TLCK, N^{α}-tosyl-L-lysine chloromethyl ketone.

0006-2960/93/0432-2542$04.00/0

consequently provide a clue to the question of how heart functionally adapts to its physiopathological environment [for a review, see Swynghedauw (1986)].

In this study, we looked for functional areas or residues within the cardiac β myosin head using monoclonal antibodies. Their epitopes were determined through bacterial expression of overlapping segments of the MHC. Antibody effects on several myosin ATPase activities were recorded. These results are discussed in terms of myosin function itself and in light of functional differences between cardiac isoforms.

MATERIALS AND METHODS

Reagents. TLCK-treated α-chymotrypsin, trypsin, and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO). *Eco*RI linkers, restriction endonucleases, *Escherichia coli* DNA polymerase Klenow fragment, and DNase I were from New England Biolabs. The DNA ligation kit was purchased from Amersham. Oligonucleotides were synthesized by Appligene (Strasbourg, France). All other reagents used in the experiments were of analytical grade.

Proteins. Myosin was prepared from bovine left ventricular myocardium (Klotz et al., 1981) and proteolyzed with chymotrypsin to produce S-1 and rod subfragments. S1 was then isolated by centrifugation at low salt (Weeds & Taylor, 1975). S1 was cleaved by trypsin to produce a mixture of the three S1 subfragments: 23, 50, and 20 kDa. Each fragment was then purified by the method of Klotz et al. (1983). F-Actin was prepared from rabbit skeletal muscle as described by Mornet and Ue (1984).

Production of Monoclonal Antibodies. Monoclonal antibodies were produced according to Köhler and Milstein (1975). Chymotryptic S1 used as antigen was from two origins: bovine ventricule or rabbit fast skeletal muscle. The procedures for mouse immunization, cell fusion, hybridoma screening and cloning, and monoclonal antibody production have been previously described (Dechesne et al., 1985). Purification of mAbs from ascitic fluid (11E10) or high-volume hybridoma cultures (other mAbs) was performed on Affi-gel Protein A columns (Bio-Rad) as recommended by the manufacturer.

Construction of Recurrent Overlapping Expression Clones. A human βMHC cDNA fragment coding for residues 1–524 was excised from the pGM2 plasmid (a generous gift of H.-P. Vosberg) by *Nco*I and *Hin*dIII cleavage. This 1585-bp fragment was subcloned into ptrc99 vector (Pharmacia). Its expression in *E. coli* strain TB1 led to one 66-kDa soluble fragment (referred to as βHC13). Shorter constructs were derived from βHC13 by deletion of 3′ fragments using unique cutter restriction enzymes. βHC12′ and βHC16 were obtained by deletion of *Eco*RI–*Hin*dIII and *Dra*II–*Hin*dIII fragments, respectively. They led to the expression of 32- and 23-kDa protein fragments spanning residues 1–248 and 1–149, respectively. The longer construct (βHC17) was obtained by in vitro ligation of the βHC13 to cLR insert (Eldin et al., 1990) encoding residues 362–1083, by a common unique *Bgl*II site. βHC17 encoding residues 1–1083 led to the expression of a 120-kDa protein fragment. These fragments were expressed by IPTG induction at 37 °C of TB1 cells, and total soluble extracts were used in Western blots.

