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A novel myosin I from bovine adrenal gland

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Abstract

A 3.5 kb cDNA clone was isolated from bovine adrenal gland cDNA library. The clone contained a full-length 3.1 kb open reading frame, encoding a novel myosin I. The deduced amino acid sequence was highly homologous to other known myosin Is in the N-terminal 2 kb region which corresponds to the myosin head domain, while no strong homology was detected in the tail region. The head-tail junction contained the Ca^{2+}-independent calmodulin binding consensus sequence, suggesting that the novel myosin I binds calmodulin. This was confirmed by calmodulin overlay which showed the binding of ^{125}I-calmodulin to the recombinant myosin I expressed in *E. coli*. Northern blots with probes from head and tail regions of this myosin I revealed that this novel myosin I is widely distributed among various tissues.

Key words: Myosin I; Primary structure; Molecular cloning; Adrenal gland

1. Introduction

Myosins are molecular motors that have at least one head domain with mechanochemical activity capable of transducing energy stored in ATP into motion along actin filaments. Myosins have been divided into two classes, i.e. myosin I and myosin II, of which the former is single headed and unable to form filaments and the latter is a double headed, filament forming dimeric conventional myosin [1]. Recently a number of unconventional myosins were found, and according to their sequence homology, the myosin superfamily is now classified into seven groups, including myosin I and myosin II [2,3]. Among these unconventional myosins, myosin I is the best characterized, although most of the information we know about it is obtained from amoeba myosin I, not from vertebrate ones.

Myosin I was first identified in *Acanthamoeba*, later it was also found in *Dictyostelium*, *Drosophila*, intestinal brush border of chicken and bovine, and there have been indications of multiple myosin I isoforms. Sequence and domain structure analyses have demonstrated strong similarities between myosin I and myosin II in the globular head region. This is the region that, in all myosins, contains the ATP-binding site and the ATP sensitive actin binding site. In contrast, the tail portion of myosin I is distinct from the rodlike, α-helical coiled coil domain of myosin II and forms non-helical structures which may function as anchoring sites of myosin I such as membrane binding and ATP insensitive actin binding sites. The sequences of myosin Is tail are divergent. Since the head region is conserved among different known myosin Is, it has been suggested that this divergence in the tail region defines the specific function of different myosin Is [1,2,4].

Although myosin I has been studied for a long time, the physiological functions of myosin I have not been well defined; however, their localization to the plasma membrane [5], their ability to interact directly with phospholipid bilayers [6], their two actin-binding sites which allow them to cross-link actin filaments [7], and their mechanochemical activity have suggested that myosin I is the motor driving contractile activity at the cell membrane, including chemotaxis, exocytosis, endocytosis and changes in cell shape [1]. In vertebrate cells, myosin I was first identified in intestinal brush border where myosin I localizes as a tether between the plasma membrane of the intestinal microvilli and the bundle of actin filaments [8]. Failure in detecting brush border myosin I (BBMI) in other tissues using DNA probe and specific polyclonal antibodies suggested that the brush border myosin I expresses in rather specialized cells. This notion is consistent with its rather specific intracellular localization as described above. Recently myosin I was also purified from bovine brain and adrenal gland, whose biochemical properties were similar to those of brush border myosin I [9], however, its primary structure is not yet identified.

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Abbreviations: BBMI, brush border myosin I; RT-PCR, reverse transcriptase coupled polymerase chain reaction; BAGMI, bovine adrenal gland myosin I; IPTG, isopropylthio-β-D-galactoside

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The antibody staining revealed that this myosin I is expressed in various tissues [10].

In this study, we isolated a new myosin I cDNA clone from bovine adrenal gland by using a cDNA fragment of chicken BBMI as a probe to screen the bovine adrenal gland cDNA library. The bacterial expressed protein of this myosin I was capable of binding calmodulin, suggesting that the holoenzyme contains calmodulin. Northern analysis revealed that this myosin I is widely distributed among various tissues.

