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A mouse brain cDNA encodes a novel protein with the protein kinase C phosphorylation site domain common to MARCKS

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We have isolated a mouse brain cDNA clone encoding a protein of 200 amino acids (M_r 20165) with partial homology with MARCKS (myristoylated alanine-rich C-kinase substrate). Two regions show similarity with MARCKS, one is the kinase C phosphorylation site domain which is supposed to bind calmodulin, and the other is the region near to the N-terminus, including the consensus sequence of myristoylation. It has a similar amino acid composition to MARCKS, but the content of alanine is not as high. It is distributed throughout the mouse brain, but the pattern is not identical with that of MARCKS. Both proteins may be members of a new protein family involved in coupling the protein kinase C and calmodulin signal transduction systems.

Myristoylated alanine-rich C-kinase substrate; Protein kinase C; Calmodulin

1. INTRODUCTION

Protein kinase C is a key enzyme of intracellular signal transduction [1]. Although the enzyme itself is well-characterized, little is known about its physiologically important substrates. One of the few examples is myristoylated alanine-rich C kinase substrate (MARCKS) [2], formerly known as 87-kDa protein [3–6]. This protein is found in a wide variety of cell types, and is phosphorylated within seconds by protein kinase C activation, in the same manner as growth factors in fibroblasts, neurotransmitters in neuronal and glial cells, as well as phorbol-esters or cell-permeating diacylglycerols. In macrophages, part of the protein is myristoylated and found associated with membranes, while the phosphorylated form is found mainly in cytosol [7]. In isolated nerve terminals, phosphorylation by kinase C leads to the translocation of MARCKS from the membrane to the cytosol [8]. It binds calmodulin, and this binding can be prevented by protein kinase C-catalyzed phosphorylation of the protein [9].

cDNA clones encoding MARCKS have been recently isolated, and sequenced [2,10]. The deduced proteins have acidic isoelectric points, and an unusual high content of alanine and glutamic acid. The sites phosphorylated by kinase C have also been determined [11]. All of these are in a 25 amino acid domain, which is extremely basic. A synthetic peptide corresponding to this domain binds calmodulin with high affinity, and phosphorylation of this peptide inhibits the binding [9]. This result strongly suggests that this domain is responsible for the main properties of MARCKS.

In this report, we describe a mouse brain cDNA which encodes a protein with the kinase C phosphorylation site domain of MARCKS. Near the N-terminus there is another region which can be identified, because it includes the consensus sequence of myristoylation. The mRNA is distributed throughout the mouse brain, but the pattern is not identical with that of the MARCKS protein [12]. Thus these two proteins may be members of a new protein family involved in coupling the protein kinase C and calmodulin signal transduction systems.

2. MATERIALS AND METHODS

The isolation of the partial cDNA clone of F52 has previously been described [13]. Total RNA was extracted by the guanidinium-CsCl method from mouse brain, and poly(A)$^+$ RNA was purified by oligo(dT) cellulose column chromatography [14]. A λgt10 mouse brain cDNA library was constructed using the method of Gubler and Hoffman [15]. To obtain full-length copies, the cDNA was fractionated on a Sepharose CL-4B column, and the top 5% was used for the library. Among several positives, the longest clone was selected and sequenced by the dideoxy chain termination method [16] using a shot-gun strategy with oligowalking.

Homology searches used the FASTA program [17]. The amino acid alignment of the homologous amino acid sequence was done using Clustal [18].