Construction of an Epitope Library. The method used was essentially as described (Stanley & Hertz, 1987) with minor modifications. Standard procedures for preparation and manipulation of plasmid DNA were used (Sambrook et al., 1989). Ten micrograms of the same 1585-bp human β myosin cDNA fragment was digested with DNase I under carefully precalibrated conditions to give fragments with a mean length approximately 100–150 bp. Fragments were repaired using *E. coli* DNA polymerase and purified by agarose gel electrophoresis. Unphosphorylated 8-, 10-, and 12-bp *Eco*RI linkers were ligated to the polished fragments in three separated ligations. The fragments were then cleaved with *Eco*RI and fractionated on a 2% agarose gel. DNA bands (100–200 bp) were excised from the gel and then extracted and precipitated with ethanol/ammonium acetate. The recovered cDNA fragments were ligated to pEX2 vectors, previously linearized by *Eco*RI and treated with alkaline phosphatase. Competent *E. coli* cells pop2136 were transformed by electroporation with the ligated products to generate a library of approximately 10^4 cells. Cells were routinely grown at 30 °C and then for 2 h at 42 °C for the expression of peptide sequences as β-galactosidase fusion proteins. Colony blots of approximately 4000 individual cells were prepared and screened as described (Stanley & Luzio, 1984). Blots were probed with a mix of preselected monoclonal antibodies (culture supernatant) and developed with an alkaline phosphatase rabbit anti-mouse second antibody with an NBT/BCIP substrate system. Each positive colony was isolated and tested with each monoclonal antibody by Western blotting. The cDNA fragments of positive clones were identified by double-stranded DNA sequencing of plasmid DNA. The sequencing template was prepared from 100 mL of overnight cultures using a standard method (Sambrook et al., 1989). Sequencing was carried out using the T7 sequencing kit from Pharmacia according to the manufacturer's protocol, and products were analyzed on 8% sequencing gels. The primers used were (5′) GGTGGC-GACGACTCCTG and (3′) TAGAGCCGAGTCGATCC, located 15 bp upstream and downstream from the *Eco*RI cloning site of the pEX2 vector, on the coding and noncoding strands, respectively.

ATPase Measurements. Actin- and calcium-activated ATPase activities were assayed by measuring phosphate release using the microassay-malachite green method (Henkel et al., 1988). S1 (0.4 μM) and myosin (0.2 μM) were preincubated with different dilutions of monoclonal antibodies (Mab/S1 ratios ranging from 2 to 3) at 4 °C for 16 h before the assay in buffer A (50 mM Tris-HCl, pH 8.0, 10 mM KCl, and 2 mM $MgCl_2$), with 0.4 μM S1 or 0.2 μM myosin, for actin-activated ATPase or in buffer B (50 mM Tris-HCl, pH 8.0, 10 mM KCl, and 5 mM $CaCl_2$) for calcium-activated ATPase. Reactions were carried out in a 50-μL final volume and started by the addition of 0.2 mM ATP. The reaction was run at 25 °C for 15 min. Actin-activated ATPase activity was measured in the presence of 20 μM F-actin.

Affinity Measurements. Monoclonal antibodies' binding to cardiac myosin and S1, in the presence or absence of 20 μM F-actin were assayed by ELISA according to the method of Friguet et al. (1985) as previously described (Cornillon et al., 1992) in buffer A containing 1% BSA.

Miscellaneous. SDS–polyacrylamide gel electrophoresis and Western blotting were performed as described by Eldin et al. (1990).

RESULTS

Mabs were raised against either bovine ventricular β or rabbit fast skeletal muscle myosin subfragment 1. Their specificities for other cardiac α and β MHC isoforms and their putative influence on different β S1 or myosin ATPase activities were investigated.

Antibody Characterization. The available antibodies were initially tested for their location on one of the three usual (23,

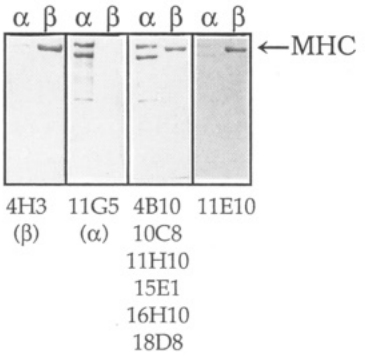

FIGURE 1: Western blots of purified cardiac myosin isoforms. Western blots of purified myosin isoforms were performed with the seven Mabs following SDS–10% PAGE size fractionation and electroblotting on nitrocellulose membranes. They were compared with two anti-α and anti-β specific controls. α isoform was extracted from bovine atrium and purified by several passages through a 4H3 (anti-β) immunoaffinity column in order to remove all contaminating β myosin. The same procedure was used to purify β isoform extracted from bovine ventricule and purified on a 11G5 (anti-α) affinity column. α myosin degradation was frequently observed from atrium extractions as shown by the double-band pattern. Only 11E10 was β-specific whereas the six other Mabs reacted with both isoforms.

