Novel Staining Pattern of Skeletal Muscle M-Lines upon Incubation with Antibodies against MM-Creatine Kinase

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ABSTRACT Incubation of chicken skeletal muscle fibers with an excess of anti-M-creatine kinase (CK) immunoglobulin G and an excess of anti-M-CK Fab fragments leads to heavy decoration of the M-line (Wallimann, T., D. C. Turner, and H. M. Eppenberger, 1977, *J. Cell Biol.* 75:297–317) and to removal of the electron-dense M-line structure (Walliman, T., G. W. Pelloni, D. C. Turner, and H. M. Eppenberger, 1978, *Proc. Natl. Acad. Sci. USA.*, 75:4296–4300), respectively. On the other hand, incubation with low concentrations of monovalent anti-M-CK Fab did not extract but rather decorated the M-line, giving rise to a distinct two-line staining pattern. A similar double-line staining pattern, although less pronounced, was also observed within the M-line of paraformaldehyde-prefixed myogenic cells, which after permeabilization were incubated with low concentrations of divalent anti-M-CK antibody. In both cases, the two decorated lines appearing in the middle of the A-band were spaced axially 42–44 nm apart and correspond most likely to the two M4 and M4′ m-bridge rows described by Sjöström and Squire (1977, *J. Mol. Biol.*, 109:49–68; 1977, *J. Microscopy.*, 111:239–278). It is concluded that the muscle-specific form of creatine kinase, MM-CK, contributes mainly to the electron density of these M4 and M4′ m-bridges within the M-line structure. This specific labeling pattern is a further demonstration that CK is an integral part of the M-line.

The electron-dense M-line in the center of the A-band is one of the striking structural features of cross-striated muscle myofibrils seen in the electron microscope. Thin sections of skeletal muscle fixed in situ after plastic-embedding reveal three to five main transverse substriations within the M-band structure (10, 14, 27). On the basis of these and additional structural details seen in well-oriented longitudinal and transverse sections, Knappeis and Carlsen (14) proposed an M-line model of which the general features have since been confirmed. According to these authors, the M-line consists of an array of m-filaments (~75-nm long) parallel to the thick filaments and linked to the thick filaments by transverse m-bridges. Each of the three (or five, depending on fiber type) principal substriations, spaced by 22 nm, seen in longitudinal sections corresponds to an array of m-bridges. Considerable progress in resolving the structural details of the complex M-line structure has been made by ultracryotomy of muscle tissue prefixed in situ (32, 33). After negative staining, ultrathin frozen sections of skeletal muscle revealed a host of details not seen before, and several more transverse substriations became apparent. Besides the three main transverse lines made of primary m-bridges, the presence of secondary m-bridges has been suggested, which led to the proposal of modified M-line models (17, 32, 33).

Parallel to the structural work, considerable effort has been made to elucidate the biochemical composition of the M-line. So far, two proteins are known to reside within the M-line. In 1968, Masaki and co-workers (23) reported on the existence of an "M-substance", which thereafter was described as the M-protein of M_r 165,000 (24). This protein, called myomesin by Eppenberger et al. (8), attracted considerable interest and its localization at the M-line has since been confirmed (7–9, 16, 18, 26, 35, 37).

Another M-line protein originally isolated from skeletal muscle (25) has been identified as the muscle form of creatine kinase (CK; MM-CK) (38). Immunochemical and electron microscopic studies have since made it clear that a small but significant amount of the total CK present in muscle is specifically bound to the M-line (40, 41, 43). Incubation of chicken skeletal fiber bundles with antibodies against MM-CK led to heavy decoration of the M-line structure without revealing finer details (43). Incubation with an excess of monovalent anti-M-CK Fab, on the other hand, led to a complete removal of the electron-dense material within the M-line, concomitantly

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releasing all the bound CK (43). For the above reasons and because most of the electron-dense M-line structure seems to be composed of m-bridge material (14), MM-CK was assigned to represent the m-bridges (40, 41, 43). This is supported by the fact that the amount of CK activity that is released from the M-line by low salt buffer and the molecular dimensions of MM-CK dimers are consistent with the number and dimensions of the m-bridges (1, 20, 25, 40, 41, 47). In addition, no CK is bound to the M-region in chicken heart muscle (42), a tissue lacking both the MM-form of CK and a clear electron-dense M-band (34).

To further investigate the localization of MM-CK in the M-line, we have incubated skeletal muscle fibers with various concentrations of anti-M-CK antibodies. We report here a novel two-line staining pattern within the M-line obtained after incubation with low concentrations of anti-M-CK Fab and immunoglobulin G (IgG). As a consequence, MM-CK is tentatively assigned to the two M4 and M4′ m-bridge substriations

