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Deduced primary structure of the β subunit of brain type II Ca^{2+}/calmodulin-dependent protein kinase determined by molecular cloning

(protein phosphorylation/synaptic regulation)

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ABSTRACT **cDNA clones coding for the β subunit of rat brain type II Ca^{2+}/calmodulin-dependent protein kinase were isolated and sequenced. The clones, including one containing the entire coding region, hybridize at high stringency to a single band of poly(A)$^+$ RNA of length 4.8 kilobases. The subunit coded for by the clones was identified by *in vitro* transcription of the cDNA followed by translation of the resulting RNA. The DNA sequence of the clones contains a single long open reading frame (1626 nucleotides) coding for a protein of 542 amino acids with a molecular weight of 60,333, the amino-terminal half of which is homologous to several other protein kinases. Potential ATP- and calmodulin-binding domains were identified. Two independent clones contain an identical 45-nucleotide deletion, relative to the clones described above, resulting in a shorter open reading frame coding for a protein of molecular weight 58,000. This suggests that the minor, 58-kDa β′ subunit of the type II Ca^{2+}/calmodulin-dependent kinase may be synthesized on a separate message.**

The type II Ca^{2+}/calmodulin-dependent protein kinase (type II CaM kinase) is one of the most abundant brain protein kinases (1–3). It is a member of a family of broad-specificity protein kinases of similar structure and substrate specificity that occur at lower concentrations in other tissues (4–6). This kinase is particularly highly expressed in forebrain neurons (3, 7), where it is present throughout the neuron and appears to be concentrated in postsynaptic densities (8–10). The properties of the kinase suggest that it can act as a calcium-triggered switch and that it could, therefore, encode certain long-lasting changes in synapses that are produced by brief bursts of synaptic activity (11, 12). The predominant forebrain form is composed of approximately nine 50-kDa α subunits and three 60-kDa β subunits, both of which bind calmodulin and appear to be catalytic. We have isolated cDNA clones that code for the β subunit and deduced its amino acid sequence from the nucleotide sequence of the coding region.

METHODS

Materials. DNA polymerase I Klenow fragment and restriction endonucleases were purchased from Boehringer Mannheim Biochemicals; oligo(dT)-cellulose, M13 hybridization probe primer, and protein A-Sepharose, from Pharmacia; [α-^{32}P]dATP and [^{35}S]methionine, from Amersham; SP6 RNA polymerase, from New England Biolabs; and plasmid pGEM-2, from Promega Biotech. Two oligo(dT)-primed rat brain cDNA libraries, constructed in the vectors λgt11 (13) and λgt10 (14), were a gift of David Anderson of Columbia University.

Screening of cDNA Libraries. The λgt11 library (2.5×10^5 plaques) was screened as described by Moon *et al.* (15) with two rabbit polyclonal antisera, one generated against whole purified type II CaM kinase (Annette) and the other against electrophoretically purified β subunit (Darcy) (16). Two cDNA clones coding for proteins that reacted with both antisera were isolated.

The λgt10 library was screened by plaque hybridization (17) with a ^{32}P-labeled 600-base restriction fragment of one of the λgt11 inserts. Conditions for prehybridization, hybridization, and washing were essentially as described by Meinkoth and Wahl (18). Hybridization was done in 50% formamide in a buffer containing 0.75 M NaCl at 42°C and was followed by washes with a buffer containing 18 mM NaCl at 42°C. Twenty-three additional cDNA clones were isolated from 4×10^5 plaques screened.

DNA Sequencing. Detailed restriction maps of the cDNAs were constructed, and appropriate restriction fragments were isolated by agarose gel electrophoresis (19) and ligated into the M13 vectors mp18 and mp19 for sequencing (20). DNA sequences were determined by the dideoxy chain-termination method (21) with either a 15-nucleotide universal primer or an 18-nucleotide β-subunit-specific primer.

Preparation of Hybridization Probes. Hybridization probes with specific activities of $1–2 \times 10^8$ cpm/μg of insert were prepared from the single-stranded DNA of M13 subclones by extension of a primer with DNA polymerase I Klenow fragment in the presence of [α-^{32}P]dATP as described by Messing (20).

Analysis of Endogenous Messages. RNA was purified from whole rat brain and from forebrain and cerebellum as described by Chirgwin *et al.* (22). Poly(A)$^+$ RNA was purified by two cycles of chromatography on oligo(dT)-cellulose (23), fractionated by electrophoresis in 1% agarose gels containing 2.2 M formaldehyde, and transferred to nitrocellulose (19). Hybridization of probes with the immobilized RNA was performed as described above for plaque hybridization.