50, and 20 kDa) S1 subdomains resulting from tryptic MHC digestion within the native myosin head (Balint et al., 1975). Seven antibodies were selected: six reacted with the N-terminal 23-kDa fragment and one with the central 50-kDa fragments (not shown). Their specificities for each of the other cardiac isomyosins were investigated by two complementary methods: immunofluorescence staining of rat atrium cryostat sections (not shown) and comparative Western blots on α and β affinity-purified bovine cardiac myosins (Figure 1). The α versus β selectivity was controlled using two anti-myosin rod antibodies [11G5 (anti-α) and 4H3 (anti-β)], which were previously well documented (Dechesne et al., 1985; Bouvagnet et al., 1987). All antibodies except one (11E10) cross-reacted with both α and β MHC isoforms. 11E10 appeared as a β-specific antibody since it stained the same fibers as 4H3 (anti-β) and recognized only the β MHC in Western blots.

Four Anti-MHC Antibodies Induced ATPase Activity Alterations. ATPase activities under either Ca^{2+}-activated or Mg^{2+}-actin-activated conditions were determined using bovine ventricular S1 or myosin in the presence of the seven affinity-purified anti-S1 antibodies. Four Mabs (10C8, 11E10, 11H10, and 16H10) altered ATPase activities, while three Mabs (18D8, 4B10, and 15E1) had no effect (Figure 2). In fact, 11E10 substantially inhibited (30–60%) the four ATPase activities measured. 10C8, 11H10, and 16H10 moderately increased actin-activated S1 ATPases by approximately 25% but had no effect or only a weak effect on the three other activities. 18D8 (as 4B10 and 15E1) had no effect on any ATPase measured. Briefly, 11E10 appeared to be a strong inhibitor of all ATPase activities, whereas 10C8, 11H10, and 16H10 moderately accelerated actin-activated S1 ATPase activity only. For all antibodies studied, inhibition or activation of Ca^{2+}-activated and actin-activated S1 ATPases plateaued at antibody/antigen ratios of 1.

Affinity measurements of each Mab for both bovine β-specific S1 and myosin with or without actin in solution indicated that the Mab affinities were similar for myosin and actomyosin at 0.12 ionic strength (Table I). In the same ionic conditions, Mab affinities for S1 were relatively weaker than for myosin. The differences were highly significant for Mabs

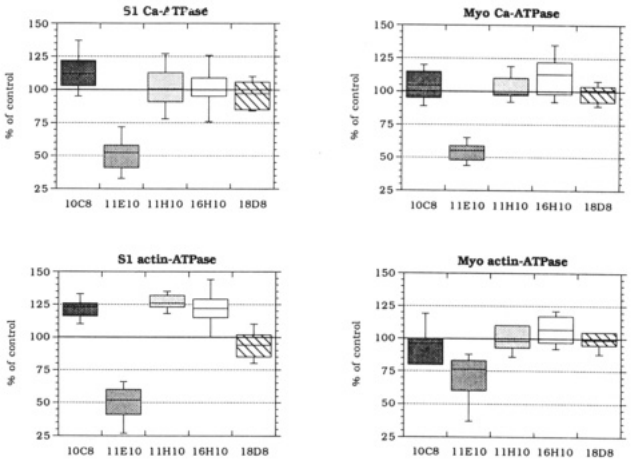

FIGURE 2: Antibody effects on myosin and S1 ATPase activity. Results are expressed as percent of control S1 or myosin ATPase activity and represented in boxes. Horizontal bar in the box corresponds to the mean data values. Boxes contain 50% of the values. Vertical bars represent minima and maxima of the data. The number of determinations for 10C8, 11E10, 11H10, 16H10 and 18D8 are respectively 12, 30, 10, 13, and 7 for S1 Ca-ATPase; 13, 26, 27, 25, and 6 for S1 actin-ATPase; 6, 18, 6, 6, and 5 for Myo Ca-ATPase; and 7, 24, 8, 11, and 6 for Myo actin-ATPase.