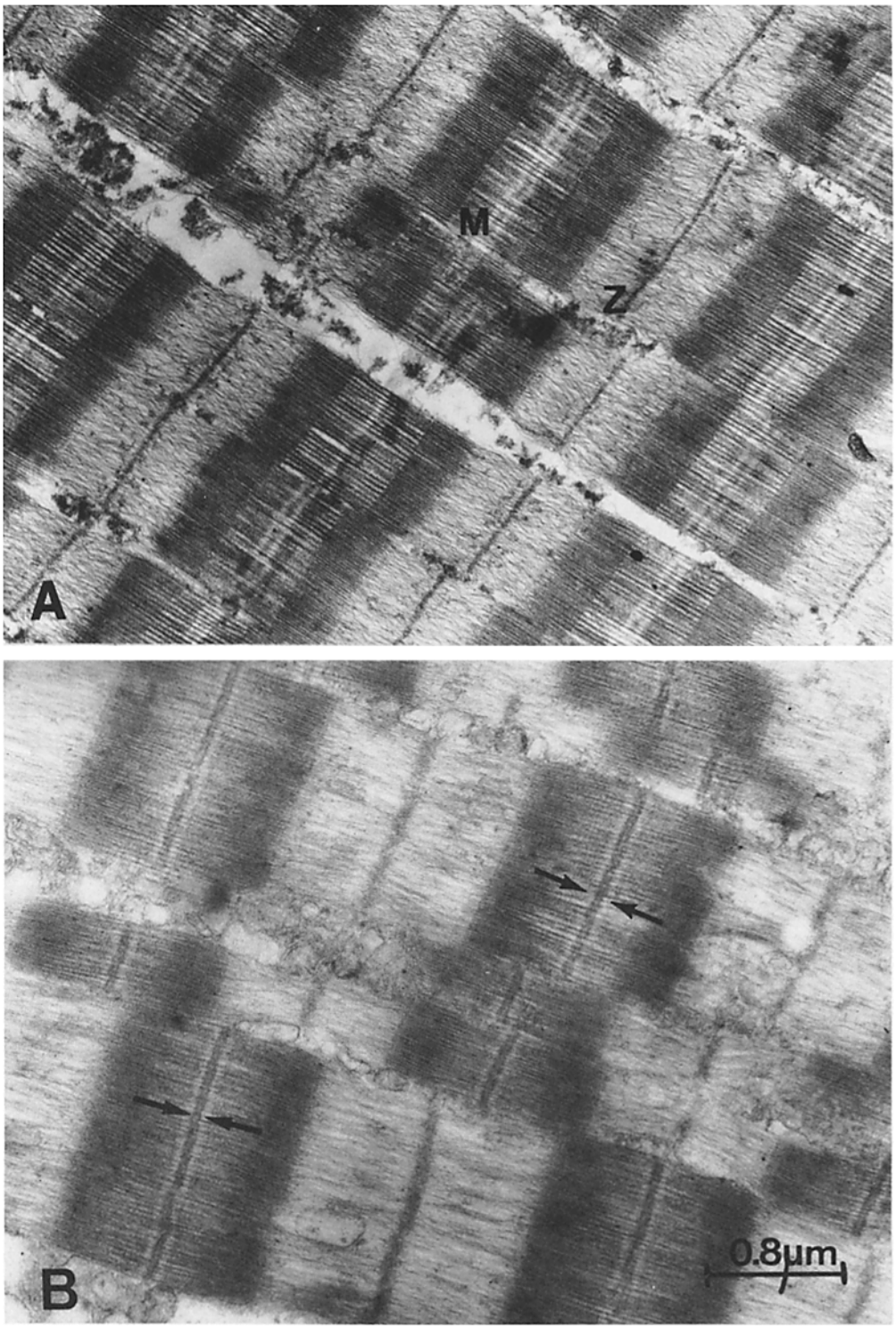

FIGURE 1 Thin sections of glycerinated chicken skeletal fiber bundles after incubation with 0.1 mg/ml of each control Fab (*A*), and anti-M-CK Fab (*B*). Note increase in electron density and appearance of a double-line staining pattern marked by arrows in (*B*). *M*, M-line; *Z*, Z-band. × 22,000.

described by Sjöström and Squire (32, 33) that correspond to the two lateral of the three main m-bridge arrays described by Knappeis and Carlsen (14), Pepe (27, 28), and others (10).

MATERIALS AND METHODS

Preparation of Monovalent Antibody Fab Fragments: Fab fragments of affinity-purified anti-M-CK IgG and of control IgG were made under identical conditions, essentially according to Porter (30) with modifications as described in references 43 and 45. Preactivated papain (Sigma Chemical Co., St. Louis, MO) was added to IgG at a ratio of 0.5 mg/100 mg IgG. After 8–12 h of digestion at 37°C in the presence of 10 mM cysteine, 1 mM EDTA, and 0.1 M P_i at pH 7.0, papain activity was blocked by the addition of freshly made P_i-buffered iodoacetic acid at pH 7.2 to give a final concentration of 20–23 mM. After 30 min at 25°C, the samples were dialyzed extensively against 0.1 M KCl, 1 mM EGTA, 5 mM EDTA, pH 7.0, centrifuged, and the supernatant was stored frozen. The extent of digestion was tested by SDS PAGE.

Incubation of Muscle Fiber Bundles with Antibody: Fresh chicken breast muscle fibers were prepared and glycerinated as described (43). After removal of glycerol by washing with 0.1 M KCl containing 1 mM EGTA, 5 mM EDTA, and 1 mM DTT at pH 7.0, small fiber bundles (0.2–0.5 mm in diameter and 0.5–1.0 cm long) were incubated for 12–24 h at 4°C either with Fab of affinity-purified anti-M-CK IgG that had been characterized by immunoprecipitation, immunodiffusion, immunoelectrophoresis, microcomplement fixation, and immunoblotting onto nitrocellulose (5, 29, 39, 41, 43, 46) or with Fab of preimmune IgG at concentrations ranging from 0.1 to 5 mg/ml. The first supernate was saved and the proteins released by the treatments were concentrated by addition of 4 vol of acetone prechilled to −10°C. The precipitate was redissolved in a small volume of SDS gel buffer (15) and subjected to either 5 or 10% PAGE using the Laemmli system (15). Unbound antibody was removed from fiber bundles by three consecutive washings with the above buffer during a 2-h period. Fibers were fixed, dehydrated, and embedded as described (41, 43). Longitudinal sections were poststained with 5% uranyl acetate in 75% ethanol for 1–3 h, followed by lead citrate. A Philips EM 300 microscope was used at 100 kV.