***In Vitro* Translation and Immunoprecipitation of Products.** A rabbit reticulocyte lysate translation system was prepared as described by Jackson and Hunt (24). RNA was translated in the presence of [^{35}S]methionine. Translation products were diluted with 3 volumes of NET (50 mM Tris, pH 7.5/150 mM NaCl/10 mM EDTA) containing 1% (vol/vol) Nonidet P-40, then precleared by stirring with one volume of protein A-Sepharose beads and 0.2 volume of rabbit anti-mouse IgG (1.5 mg/ml) for 2 hr at 4°C. The beads were sedimented, and the supernatant was transferred to a fresh tube and incubated with 80 ng of pure carrier type II CaM kinase and 2.5 μl (≈50 μg) of anti-kinase monoclonal antibody 4A11 (3) for 4 hr at 0°C. Rabbit anti-mouse IgG (7.5 μl) and protein A-Sepharose (40 μl) were added, and the reaction continued for 2 hr at 4°C

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Abbreviation: type II CaM kinase, type II Ca^{2+}/calmodulin-dependent protein kinase.

with mixing. The beads were sedimented and washed six times with NET containing 1% Nonidet P-40 and twice with NET. Immunoprecipitated proteins were released by boiling in $NaDodSO_4$ sample buffer and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis.

***In Vitro* Transcription.** The longest λgt10 cDNA insert [λ10β5-2; 1.8 kilobases (kb)] was excised with *Eco*RI and inserted into the plasmid pGEM-2, which contains the SP6 promoter. The recombinant plasmid was linearized with *Hin*dIII and transcripts were synthesized with SP6 RNA polymerase as described by Melton *et al.* (25).

RESULTS

Selection of cDNA Clones. cDNA clones coding for the β subunit of brain type II CaM kinase were identified by screening a rat brain cDNA library constructed in the expression vector λgt11 with two anti-kinase rabbit antisera. Both antisera react strongly with the β subunit and weakly with the α subunit on immunoblots (16). Two cDNA clones, both of which code for fusion proteins that react with both antisera, were isolated. The reaction was blocked by preabsorption of the antisera with pure type II CaM kinase. A λgt10 library was then screened with a 600-base restriction fragment of one of the λgt11 inserts, resulting in selection of 23 additional cDNA clones. The seven clones with the longest inserts (0.85–1.8 kb) were characterized further.

Restriction site analysis of these clones generated a consistent map 1.8 kb in length (Fig. 1). Although some of the clones extended outside the 1.8-kb region, the restriction maps in these flanking regions were not consistent. These regions may represent artifacts due to the ligation of two or more unrelated sequences during the construction of the λgt10 library (D. Anderson, personal communication). Only restriction sites and nucleotide sequences that were obtained from at least two independent clones are included in the overall restriction map (Fig. 1) and nucleotide sequence (Fig. 3).

Subunit Specificity. One cDNA clone, λ10β5-2, included the entire 1.8-kb region shown in Fig. 1 and was used to establish the subunit coded for by the cDNA clones. We first established that there is a differential distribution of messages for the two subunits between the forebrain and the cerebellum. Equal amounts of RNA from each of these two regions were translated *in vitro*. Immunoprecipitation of the products with an anti-kinase monoclonal antibody demonstrated that the α-subunit message is more abundant in the forebrain than in the cerebellum, whereas the β-subunit message is equally distributed between them (Fig. 2*A*). This is consistent with the different subunit composition of the enzymes purified from these two brain regions (16, 26). The distribution of the messages recognized by the λ10β5-2 insert was determined by blot analysis of equal amounts of forebrain and cerebellar RNA (Fig. 2*B*). The insert hybridizes primarily with a 4.8-kb band (thick arrow) that is roughly equally distributed between the forebrain and the cerebellum, as is the β-subunit message. In addition, it hybridizes with a 5.4-kb band (thin arrow) which is more abundant in the forebrain than in the cerebellum. Hybridization with this band is eliminated by washing the blot at higher stringency (18 mM NaCl, 65°C). We believe