2. Experimental

Total RNA was isolated by acid guanidine thiocyanate/phenol/chloroform method from various tissues [11]. 1 μg of total RNA from chicken intestine was used as the template for reverse transcriptase coupled polymerase chain reaction (RT-PCR) to amplify the cDNA fragment from chicken BBMI (Fig. 1Aa), using AMV reverse transcriptase (Boehringer Mannheim) and Taq polymerase (Perkin Elmer Cetus) under standard conditions. Two primers, 5′-ATGACTGGTGAAAGCGGAGCT-3′ and 5′-GCAGCTGGTAGAAGATGTGG-3′, were made to sandwich the nucleotide sequence of chicken BBMI conserved among various myosin Is but less conserved for conventional myosin (filled box in Fig. 1Aa). PCR amplified chicken BBMI cDNA probe was subcloned into pBluescript SKII(+) and the sequence was confirmed by the dideoxynucleotide chain termination method (Sequenase 2.0, USB). Chicken BBMI cDNA fragment was purified by agarose gel electrophoresis, and 25 ng of the cDNA fragment was labeled by ^{32}P, using a random labeling kit from Boehringer Mannheim. 160,000 plaques of a random primed λgt11 bovine adrenal gland cDNA library (Clontech) was screened using the cDNA probe. Hybridization for cDNA library screening was carried out at 42°C for 20–24 h in Church's buffer [12], followed by a final wash of 0.1–0.5 × SSC and 1% SDS at 42°C. λ phage DNA containing positive inserts was purified by a DEAE cellulose treatment, and the *Eco*RI fragments of the positive inserts were subcloned into SKII(+). To determine the sequence of the clones, deletion mutants were produced by exonuclease III and Mung bean nuclease digestion from both strands (pBluescript Exo/Mung DNA sequencing system, Stratagene). For the subsequent screenings with novel myosin I partial cDNA probes from bovine adrenal gland, all conditions were the same except that hybridization was carried out at 60°C.

Bacterial expressed recombinant bovine adrenal gland myosin I was obtained as follows: the myosin I clone was in-frame subcloned into *E coli* expression vector pET, then *E. coli* strain BL21 was transformed by the pET vector containing bovine myosin I. Expression was induced by adding 0.25 mM IPTG to the liquid culture of transformed BL21 cells. 4 h after induction, cells were harvested and stored at −80°C. Electrophoresis was carried out on 7.5–20% polyacrylamide gradient slab gels by using the discontinuous buffer systems of Laemmli [13]. Molecular markers used were smooth muscle myosin heavy chain (200 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), smooth muscle myosin light chain (20 kDa) and α-lactalbumin (14.2 kDa). Calmodulin overlay of the bacterial expressed bovine adrenal gland myosin I was performed using 3 μCi ^{125}I-calmodulin (NEN research products) as described by Slaughter and Means [14].

Northern blot was performed by standard methods, using 20 μg of total RNA for each rat tissue. Probes from head and tail domains of our clone (Fig. 1Ac) were purified and labeled as above.

3. Results and discussion

To prepare the cDNA probe to obtain novel myosin Is from mammalian tissue, we selected the nucleotide sequence of chicken BBMI conserved for various known myosin Is but less conserved for myosin IIs, which corresponds to 308–626 of chicken BBMI (Fig. 1Aa). Two oligonucleotide primers corresponding to this region were made and used for RT-PCR amplification of chicken intestinal RNA to produce the cDNA fragment of BBMI. The obtained PCR product (319 bp) was subcloned into pBluescipt SKII(+) and the clone was confirmed by sequencing. This chicken BBMI cDNA fragment was used as a probe to screen a bovine adrenal gland λgt11 cDNA library under reduced stringency. We obtained 32 positive clones. Among them 3 clones showed homologous sequences to myosins. One of them, clone 23 (corresponding to 120–600 in Fig. 1Ab), showed higher homology to myosin I than to myosin II according to the translated partial amino acid sequence. The clone 23 was used for further screening of the bovine cDNA library under higher stringency. Four positive clones were obtained, and among them clone 6 contained the largest insert, 3.5 kb. The complete nucleotide sequence of the 3.5 kb insert and its deduced amino acid sequence are shown in Fig. 1B. It contains 3,505 bp and has an open reading frame of 3,084 bp which encodes 1,028 amino acids with a calculated relative molecular mass of 118,012 Da. The open reading frame of this clone started with an initiation sequence consistent with the consensus initiation sequence [15]. There are about 100 bp of 5′ untranslated region and 300 bp of 3′ untranslated region in our clone, however, the polyadenylation signal was not found. Dot matrix analysis revealed that the amino acid sequence of this clone which corresponds to the head domain, i.e. the N-terminal 2/3 of the total open reading frame, was quite homologous to chicken BBMI. It has been shown that the various myosin Is are