Table I: Antibody Binding Constants[a] for Myosin and S1 in the Presence or Absence of F-Actin

	myosin		S1	
mAb	–actin	+actin	–actin	+actin
11E10	15 ± 10 (4)	20 ± 10 (3)	64 ± 22 (4)	90 ± 20 (3)
10C8	43 ± 10 (3)	32 ± 20 (3)	200 ± 20 (3)	110 ± 80 (3)
11H10	5 ± 2 (11)	7.2 ± 5 (6)	73 ± 50 (11)	18 ± 8 (5)
16H10	150 ± 40 (3)	210 ± 60 (3)	1300 ± 300 (3)	400 ± 200 (3)

[a] Dissociation constants (K_d) are expressed in nanomolar ± SD. The number of determinations is indicated in parentheses.

11E10 and 16H10. Actin fixation on S1 substantially decreased the differences between S1 and myosin.

Localization of Antibody Epitopes. Two complementary methods, using different recombinant fragments of cDNA encoding the human β myosin heavy chain, were used to localize epitopes of the seven antibodies. Antibody reactivities were tested on overlapping recombinant myosin fragments spanning different parts of the whole S1 region (Figure 3A). Six antibodies (excluding 11H10) reacted with the four recombinant fragments, indicating that the corresponding epitopes were concentrated within the MHC fragment (βHC16) containing residues 1–149. The 11H10 antibody recognized the βHC12′ MHC fragment containing residues 1–248. As noted, 11H10 also reacted with the 50-kDa tryptic MHC fragment, which probably starts after lysine residue 214 in the human β MHC sequence (Jaenicke et al., 1990; Mornet et al., 1981). Consequently, we estimated that the 11H10 epitope was located between residues 215 and 248.

Epitopes of the five anti-23-kDa MHC fragment were more precisely mapped by probing an epitope library, encoding about 20–40 amino acids, constructed from a cDNA fragment encoding residues 1–524 of the human β MHC. An example of this epitope mapping procedure is shown for 10C8, 11E10, and 16H10 antibodies (Figure 3B). The reactivity of antibody 11E10 with fragments e, d, and b demonstrated that its epitope was enclosed in a MHC region from residues 23 to 39. The 16H10 epitope (as those of 4B10, 15E1, and 18D8) was located in segment c but not in segment a. They were located within the MHC region extending from residues 85 to 106. Moreover, the 10C8 epitope present in segments a and c was estimated