Mouse brain sections were mounted on organosilane-treated microscope slides and stored at −20°C until use. Following fixation and pronase digestion, the sections were prehybridized for 2 h at 37°C in 50% formamide, 5 × SSC, 10 mM sodium phosphate (pH 7.0), 2 × Denhardt's, 0.1% SDS. Hybridization was performed at 42°C for 30 to 40 h in the above solution containing 250 μg/ml denatured salmon sperm DNA and ^{32}P-labelled DNA probe (1–1.5 × 10^6 cpm/ml). The probe used was single stranded DNA derived from

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   1  GGAGCGCAGCAGCAGTGGGCGCAGCTAGCGGGTCGGCTGCGGAGCGGGGGTGCAGCGCGC   60
  61  TTTGCCCCCCGTACCCCTCCCCCTCGGACGCAGCCCCACCCCTCTGCCCGCCGGGCCGTC  120
 121  CCAGCCGAACTATCCCCTGCGGCGCCAGCGCGGGGCGGCTCCGGGCGCCCCCAGCAGACC  180
  -1        M  G  S  Q  S  S  K  A  P  R  G  D  V  T  A  E  E  A     18
 181  CCCATCATGGGCAGCCAGAGCTCTAAGGCTCCCCGGGGCGACGTGACCGCCGAGGAGGCA  240
  19  A  G  A  S  P  A  K  A  N  G  Q  E  N  G  H  V  R  S  N  G     38
 241  GCGGGCGCTTCCCCCGCGAAGGCCAACGGACAGGAGAATGGCCACGTGAGAAGCAATGGA  300
  39  D  L  T  P  K  G  E  G  E  S  P  P  V  N  G  T  D  E  A  A     58
 301  GACTTAACCCCCAAGGGTGAAGGGGAGTCACCACCCGTGAACGGAACAGATGAGGCAGCT  360
  59  G  A  T  G  D  A  I  E  P  A  P  P  S  Q  E  A  E  A  K  G     78
 361  GGGGCTACTGGTGATGCCATCGAGCCAGCGCCCCCTAGCCAGGAAGCTGAGGCCAAGGGG  420
  79  E  V  A  P  K  E  T  P  K  K  K  K  K  F  S  F  K  K  P  F     98
 421  GAGGTCGCCCCCAAGGAGACCCCCAAGAAGAAGAAGAAATTCTCTTTCAAGAAGCCTTTC  480
  99  K  L  S  G  L  S  F  K  R  N  R  K  E  G  G  G  D  S  S  A    118
 481  AAATTGAGTGGCCTGTCCTTCAAGAGAAATCGGAAGGAGGGTGGGGGTGATTCCTCAGCC  540
 119  S  S  P  T  E  E  E  Q  E  Q  G  E  M  S  A  C  S  D  E  G    138
 541  TCCTCGCCAACAGAGGAAGAGCAGGAGCAGGGCGAGATGAGCGCCTGCAGCGACGAGGGC  600
 139  T  A  Q  E  G  K  A  A  A  T  P  E  S  Q  E  P  Q  A  K  G    158
 601  ACTGCCCAAGAAGGGAAGGCCGCTGCCACCCCTGAGAGCCAAGAGCCCCAGGCCAAGGGG  660
 159  A  E  A  S  A  A  S  K  E  G  D  T  E  E  E  A  G  P  Q  A    178
 661  GCAGAGGCTAGTGCTGCCTCCAAGGAAGGAGACACAGAAGAGGAGGCAGGGCCCCAGGCT  720
 179  A  E  P  S  T  P  S  G  P  E  S  G  P  T  P  A  S  A  E  Q    198
 721  GCAGAGCCATCCACTCCCTCGGGCCCAGAGAGTGGCCCCACACCTGCCAGTGCTGAGCAG  780
 199  N  E  *                                                       218
 781  AATGAGTAGGGGTGGGGACAGGTGGGTGATCTCTAAGCTGCAAAAACTGTGCTGTCCTTG  840
 841  TGAGGTCACTGCCTGGACCTTGTGCCCTGGCTGCCTTCTTGTGCCCAGAAAGGAAGGGGC  900
 901  TTGTTGCCCCCTCCCAGCCACGCTCCCTTTTTGCCCTCCTGTGGATTCTCCCATCAGCCA  960
 961  TTTGGTCTTACTCTTAAGGCCAGTTGAAGATGGTCCCTTACGGTTTCCCAAGTTAGGTTA 1020
1021  GTGATGTGAGATGCCCTGTCCCTACCTCCTTCCCGAGCCCCATCCCTGCAGAAGGCAATT 1080
1081  GCTGGGTTTCCAATTCTTTTCCAAGTTCGTTTTGTTTATCCTGCTTCTCAACCCCCTGAG 1140
1141  CCAGAAGTGGGGGCTTATACTCCCAAACCCTGAGTGTCCAGCCTTTCCCCTGTTGAATTC 1200
1201  TTAGTCTCTTGTGCTGTGCCTAGTGGCACCTGGGCTGGGGAGGACATTGCCCCATCTGGG 1260
1261  TTTTTATAAATGTCTTACTCAAGTTCAAACCTCCAGCTTGTGAAATCAACCATGTCCCTG 1320
1321  ACTTGATAAGCAAGTGTTAGGCTTCAGGAGGGAGGGAGGTCTGTAATGTGAAACAACTTC 1380
1381  TTGTTGTCTTTTTTTTCCCCCTCCCATTGTTGTAAATAACTTTTAATGGCCAAACCCCAG 1440
1441  ATTTGTACTTTTTTTTTTTTTCTAACTGCTAAAACCATTCTTCCACCCGGATTTACTGTA 1500
1501  ACATTTGGAAAAGGAATAAATGTCGTCCCTTTAAAAAAAAAAAAAAAAAAAAAAAAAAA 1560
1561  AAAAAAAAAAAAAAAAAAAAAAA  1584
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Fig. 1. Nucleotide and predicted amino acid sequence of a cDNA encoding the F52 protein, a protein with the phosphorylation site domain of MARCKS. The amino acid sequence of the predicted polypeptide is shown as single letter code above the nucleotide sequence.

