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Studies on the distribution of cellular myosin with antibodies to isoform-specific synthetic peptides

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Antisera were produced against two synthetic peptides having sequences specific for cellular myosin heavy chains from human macrophages (peptide IIA) and bovine brain (peptide IIB). Immunoblots of tissue extracts were made with these antibodies, and they showed that mammalian cells have at least three distinct cellular myosin heavy chain isoforms. Two of the isoforms ($MIIB_1$ and $MIIB_2$) were recognized by antibodies against the peptide IIB, and the other (MIIA) by the anti-peptide IIA antibodies. Polyclonal anti-platelet myosin antibodies recognized the MIIA isoform, but did not recognize $MIIB_1$ or $MIIB_2$. The isoforms were expressed in a tissue-specific pattern; the $MIIB_1$ isoform was found only in brain.

Nonmuscle myosin; Heavy chain isoform; Antibody

1. INTRODUCTION

Non-muscle, or cellular, myosins of the 'conventional' type (myosin II) are similar in size (200–220 kDa) to muscle myosins, but they are encoded by separate genes, and have different functions and locations. Several lines of evidence suggest that isoforms of the cellular myosin heavy chains (MHC) exist [1–6]. In chicken this evidence comes from peptide fingerprints [1] as well as from cDNA cloning studies, which demonstrated tissue-specific expression of two different MHC isoforms encoded by different genes [2,3]. In the case of mammalial cells, partial sequences of two cellular myosins have been reported, including a peptide sequence from the COOH-terminus of bovine brain MHC [4] and the sequence of a cDNA encoding the COOH-terminal one-half of a human macrophage MHC [5]. We exploited sequence differences between these MHCs and produced antisera which distinguished between the MHC isoforms. The distribution of cellular myosin isoforms was studied in extracts of homogenates of various mammalian organs by using these antibodies and commercial anti-platelet myosin antibodies.

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Abbreviations: EGTA, ethylene bis(oxylenenitrilo)tetraacetic acid; 2-ME, 2-mercaptoethanol; PMSF, phenylmethylsulfonylfluoride

2. MATERIALS AND METHODS

Anti-human platelet myosin antibody (affinity-purified rabbit polyclonal against the MHCs) and alkaline phophatase conjugated goat anti-rabbit IgG were from Biomedical Technologies Inc. and Tago Inc., respectively. Isolation, phosphorylation, and dephosphorylation of bovine brain myosin were performed as described [4]. Synthetic peptides were purchased from Multiple Peptide Systems, San Diego, CA. New Zealand white rabbits were immunized with Keyhole Limpet hemocyanin-coupled peptide (100 μg antigen). Antibodies were purified on peptide-coupled affinity columns [7]. SDS-8% polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method in [8] with a ratio of acrylamide/bisacrylamide of 30:0.2. Proteins were electrically transferred (10 V/cm for 12 h at 4°C) to polyvinylidene difluoride membranes (Millipore) and immunostained using an indirect alkaline-phosphatase method [7]. Protein concentrations were determined by the method in [9] using bovine serum albumin as a standard.

3. RESULTS AND DISCUSSION

The peptides used as antigens were selected by comparison of the amino acid sequences near the COOH-ends of three vertebrate cellular myosins, bovine brain [4], human macrophage [5], and chicken intestinal epithelium (CIE) [2] (Fig. 1). The sequences were aligned as shown by fixing the position of the serine that is the phosphorylation site for casein kinase II in brain myosin [4] above the predicted casein kinase II site in macrophage and CIE myosins. The CIE and human macrophage sequences illustrated in Fig. 1 are homologous throughout, while the brain sequence is distinct from the others near the COOH-end. Since we planned to study mammalian myosins, we selected, from the brain and macrophage MHCs, the sequences underlined in Fig. 1 as the peptide antigens.