Incubation of Myogenic Cells with Anti-M-CK Antibody: Myogenic cells derived from 12-d-old chicken embryos were cultured as described earlier (39). After the plates were rinsed with phosphate-buffered physiological salt solution (PBS), cells were fully fixed in situ with 3% paraformaldehyde in PBS (this solution was stored frozen) at pH 7.2–7.5 for 15 min. After three washes with PBS, traces of paraformaldehyde were quenched by three changes of 0.1 M glycine in PBS, pH 7.5, for 30 min. Permeabilization of cells for subsequent staining with antibodies was achieved by incubation of cells for 4 min with 0.2% Triton X-100 in PBS. After three washes with PBS, the fully fixed cells were ready for immunostaining. After PBS was aspirated from the culture plates, 100–200 μl of specific anti-M-CK serum or IgG (5–10 μg/ml) diluted 1:50 was placed on selected areas on the culture dish. After incubation for 30 min followed by three washings with PBS, 100–200 μl of goat anti-rabbit antibody diluted 1:100 (Cappell Laboratories, Inc., Cochranville, PA) was placed on the same area and the cells were incubated for another 30 min. After three washes with PBS, the cells were processed and embedded for electron microscopy as described above.

Other Methods: Proteins of the supernatants of antibody-treated muscle fibers were analyzed by PAGE in SDS (15), and CK was identified by immunoreplication by using anti-M-CK antibodies as described earlier (43).

RESULTS

After incubation of glycerinated chicken skeletal muscle fiber bundles with low concentrations (0.1 mg/ml or less) of monovalent anti-M-CK Fab, a clear double-line antibody decoration pattern at the M-line was often observed (Fig. 1 *B*). A similar double-line pattern, although somewhat more diffuse and less clearly resolved, was also seen in myogenic cells after fixation and incubation with low concentrations of anti-M-CK IgG (Fig. 2). For visualization of this pattern with the larger IgG antibodies as compared with Fab, low concentrations of anti-M-CK antibody were used in this case on paraformaldehyde-prefixed myogenic cells and the IgG bound at the M-line was

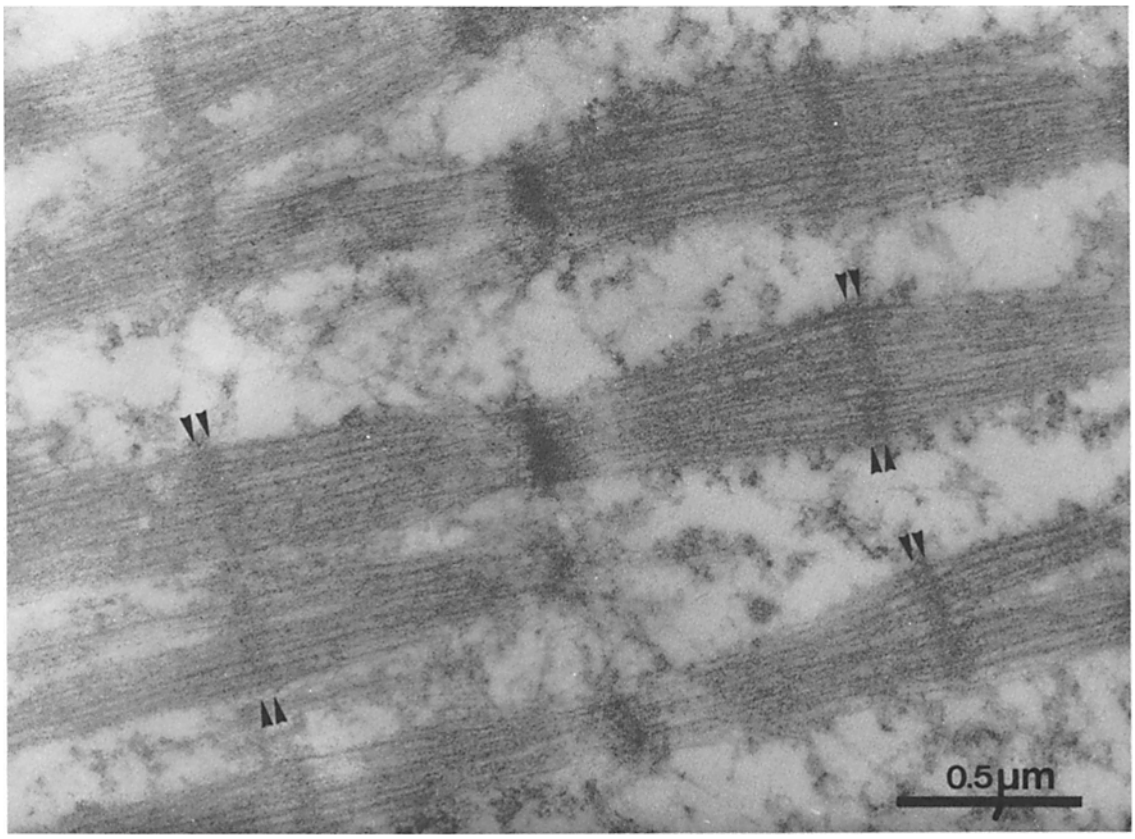

FIGURE 2 Thin section of paraformaldehyde-prefixed myogenic cells grown in culture for 7 d, after permeabilization with 0.24% Triton X-100 and subsequent staining with 1:50-diluted antiserum specific for M-CK followed by contrasting with 1:100-diluted second antibody. The appearance of a double-line staining pattern within the M-line is indicated by arrowheads. × 49,000.