a M13 clone containing the F52 insert. The sections were washed in three changes of 2 × SSC at room temperature, and then in 50% formamide, 0.5 M NaCl, 10 mM Tris-HCl, 1 mM EDTA (pH 7.4) at 37°C for about 6 h. They were dehydrated in 70% ethanol, and exposed on a Kodak X-AR 5 film.

3. RESULTS

In a previous experiment, 35 cDNA clones with specific expression patterns in mouse brain were isolated from 950 cDNA clones by Northern and in situ hybridization using the cDNA inserts as probes [13]. DNA sequence analysis of full-length clones revealed that one, F52, had an open reading frame encoding 200 amino acids (Fig. 1). A comparison of the predicted amino acid sequence with the existing data base revealed that F52 was related to myristoylated alanine-rich C kinase substrate (MARCKS). The amino acid alignment of F52, bovine MARCKS [2] and chicken MARCKS [10] is presented in Fig. 2. Similarity is observed in only two regions, from the first methionine to position 47, and from position 152 to 176 (Fig. 2). The first region includes the consensus sequence of myristoylation [19,20] around glycine 2, which may be myristoylated. The second region corresponds to the 25 amino acid domain with phosphorylation sites for protein kinase C, with conservation of three of the four serines. The domain maintains a strong basic nature, in spite of some amino acid substitutions. The overall amino acid identity is as follows: F52 and bovine MARCKS, 52%; F52 and chicken MARCKS, 52%; bovine and chicken MARCKS, 65%.

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                              20                  40                  60
    F52          MGSQSSK-APRGDVTAEEAA----GASPAKANGQENGHVRSNGDLTPKGE----------
MARCKS (BOVINE)  MGAQFSKTAAKGEATAERPGEAAVASSPSKANGQENGHVKVNGDASPAAAEPGAKEELQA
MARCKS (CHICKEN) MGAQFSKTAAKGEAAAEKPGEA-VAASPSKANGQENGHVKVNGDASPAAAEAG-KEEVQA
                 ** * ** *  *   **         ** **********  ***  *