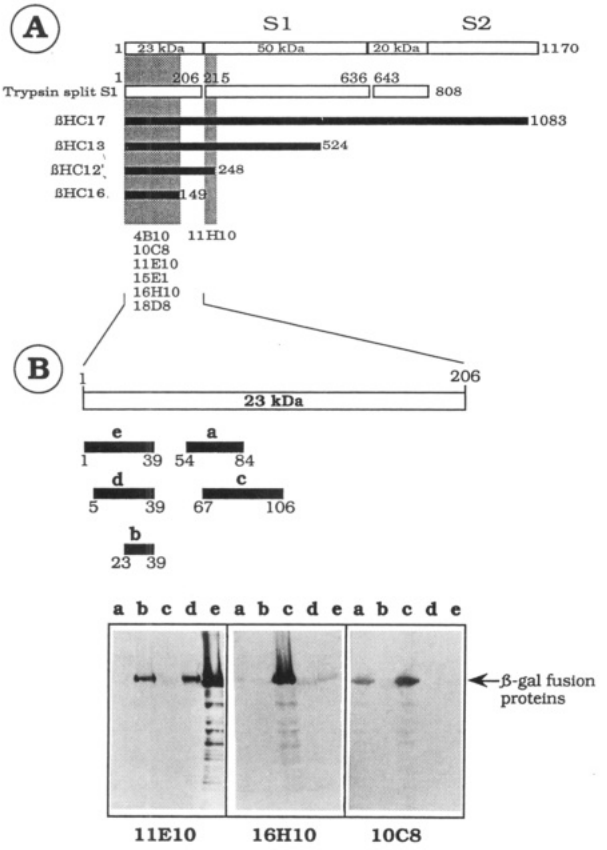

FIGURE 3: Epitope mapping with bacterially expressed human β myosin fragments. (A) Overlapping β MHC fragments expressed in *E. coli* generated by recurrent cloning. Overlapping constructs were obtained by restriction cloning as outline in the Materials and Methods and cloned in the ptrc99 expression plasmid. Fragments were expressed in *E. coli* by IPTG induction (0.5 mM). Soluble extracts were used in Western blot analysis to test mAb reactivity. 11H10 reacted with βHC17, βHC13, βHC12′, and the 50-kDa S1 tryptic fragment but not with βHC16. The six other Mabs stained all of the recombinant fragments and the 23-kDa S1 tryptic fragment. (B) Epitope mapping with β myosin short fragments expressed in *E. coli* generated by random cloning. The epitope library was constructed as outline in the Materials and Methods in the pEX2 expression plasmid. Positive clones were isolated and separately expressed as β-galactosidase fusion peptides by increasing the culture temperature from 30 to 42 °C. Fusion proteins were purified from inclusion bodies and used in Western blots to analyze Mab reactivities.

as being located between residues 67 and 84. A sequence comparison of α and β human myosin heavy chains revealed that 11E10 specificity was actually due to the five-residue LKKDV motif only present in the β MHC (Figure 4). In summary, the present epitope mapping revealed four main immunodominant regions in the N-terminal part of the β myosin head: residues 33–37 (11E10), 67–84 (10C8), 85–106 (4B10, 15E1, 16H10, and 18D8), and 215–248 (11H10) (Figure 4).

DISCUSSION

Monoclonal antibodies that react with well-defined regions of cardiac isomyosin heavy chains have been used as probes to identify MHC sequences possibly involved in isomyosin functions such as enzymatic activities and actin interaction. The chymotryptic myosin S1 heavy chain, which has actin-binding and ATPase activities, was chosen as an adequate target for producing monoclonal antibodies to putatively detect functionally important myosin areas.

Fine Delineation of Antibody Epitopes. The Mabs obtained using two different intact chymotryptic S1s from either β cardiac or fast skeletal muscles as antigens were selectively directed against the first N-terminal half of the myosin head (between residues 1 and 524). This part of the S1 molecule contains dominant antigenic determinants as previously noted (Winkelmann & Lowey, 1986; Miller et al., 1989). Our epitope mapping techniques revealed that the seven selected antibodies were localized within four different MHC subdomains containing 5–30 residues (Figure 4). These epitopes corresponded to MHC regions which are either mobile and/or accessible and/or which differ according to the myosin isoform (Westhof et al., 1984).

(i) Mabs 4B10, 15E1, 16H10, 18D8, and 10C8 bind to a mobile region that undergoes important structural changes through ATP binding (Miyanishi et al., 1988; Muhlrad, 1977).

(ii) The Mab 11H10 epitope is located close to a highly isoform-divergent (McNally et al., 1989; Babij et al., 1991) and protease-sensitive region between the 23- and 50-kDa MHC fragments, which also contains a very conserved segment (ILEAFGNAK). This MHC region is thus accessible and probably on the surface of the myosin head. However, no isoform-specific antibody has been obtained in this S1 area, particularly within the difference cluster between the cardiac isomyosins (McNally et al., 1989).