The anti-peptide IIA and anti-peptide IIB antibodies

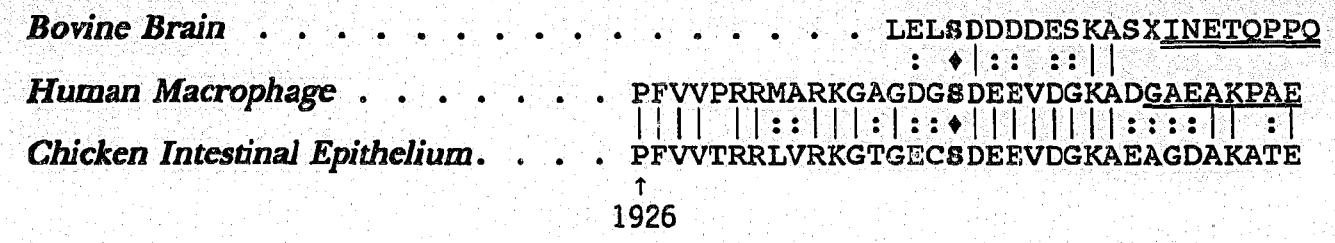

Fig. 1. Amino acid sequences near the COOH-terminus of cellular myosin heavy chains. (|) designate identical amino acids; (:) conservative replacements; and (♦) the casein kinase II site. Peptide antigen IIA is underlined and IIB is doubly underlined. The proline at the left of the CIE sequence is residue no. 1926 in the heavy chain sequence [2].

reacted only with cellular MHCs; neither recognized muscle-type MHCs. The location of the epitope(s) for the anti-peptide IIB antibodies within the MHC was confirmed to be within 5 kDa of the COOH-end [4,10] by autoradiography of an immunoblot of a partial chymotryptic digest of ^{32}P-labeled brain myosin (data not shown). In addition, it was found that the phosphorylation status did not affect the antibody reactivity although the peptide antigen and the casein kinase II phosphorylation site are adjacent in the sequence.

The antibodies were then used to assess the tissue distribution of the cellular MHC isoforms; tissue extracts were electrophoresed under conditions that resolved the heavy chains into multiple bands, and immunoblotted. Fig. 2A shows an immunoblot of a rat cerebellar extract; one light, fast moving band (MIIA) was stained by anti-IIA (lane 1). Two stronger bands (designated $MIIB_1$ and $MIIB_2$) were stained by anti-IIB (lane 2), while a mixture of anti-IIB and anti-IIA stained all three bands (lane 3). Anti-platelet myosin antibodies stained a single band that corresponded to MIIA (lane 4). Neither $MIIB_1$ nor $MIIB_2$ was stained by the anti-platelet myosin antibodies. Bovine cerebellar cortex extracts gave patterns and staining intensities very similar to those of rat cerebellum (Fig. 2B, lanes 1–3). The peptide IIB sequence was derived from bovine myosin and these results indicate that the rat cellular MHCs must have the same or similar sequences at their COOH-ends. As with the rat, the anti-platelet myosin antibodies did not bind the bovine $MIIB_1$ or $MIIB_2$ bands, but did stain the band corresponding to the MIIA isoform (not shown).

Electrophoretic resolution of the bands was not due to the phosphorylation state, because phosphorylation of the purified bovine brain myosin with casein kinase II or dephosphorylation with phosphatases did not change the patterns seen in Fig. 2B (not shown). Therefore these bands appear to be isoforms that differ slightly in size. Perhaps alternative splicing of the mRNA, as seen in both human smooth muscle myosin [11] and *Drosophila* non-muscle myosin [6], is responsible for generation of the $MIIB_1$ and $MIIB_2$ isoforms;