contrasted by a diluted second antibody (Fig. 2). However, the double-line pattern was also observed without a second antibody (not shown). The two heavily decorated transverse lines each ~21–22 nm off center from the middle of the A-band were always separated by ~42–45 nm (Fig. 1 *B*, as pointed out by arrows; Fig. 2, arrowheads; Fig. 3 *A*, arrows). No such decoration was observed with Fab from control IgG (Fig. 1 *A*) or with control IgG (not shown). The double-line staining pattern is shown at higher magnification in Fig. 3 *A*. As reported earlier (43) incubation of fibers with higher concentrations of anti-M-CK IgG (~1 mg/ml) led to such a heavy decoration of the M-line structure that a double-line staining pattern could not easily be resolved (43). However, in some cases, hints of a double-line could even be seen after heavy labeling with anti-M-CK IgG as shown in Fig. 3 *B*. Incubation of myofibrils with an excess of anti-M-CK IgG makes the M-line-bound CK unextractable by low ionic strength buffer (5 mM Tris/HCl, pH 7.8; 25, 41), which in fibers treated with control IgG dissociates the M-line-bound CK and concomitantly extracts most of the electron density from the M-line structure (25, 41).

On the other hand, incubation of fiber bundles with higher concentrations of anti-M-CK Fab led to a removal of the visible M-band by extracting most of the electron density of this structure (Fig. 4 *b*; 43). Figure 4 *b* illustrates the extent of removal of M-band material after incubation with 1.2 mg/ml of affinity-purified anti-M-CK Fab. Some remnants of M-band material are still visible. The same amount of preimmune Fab has no obvious effect on the M-band (Fig. 4 *a*). At this concentration of anti-MM-CK Fab, no effect on the appearance of the Z-band is observed (Fig. 4 *a*, *b*). The major protein released under these conditions by specific anti-M-CK Fab has an M_r of 40,000 and an electrophoretic mobility on SDS polyacrylamide gels (15) that is slightly faster than that of actin (Fig. 5 *A*, lane *3*) and corresponds with the position of the muscle form of creatine kinase, MM-CK (Fig. 5 *A*, lane *6*). Besides