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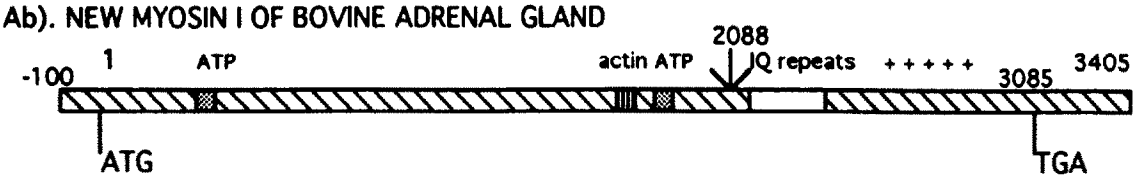
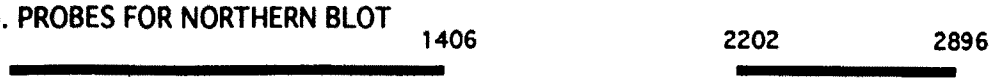
Fig. 1. Nucleotide and deduced amino acid sequence of the novel myosin I. (Aa) The PCR amplified cDNA fragment from Chicken BBMI as indicated by the filled box. This cDNA fragment was used as the probe to screen bovine adrenal gland library. The partial cDNA sequence of chicken BBMI was numbered according to our clone. (Ab) The diagram of isolated new myosin I from bovine adrenal gland. The A of start codon ATG was numbered 1. The stop codon TGA is at positions 3,085–3,087. The arrow indicates the end of the myosin globular head domain. ATP binding sites, actin binding site, IQ motifs and the net positive charges at tail region are indicated. (Ac) The two restriction-enzyme-digested fragments from our myosin I clone head and tail region, respectively, which were used as probes for northern blot. (B) Nucleotide sequence of the myosin I from bovine adrenal gland and its deduced amino acid sequence (GenBank Accession No. U03420). The amino acid sequence of the myosin I is shown below the nucleotide sequence. The start codon at position 1–3 is underlined. Amino acid residues corresponding to the consensus ATP binding domains are double underlined. The putative actin binding site at amino acid 576–595 is dash underlined. The consensus amino acid residues of IQ motif at the head tail junction were marked with an asterisk (*) under them.

Aa). CHKBBMI

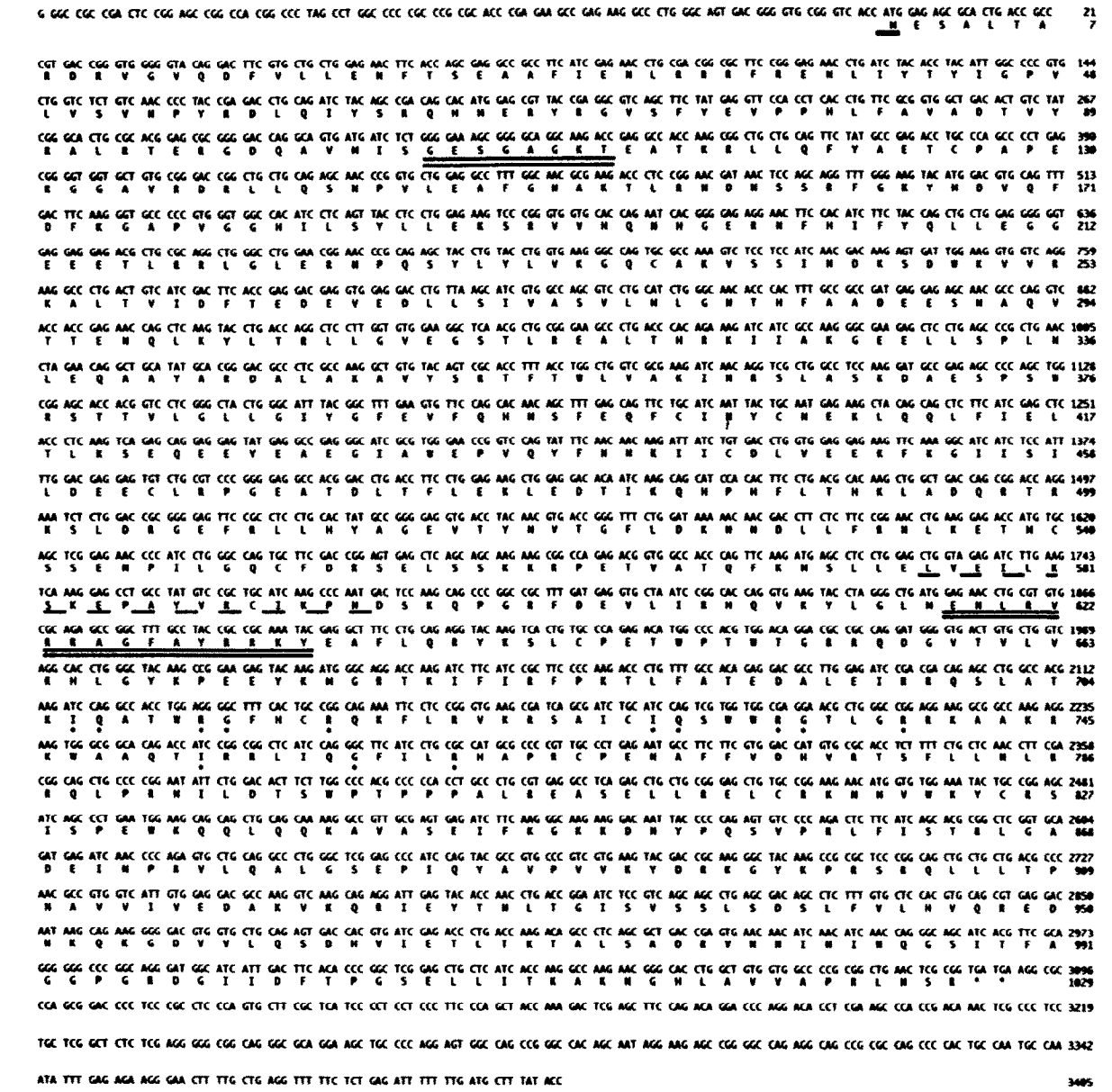
Ab). NEW MYOSIN I OF BOVINE ADRENAL GLAND