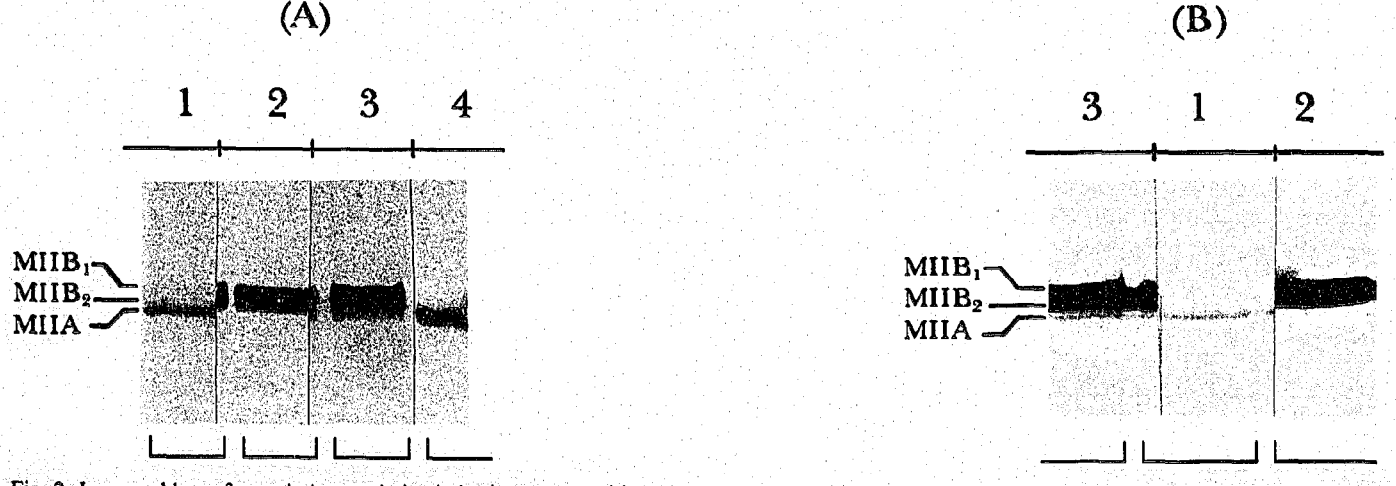

Fig. 2. Immunoblots of myosin heavy chains in brain extracts with anti-peptide IIA, anti-peptide IIB, and anti-platelet myosin antibodies. Rat and bovine brains were homogenized in a solution containing 10 mM imidazole-HCl (pH 7.0), 5 mM EGTA, 5 mM EDTA, 5 mM 2-ME, 1 mM PMSF, 1 mM benzamidine-HCl, and 0.1 mg/ml each of pepstatin A, leupeptin, aprotinin, and soybean trypsin inhibitor. After centrifugation at 100 000 × *g* for 30 min, the supernatants were discarded and the precipitates were extracted with 10 mM $MgCl_2$, 10 mM ATP, 0.3 M NaCl, 25 mM Tris-HCl (pH 7.5), 1 mM EGTA, 5 mM 2-ME and protease inhibitors as indicated above. The resultant 'Mg^{2+}-ATP/NaCl' extracts (40 μg/lane) were subjected to SDS-PAGE followed by immunoblotting. (A) The extracts from rat cerebellum; and (B) the extracts from bovine cerebellar cortex. Lanes 1, 2, 3, and 4 are blots produced with anti-IIA, anti-IIB, anti-IIA + anti-IIB, and anti-platelet myosin antibodies, respectively. ⌊⌋ indicate the width of the individual sample lanes.

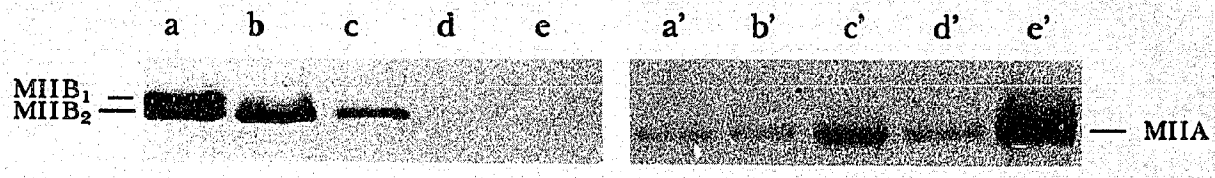

Fig. 3. Immunoblots of the Mg^{2+}-ATP/NaCl extracts from various rat tissues with anti-peptide IIB and anti-peptide IIA antibodies. The Mg^{2+}-ATP/NaCl extracts from rat cerebrum, cerebellum, kidney, thymus and platelets were prepared as described in Fig. 2 and aliquots (30 μg/lane) were subjected to SDS-PAGE followed by immunoblotting with anti-IIB (a–e) and anti-IIA (a'–e') antibodies. Lanes a to e as well as a' to e' represent the extracts from cerebellum, cerebrum, kidney, thymus, and platelets, respectively.

MIIA is presumably the product of a separate gene.

We then examined the distribution of MIIA and MIIB isoforms in other rat tissues. As shown in Fig. 3, the $MIIB_1$:$MIIB_2$ ratio varied in the tissues examined. Cerebrum showed a lower $MIIB_1$: $MIIB_2$ ratio than did cerebellum (lanes a and b). Kidney contained a single anti-IIB positive band that migrated to the same position as the $MIIB_2$ band of the brain extracts (lane c). The thymus and blood platelet extracts showed no visible bands with anti-peptide IIB (lanes d and e). On the other hand, anti-peptide IIA detected single MIIA bands that migrated to the same position in all tissue extracts (lane a'–e'). The affinity of anti-peptide IIA antibodies for human platelet myosin was shown, by ELISA, to be similar to that of anti-peptide IIB antibodies for bovine brain myosin. These results indicate that myosins from brain and blood platelets are predominantly the two different isoforms, MIIB and MIIA, respectively, and that tissues such as the kidney contain similar amounts of both. This conclusion is consistent with that drawn by Burridge and Bray [1] from peptide fingerprint studies. The isoform-specific antibodies described here open up the possibility to study differences in subcellular distribution and function of the cellular myosin isoforms.

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