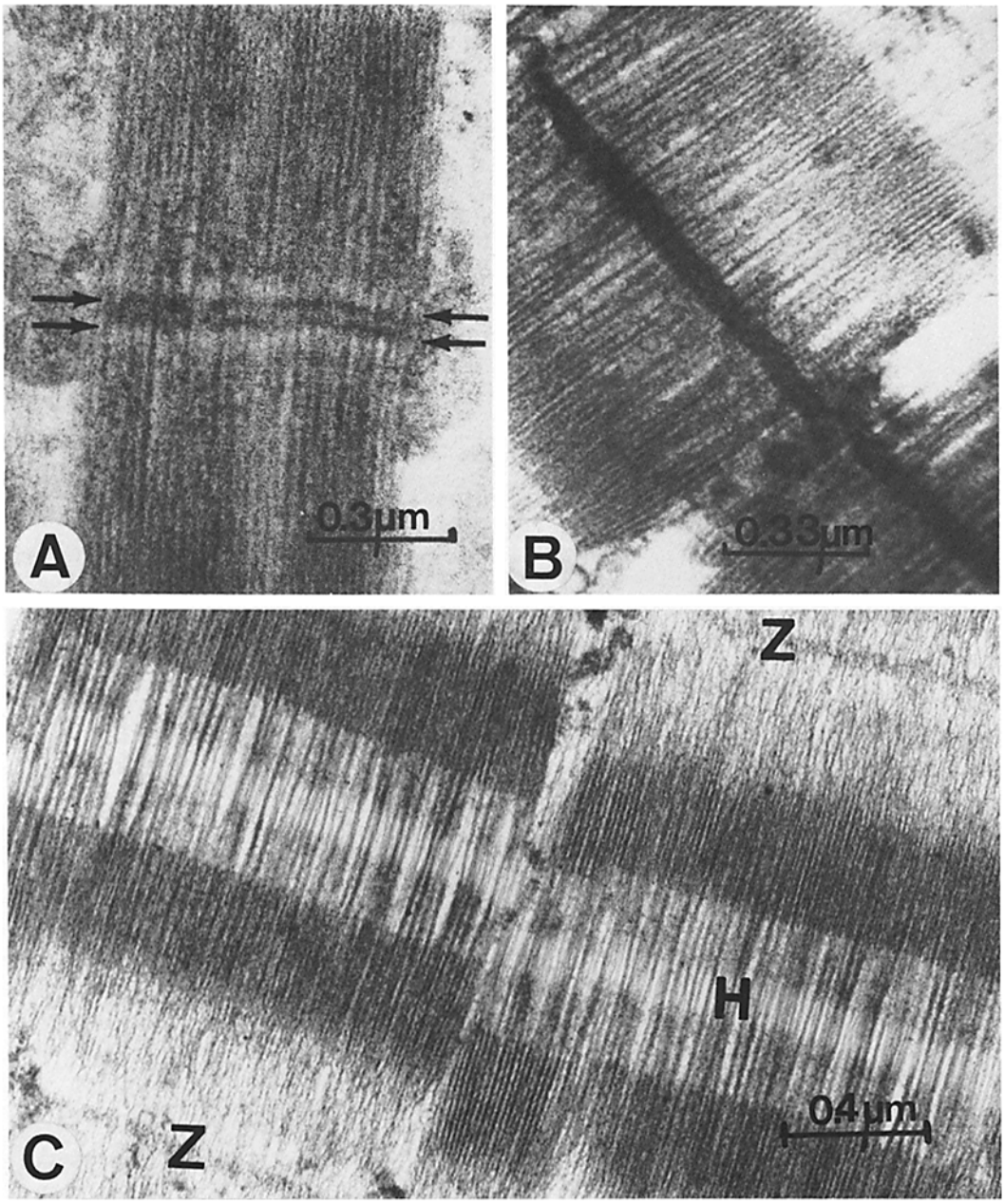

FIGURE 3 Higher magnification of thin sections of glycerinated chicken skeletal fiber bundles after incubation with anti-M-CK Fab (*A*, *C*) and anti-M-CK IgG (*B*). Double-line staining pattern (arrows) at the M-line obtained after incubation with 0.1 mg/ml of monovalent anti-M-CK Fab (*A*); removal of electron-dense M-line material after incubation for 36 h with 5 mg/ml of anti-M-CK Fab (*C*); and heavy decoration of the M-line after incubation with 2 mg/ml of anti-M-CK IgG (*B*). *Z*, Z-band, *H*, H-zone. (*A*) × 64,000. (*B*) × 60,000. (*C*) × 45,000.

actin, several minor protein components were also washed out by this treatment. However, the same minor bands were also present in supernates of fibers incubated with relaxing buffer (Fig. 5*A*, lane *1*) and control Fab (Fig. 5*A*, lane *2*). The latter treatments also removed some MM-CK, but the amount of MM-CK released specifically by anti-M-CK Fab was significantly larger (Fig. 5*A*, lane *3*; Fig. 5*B*, lane *2*). The 40,000-M_r protein released by anti-M-CK Fab was unambiguously identified as MM-CK by agarose immuno-overlay gels by means of specific anti-M-CK antibodies as shown in Fig. 5*B*, lanes *4–7*. The only protein released in significantly larger amounts by anti-M-CK Fab as compared with the control treatments was indeed undegraded, enzymatically active MM-CK (Fig. 5*A*, lane *3*) (43). In addition, some unidentified high molecular weight material not entering the gel seemed to be present in supernates of anti-M-CK Fab (1.2 mg/ml) treated fibers (Fig. 5*A*, lane *3*). Somewhat less of this unidentified material was seen after treatment with control Fab and relaxing buffer (Fig. 5*A*, lanes *1* and *2*). Treatment of fibers with 1 mg/ml of anti-myomesin Fab did not release a significant amount of CK nor was a 165,000-M_r protein released as judged by this method (Fig. 5*A*, lane *5*). In contrast with anti-M-CK Fab, anti-myomesin Fab, even at a higher concentration, was shown not to extract but rather to decorate the M-band (8, 35). If both