Ac). PROBES FOR NORTHERN BLOT

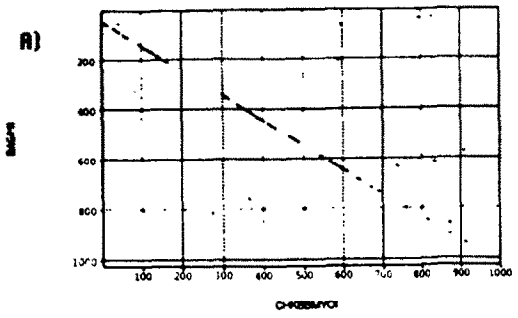


B).



quite homologous to each other in the head domains, therefore, the results suggest that the protein encoded in this clone belongs to myosin I family. In contrast, the C-terminal tail portion showed virtually no homology to that of the chicken BBMI (Fig. 2A). Furthermore, even in the head domains, there is a region with virtually no homology between the bovine adrenal gland myosin I (BAGMI) and chicken BBMI. This region corresponds to a.a. 200–350 in which few consensus myosin I sequences are found among known myosin Is (Fig. 2C). A large difference in the structure at the central portion of the head domain of BAGMI suggests its unique motor properties. These results clearly indicate that the clone 6 is a new mammalian myosin I isoform.

Analysis of the deduced amino acid sequence of BAGMI revealed that it is a novel gene product different from chicken and bovine BBMI. It contained 3 domains, an N-terminal motor domain (1–700), a head-tail junction domain (700–760) and a C-terminal domain (760–1028). Consensus ATP binding sequences [16,17], as well as the sequence important for ATP sensitive actin binding [18], were found in the head motor domain (Fig. 1). At the head-tail junction domain, 2 well conserved and 1 less conserved IQ motifs, putative calmodulin/light chain binding motifs, were found (Fig. 1 and Fig. 2B). This motif has been proposed as the Ca^{2+}-independent calmodulin binding site of neuromodulin [19]. Three consecutive repeats of IQ motif are present in chicken



B)

Calmodulin-binding region from neuromodulin	KAHKAATKIQASFRGHITRKKLKGEKKGD
Chicken BBMI (654-724)	RVAELATLIQKMFRGWCCRKYRYQ LMRKSQILISAWFRGHMCRNRYK QMKRSVLLIQAYARGWKTRRMYRRY
BAGMI (699-768)	RRQSLATKIQATWRGFHCRQKFL RVKRSAICIQSWWRGTLGRRKAA KRKWAAQTIRRLIQGFILRHAPR

C)

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BOVAGMI   MESALTARDRVGVQDFVLLENPTSEAAFIENLRRRFRENLIYTYIGPVLVSVNPYRDLQIYSRQHMERYRGVSFYEVPPHLFAVADTVYRALRTERGDQAVMISGESGAGKTEATKRLLQFYAETCPAPE  130
BOVBBMI      mtllegsVGVeDlVLLEplegE-slIrNLqlRyekkeIYTYIGhVLVSVNPYqqLpIYdlefvakYRdytFYElkPHiyAlAnmaYqaLRdrdrDQcilItGESGAGKTEAsKlvmsyvAavCgkgE
RATBMI    vkSsLld-nmiGVgDtVLLEpl-nEetFIdNLkkRFdhNeIYTYIGsVviSVNPYRsLpIYSpekvEdYRnrnFYElsPHiFAlsDeaYRsLRdqdkDQcilItGESGAGKTEAsKlvmsyvAavCgkga
Myosin I          .  .GV DLVML.  .SE. I ENLKKRY  ..IYT.IGPVLISVNPY     Y      Y.  .. E. PH.YA A  AYR  ..    QC..ISGESGAGKTEASR .MQY AA .G.
Myosin II  .. NP.  . .EDM. ..      VL NL  RY     TYSGLF V  NPYK    Y.   .   G .R E .PH P   D AY   L...  QS LITGESGAGKTENTK   QY A  ...