                              80                  100                 120
    F52          ------------------GESPPVNGTDEAAGAT--------------GDAIEPAPPSQ-
MARCKS (BOVINE)  NGSAPAADKEEPAAAGSGAASPAAAEKDEPAAAAPDAGHPVEKEAPVEGGAAEPGSPTAA
MARCKS (CHICKEN) NGSAPAEE----------------TGKEEAASSEPAS----EKEAA-EAESTEPASP--A
                                        * *                       **  *

                              140                 ▼   ▼   ▼   ▼       180
    F52          EAEAKGEVAPKETP-----------------KKKKKFSFKKPFKLSGLSFKRNRKEGG--
MARCKS (BOVINE)  EGEAASAASSSSSPKAEDGGHALAQQRDAEKKKKKRFSFKKSFKLSGFSFKKNKKEAGEG
MARCKS (CHICKEN) EGEA--------SPKTEEGATP-SSSSETPKKKKKRFSFKKSFKLSGFSFKKNKKEAGEG
                 * **          *                  **** ***** ***** *** * ** *

                              200                 220                 240
    F52          ----GDSSASSPTEEEQEQGEMSACSDEGTAQEGKAAATPESQEPQAKGAEASAASKEGD
MARCKS (BOVINE)  GEAEGAAGASAEGGKDEASGGAAAAAGEAGAAPGEPTAAPGEEAAAGEEGAAGGDPQEAK
MARCKS (CHICKEN) AESEGGAAAAAEGGKEEAAAAAPEAAGGEEGKAAAEEASAAAAGSREAAKEEAGDSQEAK
                     *   *                          *                   *

                              260                 280                 300
    F52          TEEEA----------GPQAAE-----------------------------------------
MARCKS (BOVINE)  PEEAAVAPEKPPARRGAKAVEEPSKAEEKAEEAGVSAAGAAGCEAPSAAGPGCPRAGGAP
MARCKS (CHICKEN) SDEAA--PEKATGE------EAPAAEEQQQQQQQEKAAEEAGAAATSEAGSG--EQEAAP
                   * *                 *

                              320
    F52          ---------------PSTPSGPESGPTPASAEQNE
MARCKS (BOVINE)  REEAAPPRASSACSAPSQEAQPECSPEAPPAEAAE
MARCKS (CHICKEN) AEEPAAAR----------QEAPSESSPEGP-AEPAE
                                 *  *    **  *
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Fig. 2. Comparison of the amino acid sequence of F52, bovine and chicken MARCKS. Identical residues are indicated by asterisks. Putative phosphorylation sites are indicated by arrowheads. Note that a high degree of conservation is only observed in the phosphorylation site domain and the region near the N-terminus.

The predicted molecular weight of F52 is 20165, which is smaller than the predicted molecular weights of 31979 and 27728, for bovine and chicken MARCKS, respectively. F52 has a high proportion of alanine (15.5 mol%), but this is not as high as the 28.4 and 27.0% found in bovine and chicken MARCKS. F52 has other features of the amino acid composition of MARCKS. All proteins show a high amount of glutamic acid and glycine.

An in situ hybridization experiment was performed to determine the expression sites of F52 in mouse brain. Although F52 is expressed throughout the mouse brain, it is enriched in the following regions: dentate gyrus, anterior olfactory nucleus, primary olfactory cortex, entorhinal cortex, medial preoptic area, and dorsomedial hypothalamic nucleus (Fig. 3).

4. DISCUSSION

One approach to understand the consequences of the activation of protein kinase C is to characterize cellular substrates of protein kinase C, of which MARCKS is the best example. Phosphorylation by protein kinase C regulates its binding capacity to calmodulin; it has a myristoylation site, explaining its localization in the membrane of macrophages or nerve terminals. The domain with phosphorylation sites is highly basic and has a general α-helical conformation, similar in certain respects to the calmodulin-binding regions of other proteins [21].