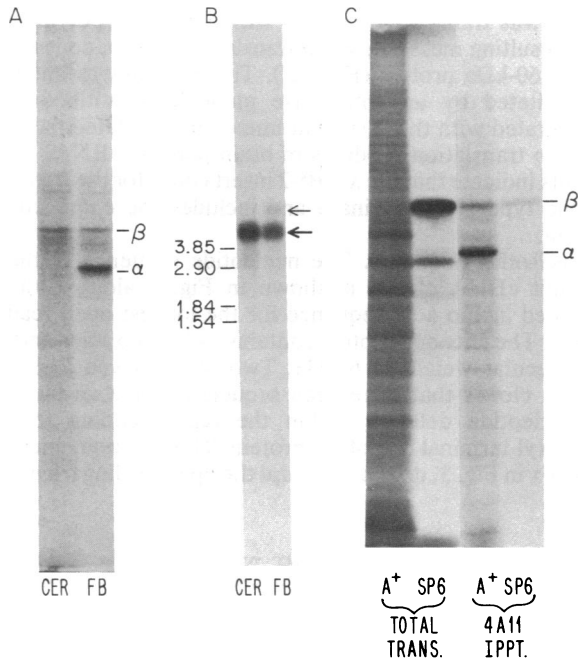

FIG. 2. Demonstration of subunit specificity of the type II CaM kinase cDNA clones. (*A*) Fifteen micrograms of cerebellar (CER) and forebrain (FB) total RNA was translated *in vitro* in 90 μl of a rabbit reticulocyte lysate translation mixture, containing 63 μCi (1 Ci = 37 GBq) of [^{35}S]methionine, for 90 min at 30°C. The translation products were immunoprecipitated with monoclonal antibody 4A11. Positions of the α and β subunits are marked. (*B*) Five micrograms of cerebellar (CER) and forebrain (FB) poly(A)$^+$ RNA was resolved in a 1% agarose gel containing 2.2 M formaldehyde and transferred to a nitrocellulose filter. The filter was probed with 0.5 μg of a ^{32}P-labeled single-stranded λ10β5-2 probe (specific activity 2×10^8 cpm/μg of insert). Major and minor bands are indicated by thick and thin arrows, respectively. Size markers used were *Escherichia coli* and sea urchin ribosomal RNAs. (*C*) Ten micrograms of forebrain poly(A)$^+$ RNA was translated *in vitro* in 200 μl of a rabbit reticulocyte lysate translation mixture, containing 80 μCi of [^{35}S]methionine, for 90 min at 30°C. Two microliters of the total translation product (A$^+$, TOTAL TRANS.) was loaded directly on a $NaDodSO_4$/polyacrylamide gel and the remainder (198 μl) was immunoprecipitated with monoclonal antibody 4A11 (A$^+$, 4A11 IPPT.) prior to loading on the gel. One-tenth microgram of the SP6 *in vitro* transcript of cDNA insert λ10β5-2 was translated *in vitro* in 20 μl of a rabbit reticulocyte lysate translation mixture, containing 8.0 μCi of [^{35}S]-methionine, for 90 min at 30°C. Two microliters of the total translation product (SP6, TOTAL TRANS.) was loaded directly on the gel, and 0.4 μl of the translation product was immunoprecipitated with monoclonal antibody 4A11 (SP6, 4A11 IPPT.) prior to loading on the gel. The 45-kDa protein present in the total translation products is an endogenous reticulocyte protein labeled in the absence of exogenous RNA (24).

the 5.4-kb message codes for the α subunit, since it has the same size and distribution as a message recognized by two α-subunit-specific oligonucleotide probes (data not shown). These results suggest that the clones code for the β subunit. More definitive evidence was obtained by examining the translation product coded for by the λ10β5-2 insert. The

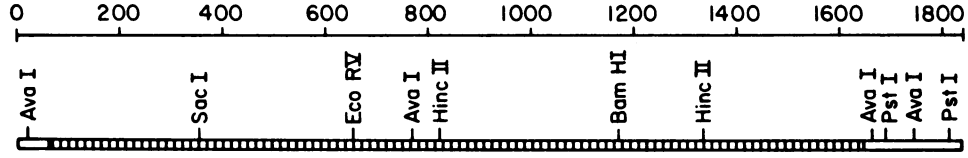

FIG. 1. Composite restriction map of seven independent type II CaM kinase cDNA clones. Region of vertical stripes indicates the position of the longest open reading frame. Scale gives length in base pairs.

insert was transcribed *in vitro* with SP6 RNA polymerase. The resulting message, when translated *in vitro*, coded for a single 60-kDa protein (Fig. 2*C*). This protein was immunoprecipitated by an anti-kinase monoclonal antibody and comigrated with the β subunit immunoprecipitated from the *in vitro* translation products of brain poly(A)$^+$ RNA. These results indicate that the λ10β5-2 insert codes for the β subunit of the type II CaM kinase and includes the entire coding region.