BOVAGMI   RGGAVRDRLLQSNPVLEAFGNAKTLRNDNSSRFGKYMDVQFDFKGAPVCGHILSYLLEKSRVVHQNHGERNFHIFYQLLSGGEEETLRRLGLERNPQSYLYLVKGQCAKVSSINDKSDWKVVRKALTVID  260
BOVBBMI   qvnsVkeqLLQSNPVLEAFGNAKTiRNnNSSRFGKYMDieFDFKGfPlGGvItnYLLEKSRVVkQleGERNFHIFYQLLsGadaqllkaLkLLERdtggYaYL-npdtarVdgmdDdanfKVlqsAmfVIg
RATBMI    evnqVkeqLLQStPVLEAFGNAKTVRNDNSSRFGKYMDieFDFKGdPlGGvIsnYLLEKSRVVkQprGERNFHvFYQLLsGasEELhkLkLERdfsrYnYL-sldsAXVngvdDaanfrtVRnAmqivg
Myosin I    . VK..IL.SNP LEAFGNAKT RNNNSSRFGKYMEIQFD  G.P GG.I.NYLLEKSRVV.Q  GERNFHIFYQLL.G ....  .L.....  . ... G.  . . . . A . .G
Myosin II     LE QI. .NP.LEAFGNAKTVRNNNSSRFGKFIRIHF  .G.   . I. YLLEESRV .Q  .ER.YHIFYQ  . . . . .  . .  . .  . . DEE  . A I

BOVAGMI   FTEDEVEDLLSIVASVLHLGN----THFAAD----EESNAQVTTENQLKYLTRLLGVEGSTLREALTHRKIIAKGEELLSPLNLEQAAYARDALAKAVYSRTFTWLVAKINRSLASKDAESPSWRSTTVL  382
BOVBBMI   FsdeEirqvLevaAlVLkLGNvelinefqAn----gvpeagirdgrgvqeigeLvGlnsveLerALcaRtmetakEKvvttLNViQAqYARDALAKniYSRlFnWLVnrINeSi--Kvgtgek-R--kVm
RATBMI    FsdpEaEswLevVAaVLkLGN----ieFkpesrmngldeskikdkNeLKeiceLtsidqvvLerAfsfRtveAKqEKvattLNVwQAyYARDALAKnlYSRlFsWLVnrINeSi--K-Aqtkv-R-kkVm
Myosin I          .  L. GN.  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  QA YARDALAK. YSR.FDW V  N.S  ...  .  .  .
Myosin II  F  E  ...  .  GN.  .  F  .  E  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  P. G.  V  ..QV  .AL K. Y  F  V  .N

BOVAGMI   GLLGTYGFEVFQHNSFEQFCINYCNEKLQQLFIELTLKSEQEEYEAEGIAWEPVQYFNNKIICDLVEEKPKGIISILDEECLRPGEATDLTFLEKLEDTIKQHPHFLTHKLADQR--TRK---SLDRGEF  507
BOVBBMI   GvLdIYGFEileNSFEQFvINYCNEKLQQvFIEmTLKeEQEEYkrEGIpWvKVeYFdNgIICnLiEhnqrGIlamLDEECLRFOvvsDaTFLaKLnqlfakHaNy-esKvtqna--qRqydhSmglacF
RATBMI    GvLdIYGFEiPedNSFEQFiINYCNEKLQQiFIELTLKeEQEEYirEdIeWthidYFNNaIICDLiEnntnGIlamLDEECLRFGtvTDeTFLEKLnqvcatHqHFesrnakcsRfIndt---tLphscF
Myosin I  G LDIYGFEIF  N FEQF IN. NEKLQQ FI  TLK.EQEEY. EGI W  .  ..N    DLIE....GI   LD  C.  ..  D.. . K .  . H.H  .  .  .  .  .F
Myosin II  GVLDI GFEIF  NS.EQL IN  NEKLQQFFNHMF LEQEEY  EGI W  . G D    LI E   I  L E    P  D    KL  ...  .  .  .  .E F