(iii) The β-specific Mab 11E10 reacts with the β-specific residues very close to the S1 N-terminal end. This MHC regions, which is trypsin-sensitive in the presence of ATP, is presumably mobile and located on the surface of the myosin head (Winkelmann & Lowey, 1986). Large amino acid sequence variations between numerous myosin isoforms have been reported in this MHC region.

None of the seven Mabs recognized MHC regions containing "sensitive" residues such as Trp-130 [labeled with an azidoaryl-ATP analogue (Szilagyi et al., 1979) and with NANDP (Sutoh, 1987)], Ser-180 [lying within one of the ATP "consensus" sequences, labeled by MgADP-vanadate (Cremo et al., 1989)], or Ser-324 [labeled with NANTP (Nakayame et al., 1985)] or a 10-kDa peptide derived from the C-terminus end of the 50-kDa fragment, labeled with Bz_2ATP (Mahmood et al., 1987)). These residues involved in cross-linking are easily reached within the S1 core near the nucleotide pocket because of the small size fo the various ATP analogs. Since the same residues were inaccessible for antibody production, they must be buried. This demonstrates the intrinsic differences between the immune response and the myosin sensitivity to ATP analogs. However, this does not explain the steady lack of production of any antibody against the 400 residues of the S1 C-terminal sequence. This MHC segment, which appears (at least with respect to the 20-kDa domain) to be very elongated and accessible in the most recent images of myosin heads (I. Rayment, personal communication, 1992), contains two highly mobile and variable regions: residues 618–630 (the connecting region near the 50/20 junction interacting with actin; Chaussepied et al., 1986) and 795–820 (the light/heavy chain interacting site). Destabilization of the native conformation of this myosin segment and its self-aggregation, observed when the LCs are removed (Schaub et al., 1986), could partly explain the poor immunogenicity of this MHC region.

Identification of Sites on the β Myosin Head That Are Critical for ATPase Activity. Four of the seven anti-MHC Mabs tested appeared to have epitopes at or near "functional" MHC areas, since they partially modified some myosin enzymatic activities (Figure 2). The involvement of epitope residues in conformational changes in the overall enzymatic process of the myosin molecule could depend on Mab size and