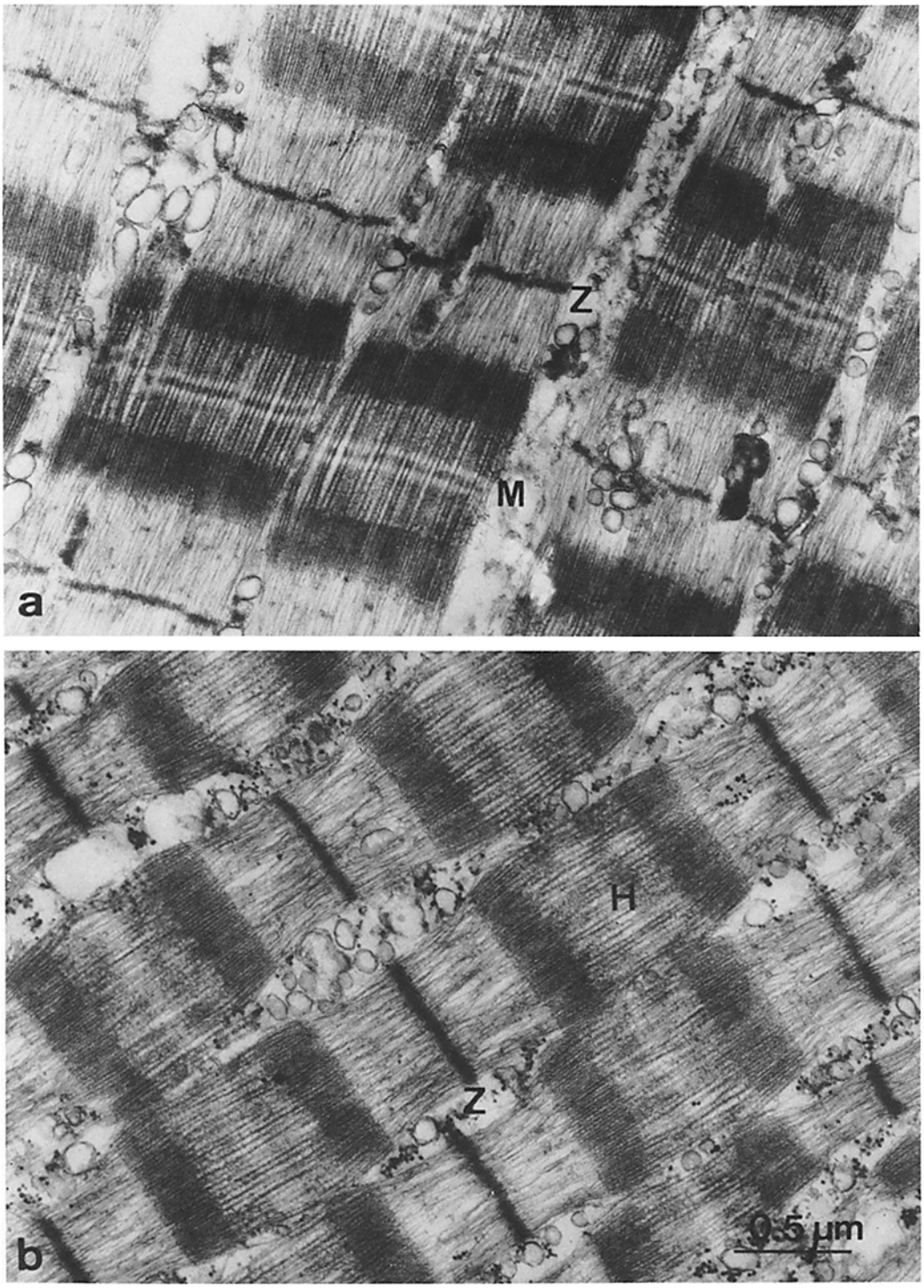

FIGURE 4 Thin sections of glycerinated chicken skeletal fiber bundles after incubation with 1.2 mg/ml of each control Fab (*a*) and anti-M-CK Fab (*b*). Note the disappearance of electron density of the M-line structure (*b*). *M*, M-line; *Z*, Z-band, *H*, H-zone. × 24,000.

antibodies were used simultaneously, somewhat less CK was removed as judged by the intensity of staining of the CK protein band (Fig. 5*A*, lane *5*). Upon incubation of fiber bundles with very high concentrations of affinity-purified anti-MM-CK Fab (5 mg/ml) and incubation over prolonged periods of time (36 h), some Z-band material was also lost concomitantly with the complete extraction of the electron density of the M-band (Fig. 3*C*). Gel analysis of proteins released into the supernatant show in addition to the proteins released by lower concentrations of anti-M-CK Fab (mainly MM-CK) (Fig. 5*A*, lane *3*) an increase in high molecular weight material that does not enter the gel and an extra protein band at ~85,000 M_r (not shown).

DISCUSSION

Double-Line Decoration at the M-Line by Anti-M-CK Antibody

It should be pointed out that an excellent preservation of the rather complex M-line substructures is achieved only by prefixation, preferentially in situ, of muscle fibers before processing of the tissue for conventional electron microscopy (10, 17, 27, 28) or ultracryotomy (32, 33). If the fibers have to be glycerinated and washed to remove the bulk of soluble sarcoplasmic MM-CK before incubation with antibodies, the detailed fine structure, e.g., M6-M9 substriations seen in cryosections (32, 33), is no longer resolved. Most remarkable is the clear double-line staining pattern within the M-band, obtained after incubation with low concentrations of anti-M-CK antibodies.

In contrast with high concentrations (≥1 mg/ml) of monovalent anti-M-CK antibody by which CK and concomitantly the electron-dense M-line are removed, incubation with low concentrations (0.1 mg/ml) of the same reagent led to a double-line staining pattern within the M-line. Obviously, under these conditions, the concentration of antibody is not sufficient to dissociate the whole M-line structure, and the Fab fragments remaining bound to CK in situ are visualized in the electron microscope as a double-line staining within the M-line. Because of the smaller size of Fab as compared with IgG, the two transverse lines are better resolved by using monovalent anti-