BOVAGMI   RLLHYAGEVTYNVTGFLDKNNDLLFRNLKETMCSSENPILQGCFDRSE--LSSKKRPETVATQFKMSLLELVETLKSKEPAYVRCIKPNDSKQPGRFDEVLIRHQVKYLGLMENLRVRRAGFAYRRKYEA  635
BOVBBMI   RicHYAGkVTYNVnsFlDKNNDLLFRdLsqaMwKarhPILrslPpegdpkqaSlKRPpTagaQFKaSvttLmknLySKnPnYiRCIKPNshqQrGhFsfeLvsvQaqYLGLiENvRVRRAGyAYRqaYgs
RATBMI    RiqHYAGkVlYqVeGFvDKNNDLLyRdLsqaMwKaghaliksIPpegnpakvniKRPpTagsQFKaSvatLmknLqtKnPnYiRCIKPNDkKaahiFsEsLvcHQirYLGLiENvRVRRAGyAfRqaYEp
Myosin I   I HYAG V Y   GF  KN D LF DL  .  .  .  F  .  .  .  K P T G   K S   L   L   PHYIRCIKPN   A  .  .  Q  YLGL ENVRVRRAG   R
Myosin II  . HYAGTV.Y  .  K. DPL..  .  .  .  .  .  .  .  .  .  .  T  .  .  .  L  L T PNFVRCIIPN.  .  .  .  L  CNGVLEGIRICR.G P R

BOVAGMI   FLQRYKSLCPETWPTWTGRRQDGVTVLVRHLGYKPEEYKMGRTKIFIRFPKTLFATEDALEIRRQSLATKIQATWRGFHCRQKFLRVKRSAICIQSWWRGTLGRRKAAKRKWAAQTIRRLIQG-----FI  760
BOVBBMI   FLeRYrlLsrsTWPtWnGgdQeGVekvlgeLsmssEElafGkTKIFIRsPKTLFylEeqrrlRiQqLATlIQkTyRGwrCRthyqlmrkSqIvIsSWfRGnmqkkhyrKmKasAllIqafvrGwkarkny
RATBMI    cLeRYKmLCkqTWPtWkQparsGVeVLfneLeipvEEYsfGRsKIFIRnPrTLFqlEDlrkqRledLATlIQKlyRGWkCRthFLLmKRSqvvIaaMyRryaqqKryqqiKssAlvIqsyIrGwkark-I
Myosin I  F  RY      TW  W.                    GKTK.FIR P  .F LE
Myosin II  F. RY  L.              K A          G TK FF  G  L   E  R

BOVAGMI   LR---HAPRCPENAFFVDHVRTS-FLLNLRRQLPRN-ILDTSWPTP--PPALREASELLRELCRKNWWKYCRSISPDWKQQLQQKAVASEIFKGK--KDNYPQSVPRLFISTRLGADEINPRVLQALGS  880
BOVBBMI   rkyf-RsgaaliSnFiykswvqkFLLgLkndLPspsILDkWPeay-PkyfntAnheLqrLfhqwkckKfrdqlSRkqvevLreKlcASEIFKGK--KasYPQSVPipFhgdyiGlqr-NPqkklLkgg
RATBMI    LRelkHqkRCkEaAttiaayvhgtqarkeRRrLkdearnkhaiavivwAfwlgSkarRELkIRKeearrk-havavivwWalgLkvrreyrkfPranagKkiYeftlqRi-vqkyL-1-Emknk-mpaLap

BOVAGMI   E--PIQYAVPVVKYDRKGYKPRSRQ-LLLTPNAVVIVEDAKVKQRIEY-TNLTGISVSSLSDSLFVLHVQREDNKQKGDVVLQSDHVIETLTKTALSADRVNNININQGSITFAQGPGRDGIIDFTPGSE  1007
BOVBBMI   eegPIlmAetVVKvnRgnaKtsSRi-LLLT-kghVIitDmKnpQaktv-ipLnslagvSvt-S-FkdglfslhlseisavgskgefllvsehvieL-ltkicratldatqmqlpvtvteefsvkFkeGS-
RATBMI    idknwpsrpylfldsth--KelkRifhLw--rckkyrdqftdqQkliYcekLeaseelfkdkkaLypesV--gqpfQgwyleinknpkykkL-KdAi-eekiiiaevvn-kInrAnGkstsrIfllT-nnn

BOVAGMI   LLITKAKNGHLAVVAPRLNSR*                                                                                                                  1028
BOVBBMI   Lrv-KvlqQpgggqtgkLs*
RATBMI    LLIadqKsGqikaevPlvd*
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Fig. 2. Amino acid sequence homology of bovine adrenal gland myosin I (BAGMI) with other vertebrate myosin Is. (A) Dot matrix of BAGMI vs chicken BBMI. (B) Alignment of the IQ motifs in BAGMI and chicken BBMI. Conserved residues are shown in bold type. (C) Amino acid alignment of bovine adrenal gland myosin I (BOVAGMI), bovine brush border myosin I (BOVBBMI) and rat brain myr 1 (RATBMI) heavy chains. Amino acids of BOVBBMI and RATBMI identical to those of BOVAGMI are indicated in capital letters. The consensus sequences of myosin I and myosin II are shown below the aligned sequences for the head region.