F52 resembles chicken and bovine MARCKS with strong conservation of a phosphorylation site domain. It is likely that this is functionally important, and F52

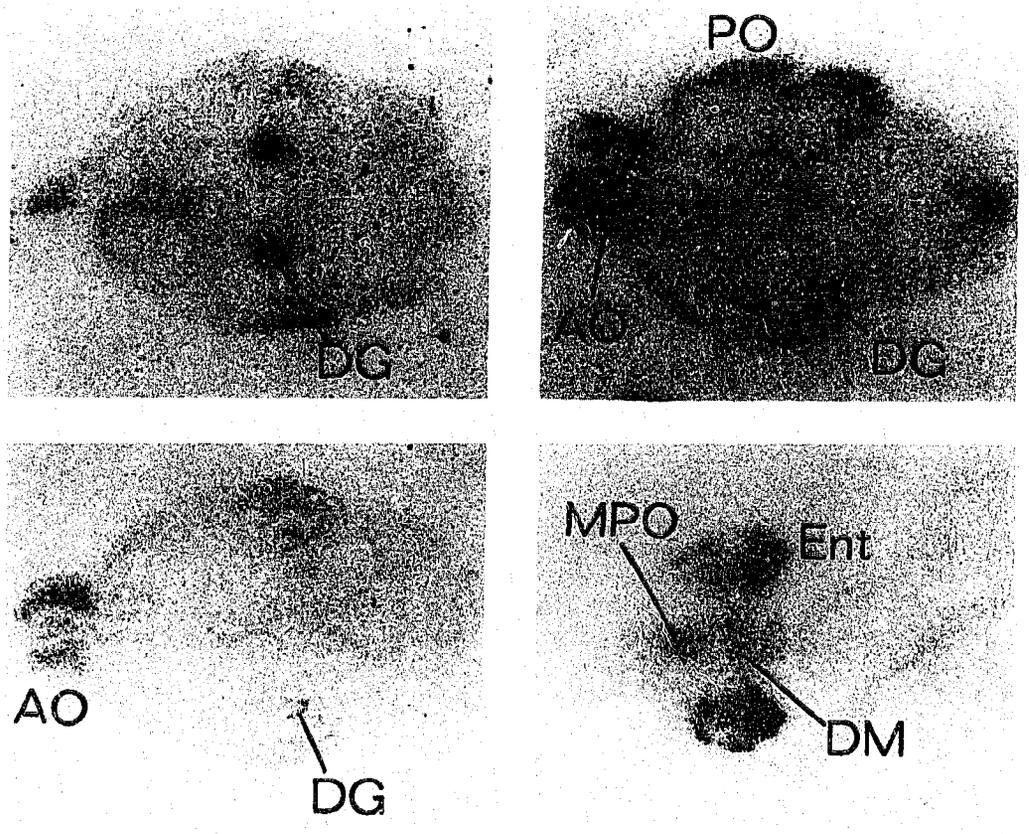

Fig. 3. In situ hybridization analysis of F52 in the mouse brain. The probe used is the partial cDNA clone of F52 which was also used for the isolation of the full-length copy. Abbreviations are as follows: AO, anterior olfactory nucleus; Ent, entorhinal cortex; DG, dentate gyrus; DM, dorsomedial hypothalamic nucleus; MPO, medial preoptic area; PO, primary olfactory cortex.

is also a calmodulin-regulating protein. The divergence of the rest of the structure from that of MARCKS suggests that the rest is probably not relevant to this function. This is supported by existence of another homologous region at its N-terminus, which includes the consensus sequence of myristoylation. This suggests that F52 also could be localized to the membrane.

MARCKS and F52 are distributed throughout the brain, but the enriched regions are not identical [12]. Both are enriched in primary olfactory cortex, entorhinal cortex and hypothalamus. While MARCKS is enriched in amygdaloid complex, F52 is enriched in anterior olfactory nucleus and dentate gyrus. Although it may be misleading to compare the data of different techniques (MARCKS, immunocytochemistry; F52, in situ hybridization) and of different species (MARCKS, rat; F52, mouse), the cellular subpopulations which express F52 seem different from those which express MARCKS.

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