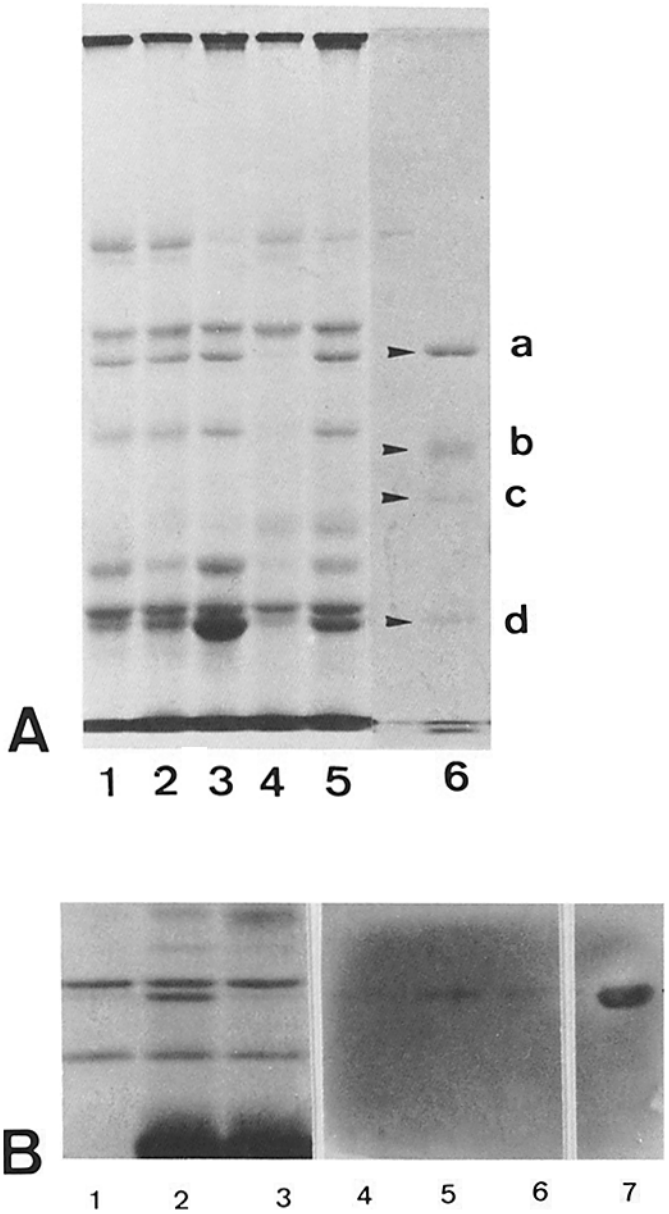

FIGURE 5 *A* SDS/5% PAGE of proteins released from fiber bundles. Fiber bundles were suspended in 0.5 ml of treatment solution for 12 h at 4°C; proteins in the supernates were precipitated with 2 ml of acetone (−10°C), redissolved in 20 μl of gel sample buffer, (15), and applied to the gel. Supernates after incubation with relaxing buffer (lane *1*); +control Fab (1.2 mg/ml, lane *2*); +anti M-CK Fab (1.2 mg/ml, lane *3*); +anti-myomesin (1 mg/ml, lane 4); and + a combination of both anti-M-CK Fab and anti-myomesin (lane *5*). M_r standards (lane *6*) were rabbit muscle phosphorylase (*a* = 92,000), bovine serum albumin (*b* = 68,000), rabbit muscle pyruvate kinase (*c* = 57,000) and chicken MM-CK (*d* = 40,000). *B* SDS/10% PAGE of proteins released from fiber bundles. Identification of released MM-CK by immunoreplication. Supernates after incubation with relaxing buffer (lane *1*); +anti-M-CK Fab (1.0 mg/ml, lane *2*); and +control Fab (1.0 mg/ml, lane *3*). All were stained for protein by Coomassie Blue (lanes *1–3*). Only the lower part of the 10% polyacrylamide gel where actin, CK, and Fab bands are seen is shown. Immunoreplicas of the same part of the gel exactly duplicating lanes *1–3* not fixed and stained for protein but instead overlaid with an agarose gel containing anti-MM-CK antiserum are shown in lanes *4–6*. Parallel MM-CK control, electrophoresed under the same conditions, is shown after immunoblotting (lane *7*). The precipitin bands formed after 12 h of incubation at 4°C were stained with Amidoblack after extensive washing of the replica with PBS (for details see reference 43).

bodies, although a double-line staining pattern was also observed with low concentrations of anti-M-CK IgG. The position and spacing of the two heavily stained transverse stripes correspond to that of the M4 and M4′ primary m-bridge lines assigned by Squire and collaborators (12, 27, 28) that correspond to the two lateral of the three main m-bridge arrays separated by ~40-nm as reported by Knappeis and Carlsen (10) and by Pepe (27, 28).

At this point in our study, the results clearly show that MM-CK is associated with the two off-center M-4 and M-4′ m-bridge arrays but nevertheless may or may not be associated with the M1 m-bridge array as well. Since high concentrations of monovalent anti M-CK Fab remove most of the M-line structure, it is conceivable that at low concentrations of Fab, e.g., under the conditions where the double-line staining is seen, some CK is also dissociated, e.g., from the very center m-bridge array, and that this may be the reason why the double-line staining pattern is seen that clearly. The notion that the M1 m-bridge array may be composed of MM-CK as well is supported by the fact that earlier calculations concerning the amount of CK released by low salt treatment (1 CK per 20 myosin) indicated that CK could account for the number of all three m-bridge arrays if one CK dimer were representing one-half of an m-bridge (40, 41). However, recent, more direct measurements of the amount of CK bound at the M-line (~1 CK dimer per 35 myosin) tend to support a model in which only two rows of m-bridges consist of CK (31 and Wallimann, T., T. Schlösser, and H. M. Eppenberger, manuscript in preparation). An assignment of MM-CK to M4 and M4′ m-bridges only and not to the center M1 array is also supported by very recent studies on immunolabeled, ultrathin frozen sectons on which a double-line pattern with prefixed muscle tissue and anti MM-CK IgG similar to that reported here was observed (36).