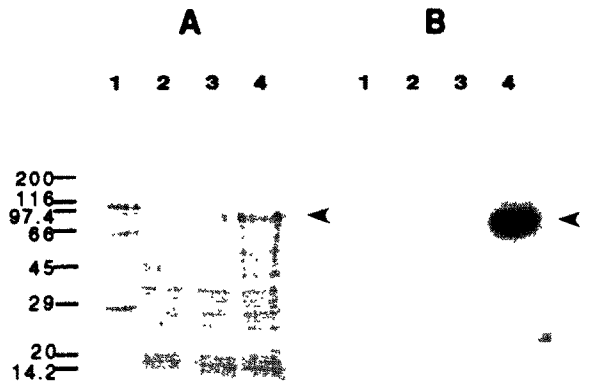

Fig. 3. Calmodulin overlay assay of bacterial expressed recombinant BAGMI. Lanes 1, molecular weight standards; lanes 2, untransformed BL21 cells crude extract; lanes 3, BL21 cells transformed with pET containing BAGMI before IPTG induction; lanes 4, BL21 cells transformed with pET containing BAGMI 4 h after IPTG induction. (A) Gel stained with Coomassie blue. (B) Autoradiogram of the gel overlaid with ^{125}I-labeled calmodulin. Molecular weight markers (in kDa) are shown to the left of the gel. Arrows indicate the induced recombinant BAGMI (estimated about 105 kDa).

BBMI and it has been shown that calmodulin serves as light chain subunits for chicken BBMI [8]. Therefore, this region of BAGMI may play a role in Ca^{2+}-independent calmodulin binding of BAGMI. In fact, the calmodulin overlay of the bacterial expressed recombinant BAGMI, which has an estimated molecular weight of 105 kDa, revealed that calmodulin binds expressed BAGMI (Fig. 3). The C-terminal tail region has a net positive charge of +15 (Fig. 1Ab), which has been found in most myosin Is to be a putative phospholipid binding site. This region may serve as membrane anchoring site. However, since the primary struture of BAGMI shows practically no homology with known myosin Is in the tail region, it is likely that its properties and specificity of membrane binding are quite different from other known myosin Is, although more detailed information requires further study. On the other hand, GPA (glycine-proline-alanine rich domain) and SH3 domains found in amoeba myosin Is tails which are assumed to serve as ATP-insensitive actin binding sites [1] were not found in BAGMI, suggesting this protein does not have an actin anchoring site. Recently it was reported that a 116 kDa myosin I like protein was purified from bovine adrenal gland and brain, although its primary structure is still unknown [9]. The reported 3 peptide sequences of this 116 kDa protein (APLGGGRVPWIW, ANLXYAGG-VXW and LTVISFTTEXEVE) were not found in BAGMI, suggesting this BAGMI is distinct from the 116KDa myosin I like protein. While this study was progressing, a cDNA of new myosin I isoform, myr 1, was isolated from rat brain [20].

Comparison of the amino acid sequence of BAGMI with those of bovine BBMI [21] and myr 1 revealed that BAGMI is highly homologous to bovine BBMI and myr 1 at the head motor domain, except the 150 a.a. residue span as described above (50% identity, 72% similarity to bovine BBMI; 51% identity, 75% similarity to myr 1), while much less homology was found in other domains (similarity less than 40% in both cases) (Fig. 2C).

To address the tissue distribution of BAGMI, northern blot analysis of RNA from various tissues was performed. Two different probes, one from the rather conserved head region and the other from non-conserved tail region, were used (Fig. 1Ac, Fig. 4). A band with an apparent size of 4.3 kb was detected in all the tissues tested by both probes. High levels of transcripts were detected in lung, kidney and heart, but the message level in brain and intestine was much lower. This is distinct from chicken BBMI and myr 1 whose message levels are high in intestine and brain, respectively [8,20]. These results suggest that BAGMI is widely distributed among various tissues in contrast to BBMI. According to its specific localization and limited tissue distribution, BBMI is likely rather to be a structurally improtant protein than to be involved in cell movement and/or vesicle movement. The wider tissue distribution of BAGMI suggests that it is involved in general cellular functions. The