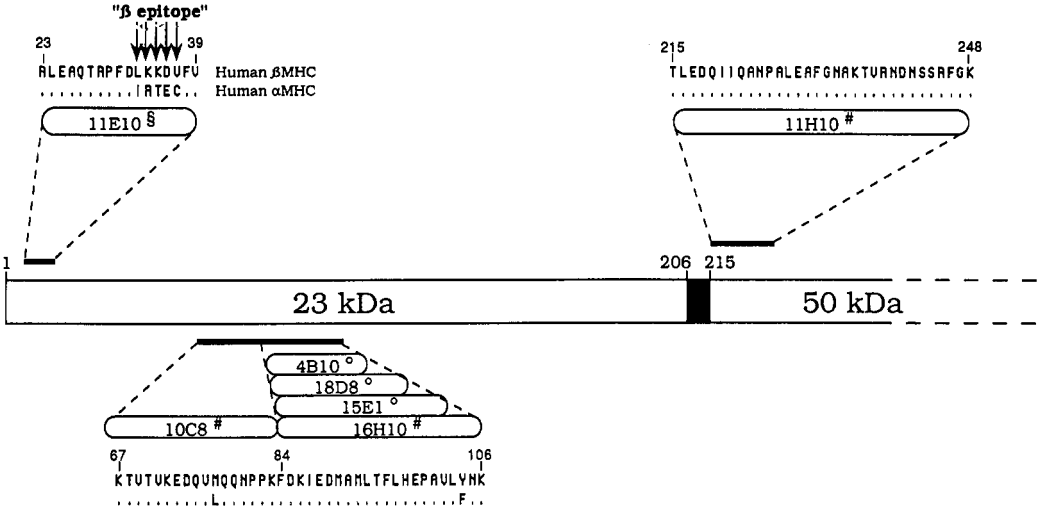

FIGURE 4: Epitope locations of the seven anti-S1 monoclonal antibodies. Amino acid residues contained in each epitope are represented with comparisons of α and β MHC. The human α isoform sequence was from Matsuoka et al. (1991) and the human β isoform sequence was from Jaenicke et al. (1990). Antibody effects on ATPase activities are indicated with the following symbols: §inhibition of all ATPase activities, #activation of acto-S1 ATPase, and °no effect.

their influence on myosin ligand accessibility (ATP, actin). The present results indicate that none of the four "informative" antibodies prevented accessibility or fixation to the actin filament since their affinities for myosin were actin-insensitive (see Table I). They confirm that the main acto–myosin interface is probably located in the C-terminal part of the myosin head. No direct information on the acto–myosin interface was obtained from the present Mab set.

Since fixation of Mabs 10C8, 11H10, and 16H10 only modified S1 actin ATPase activity with no effect on the other three activities measured, the corresponding epitope residues were probably not directly involved in ATP fixation itself. Fixation of the three Mabs could subtly alter the overall ATPase activity process by modifying (or reducing) the mobility of certain MHC residues contained in the related epitopes. Previous reports indicate that the three epitopes analyzed contain a few residues implicated in the communication pathway between ATP and actin binding sites (Botts et al., 1984): (i) Mabs 10C8 and 16H10 react with a region that is reported to be involved in the energy transduction process associated with structural changes (Miyanishi et al., 1988), in the vicinity of the reactive lysyl residue (Lys-83) whose chemical blocking suppresses ATP hydrolysis. (ii) Mab 11H10 reacts with the same MHC area as that reported for another antibody inhibiting acto-S1 ATPase activity in chicken skeletal muscle (DanGoor et al., 1990). Although both antibodies have unexplained opposite effects on ATPase activity, this confirms the clear involvement of this MHC area in actin-activated myosin ATPase activity. Note also that the conformation of this region is modified when trypsin cleavage occurs at the 23/50-kDa junction (Chen et al., 1987) and that the 11H10 epitope contains or is near Ser-243, which has recently been labeled with MgATP-vanadate. Allof these results suggest structural interdependence of the 23- and 50-kDa regions with spatial proximity between the first 30 N-terminal residues of the 50-kDa region and the nucleotide fixation site.

The affinity measurements with Mab 11E10 and its clear-cut inhibitory effects on all four measured enzymatic activities are of potential interest. Obviously indirect steric blockage due to antibody size could explain the effects of Mab 11E10 fixation on myosin activities. However, sensitivity of the N-terminal part of S1 for ATPase activities was previously observed using two different Mabs and their Fab fragments directed against the first 40 N-terminal residues of chicken skeletal myosin (Winkelmann & Lowey, 1986). It has also been reported that one Mab directed against the rabbit skeletal muscle MHC N-terminal end has no effect on myosin activities (Dan-Goor et al., 1990). These different results emphasize the crucial role of the N-terminal myosin segment in ATP fixation and its consequent hydrolysis. The present results also indicate that a few or all five MHC residues (33–37) of the Mab 11E10 epitope can directly or indirectly alter ATPase activities of β-specific cardiac isomyosin, thus indicating a functional role for one of the seven difference clusters between cardiac α and β MHC isoforms (McNally et al., 1989; Matsuoka et al., 1991). This provides experimental support for the hypothesis that these clusters of nonidentical amino acid residues are partially responsible for the observed functional differences between α and β myosin isoforms.

ACKNOWLEDGMENT

The authors thank Professor H.-P. Vosberg for providing the pGM2 clone and the ptrc99C expression vector.

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