It is of importance to mention that a similar double-line staining pattern, although somewhat more diffuse, was also obtained with divalent anti-M-CK antibody (IgG) in prefixed myogenic cells (Fig. 2). Under these conditions, the M-line structure is assumed to have been stabilized by glutaraldehyde, and anti-M-CK IgG has been shown earlier not to dissociate CK from the M-line but rather to render it inextractable to low salt treatment (25, 41). Thus, an artifactual double-line staining due to preferential dissociation by the monovalent anti-M-CK Fab of the M1 m-bridge array at the very center of the M-line is unlikely. The fact that lower concentrations of anti-M-CK antibodies, both Fab and IgG, seem to predominantly stain only two (M4 and M4′) of the three main primary m-bridge arrays present in chicken pectoralis muscle is an indication that the very center M1 m-bridge array is most likely composed of different M-line protein constituents. This was suggested earlier on the basis of structural differences seen between the M4 and M4′ and the M1 m-bridges (17). From this work it is not possible to say anything about the composition of the other m-bridge arrays. However, myomesin (8) and additional M-line proteins yet to be discovered may fill this gap (36). The existence of additional M-line proteins may also help to explain the lack of consistency of in vitro interaction and reconstitution experiments performed with myosin and purified fractions of the two known M-line proteins (3, 13, 18–21, 47).

M-Line Removal by Monovalent Antibody

In addition to the specific double-line staining of the M-line by well-characterized antibodies against M-CK (Fab as well as IgG) and the effect of excess anti-M-CK IgG on preventing subsequent M-line extraction by low salt (41, 43), the removal by an excess of monovalent anti-M-CK Fab fragments of the electron-dense M-line structure (43) indicates that CK is directly involved in the architecture of the M-line structure (40). Removal of M-CK from the M-line by monovalent antibodies would indicate a relatively weak association of this protein within the M-line as compared with myomesin, the second known M-line protein (8, 18, 21, 37), since monovalent antibodies against myomesin did not remove but rather decorate the M-line (8, 35).

It is shown that the major protein species that was specifically released from the M-line of myofibrils in significant amounts by incubation of muscle fiber bundles with 1.2 mg/ml of anti-M-CK Fab is indeed undegraded and enzymatically active MM-CK, which was identified by gel electrophoresis of the supernates followed by immuno-overlays. No significant amount of 165,000 M_r myomesin was released under these conditions by either anti-M-CK Fab or anti-myomesin Fab (Fig. 5). In addition, the amount of CK released by anti-CK Fab as measured by enzyme activity (43) is similar to that released by low salt M-line extraction (25, 41, 43), and thus is consistent with reports that MM-CK dimers may represent m-bridges (1, 20, 25, 40, 41, 47).

A similar dissociation effect has been reported with antibodies specific for the regulatory light chains of scallop (44, 45) and the dinitrobenzoic acid-light chains of rabbit myosin (11). It is thought that M-CK and myosin light chains are removed by antibody-induced conformational changes which then lead to a weakening of the binding of M-CK to the M-line structure and of the myosin light chain subunits to the heavy chains, respectively.

The dissociating effect on the M-line was found when the incubations of fiber bundles were performed over a 24-h period at concentrations of 1 mg/ml or more of the affinity-purified anti-M-CK Fab. Depending upon the thickness of the fiber bundles and the anti-M-CK Fab concentration, a radial gradient of removal of the electron-dense M-line material was occasionally observed, e.g., electron-dense M-line material was completely removed near the periphery of the fiber bundles and the double-line staining pattern appeared towards the center.

The fact that with high concentrations of anti-CK Fab most of the electron density of the M-line is dissociated even though with low concentration of Fab, only two of the three most prominent m-bridge arrays are stained may indicate that the integrity of the M-line structure depends in part on the presence of CK and the proper interaction of CK with the other M-line protein(s). That is, once a certain amount of CK is dissociated from the M-line the whole structure is disrupted.

Function of M-Line Bound CK

The fact that MM-CK is more easily dissociated from the M-line structure by treatment with low salt and monovalent antibodies than myomesin makes a sole structural function, e.g., positioning, alignment, and connection of thick filaments, less likely for this protein. However, a dual structural and enzymatic function should not be excluded. In heart myofibrils of some mammals such as rat, incorporation of CK into the M-line and the concomitant appearance of an electron-dense M-line structure are delayed until some days after birth (4). During this early postnatal period, a rapid increase in cardiac muscle contractility (12) and a marked enhancement of the

major systems involved in ATP metabolism (2) have also been shown to take place. It is therefore likely that the presence of M-line bound MM-CK is closely related to the physiological maturation of the contractile apparatus by increasing the efficiency of ATP regeneration via phosphorylcreatine within the myofibrillar microcompartment. Recent evidence suggests a physiological role of M-line bound CK as a potent intramyofibrillar ATP-regenerating system. With a combined pH-stat assay, it was shown that M-line bound CK is enzymatically active and sufficient in amount to regenerate all the ATP hydrolysed during in vitro contraction of myofibrils (31).

In conclusion, the double-line staining pattern observed at the M-line after incubation of chicken skeletal muscle fiber bundles with low concentrations of specific anti-M-CK antibodies indicates that the two off-center M4 and M4′ primary m-bridge structures contain MM-CK. Besides the other M-line protein, myomesin, the presence of additional protein constituents is postulated to make up for all the structural components of the complex M-line structure. A more precise localization of MM-CK within the M-line structure is being attempted by using monoclonal antibodies against M-CK, which have been shown by the indirect immunofluorescence technique to stain the M-line of myofibrils (L. Cerny, personal communication).

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