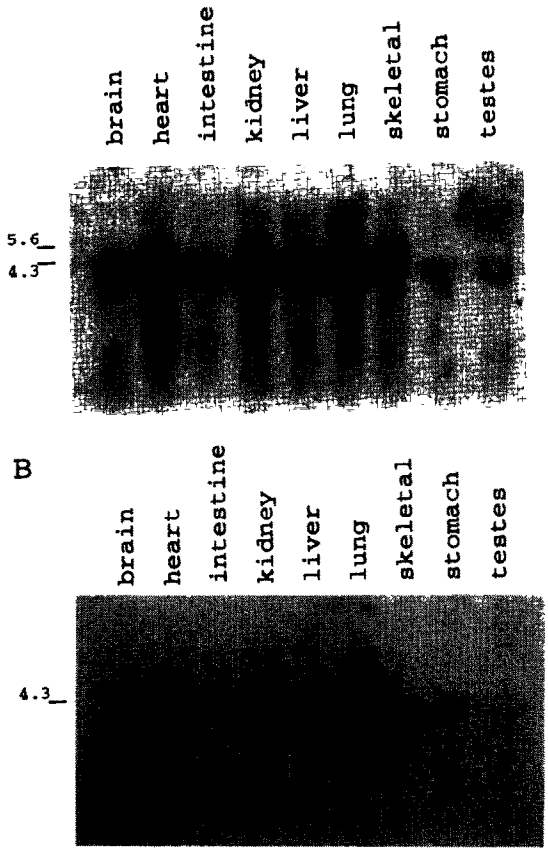

Fig. 4. Northern blot analysis of bovine adrenal gland myosin I in adult rat tissues. (A) Hybridization with BAGMI head probe (Fig. 1Ac). (B) Hybridization with BAGMI tail probe (Fig. 1Ac). The respective tissues are indicated above each lane. Size standards are in kb.

fact that it was found from adrenal gland where a lot of endocytosis and exocytosis processes are carried out suggests that it may function for vesicular movement, although further study is required to clarify the physiological function of BAGMI. The head probe hybridized with a second band with an apparent size of 5.6 kb in heart and skeletal muscle (Fig. 4A). We do not yet know whether the higher molecular weight band corresponds to mRNA of conventional myosin or another unconventional myosin.

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References

[1] Pollard, T.D., Doberstein, S.K. and Zot, H.G. (1991) Annu. Rev. Physiol. 53, 653–681.
[2] Cheney, R.E., Riley, M.A. and Mooseker, M.S. (1993) Cell Motil. Cytoskeleton 24, 215–223.
[3] Goodson, H.V. and Spudich, J.A. (1993) Proc. Natl. Acad. Sci. USA 90, 659–663.
[4] Hammer III, J.A. (1991) Trends Cell Biol. 1, 50–56.
[5] Fukui, Y., Lynch, T.J., Brzeska, H. and Korn, E.D. (1989) Nature 341, 328–331.
[6] Adams, R.T. and Pollard, T.D. (1989) Nature 340, 565–588.
[7] Pollard, T.D. and Korn, E.D. (1973) J. Biol. Chem. 248, 4682–4690.
[8] Garcia, A., Coudrier, E., Carboni, J., Anderson, J., Vandekerkhove, J., Mooseker, M., Louvard, D. and Arpin, M. (1989) J. Cell Biol. 109, 1895–2903.
[9] Barylko, B., Wanger, M.C., Reizes, O. and Albanesi, J P. (1992) Proc. Natl. Acad. Sci. USA 89, 490–494.
[10] Wagner, M.C., Barylko, B. and Albanesi, J.P. (1992) J. Cell Biol. 119, 163–170.
[11] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[12] Church, G.M. and Gilbert, W. (1984) Proc. Natl. Acad. Sci. USA 81, 1991–1995.
[13] Laemmli, U.K. (1970) Nature 227, 680–685.
[14] Slaughter, G.R. and Means, A.R. (1987) Methods Enzymol. 139, 433–444.
[15] Kozak, M. (1987) Nucleic Acids Res. 15, 8125–8148.
[16] Walker, J.E., Saraste, M., Runswick, M.J. and Gay, N.J. (1982) EMBO J. 1, 945–951.
[17] Burke, M., Rajasekharan, K.N., Maruta, S. and Ikebe, M. (1990) FEBS lett. 262, 185–188.
[18] Warrick, H.M. and Spudich, J.A. (1987) Ann. Rev. Cell Biol. 3, 379–421.
[19] Chapman, E.R., Au, D., Alexander, K.A., Nicolson, T.A. and Storm, D.R. (1991) J. Biol. Chem. 266, 207–213.
[20] Ruppert, C., Kroschewski, R. and Bahler, M. (1993) J. Cell Biol. 120, 1393–1403
[21] Hoshimaru, M. and Nakanishi, S. (1987) J. Biol. Chem. 262, 4625–14632.