Nucleotide Sequence. The nucleotide sequence of the β-subunit cDNA clones is shown in Fig. 3 along with the deduced amino acid sequence for the longest open reading frame. The encoded protein contains 542 amino acids and has a molecular weight of 60,333. Two of the seven β-subunit cDNA clones that have been sequenced have an identical 45-nucleotide deletion within the region coding for the carboxyl-terminal half of the protein. This deletion, enclosed in a box in Fig. 3, does not disrupt the open reading frame and may represent an alternatively spliced β-subunit message (see *Discussion*).

Sequence Homologies. The amino acid sequence deduced from the β-subunit cDNA clones was compared to the sequences in the National Biomedical Research Foundation protein sequence data base.* The top nine alignment scores were all for protein kinases or protein kinase-related proteins. The homologies are all within a sequence of about 300 amino acids in the amino-terminal half of the β subunit. When properly aligned (Fig. 4), the β subunit is identical with corresponding amino acids of the γ subunit of phosphorylase *b* kinase, myosin light chain kinase, and the catalytic subunit of cAMP-dependent protein kinase at 38%, 31%, and 27% of their amino acid positions, respectively. If conservative

*Protein Identification Resource (1986) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 7.0.

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 -62    GG AGCCGGAGTC GCCGCCGCCC GAGCGCAGCC GAGCGCACGC CGAGCCCAAT CGCCACCGCC

   1  ATG GCC ACC ACG GTG ACC TGC ACC CGT TTC ACG GAC GAG TAC CAG CTA TAC GAG GAT ATT GGC AAG GGG GCT TTC TCT GTG GTC CGA CGC
   1  Met Ala Thr Thr Val Thr Cys Thr Arg Phe Thr Asp Glu Tyr Gln Leu Tyr Glu Asp Ile Gly Lys Gly Ala Phe Ser Val Val Arg Arg
                                                 *
  91  TGT GTC AAG CTC TGC ACC GGC CAT GAG TAT GCA GCT AAG ATC ATT AAC ACC AAG AAG CTG TCA GCT AGA GAT CAC CAG AAG CTG GAG AGG
  31  Cys Val Lys Leu Cys Thr Gly His Glu Tyr Ala Ala Lys Ile Ile Asn Thr Lys Lys Leu Ser Ala Arg Asp His Gln Lys Leu Glu Arg

 181  GAG GCT CGG ATC TGC CGC CTG CTG AAG CAT TCC AAC ATT GTA CGC CTC CAT GAC AGC ATC TCT GAA GAG GGC TTC CAC TAC CTG GTC TTC
  61  Glu Ala Arg Ile Cys Arg Leu Leu Lys His Ser Asn Ile Val Arg Leu His Asp Ser Ile Ser Glu Glu Gly Phe His Tyr Leu Val Phe

 271  GAC CTG GTC ACT GGT GGG GAG CTC TTT GAA GAC ATT GTG GCG AGA GAG TAC TAC AGT GAG GCT GAC GCC AGT CAC TGT ATC CAG CAG ATC
  91  Asp Leu Val Thr Gly Gly Glu Leu Phe Glu Asp Ile Val Ala Arg Glu Tyr Tyr Ser Glu Ala Asp Ala Ser His Cys Ile Gln Gln Ile

 361  CTG GAG GCT GTT CTC CAT TGT CAC CAA ATG GGG GTC GTC CAC AGA GAC CTC AAG CCT GAA AAC CTC CTG CTC GCC AGC AAA TGC AAA GGG
 121  Leu Glu Ala Val Leu His Cys His Gln Met Gly Val Val His Arg Asp Leu Lys Pro Glu Asn Leu Leu Leu Ala Ser Lys Cys Lys Gly

 451  GCC GCA GTG AAA CTG GCA GAC TTC GGC CTG GCC ATC GAG GTT CAG GGA GAC CAG CAG GCA TGG TTT GGA TTT GCG GGA ACA CCA GGC TAC
 151  Ala Ala Val Lys Leu Ala Asp Phe Gly Leu Ala Ile Glu Val Gln Gly Asp Gln Gln Ala Trp Phe Gly Phe Ala Gly Thr Pro Gly Tyr

 541  CTG TCT CCC GAA GTT CTT CGG AAG GAG GCC TAT GGC AAA CCA GTG GAT ATC TGG GCA TGT GGG GTG ATC CTG TAT ATC CTG CTG GTG GGA
 181  Leu Ser Pro Glu Val Leu Arg Lys Glu Ala Tyr Gly Lys Pro Val Asp Ile Trp Ala Cys Gly Val Ile Leu Tyr Ile Leu Leu Val Gly

 631  TAC CCA CCT TTC TGG GAT GAG GAC CAG CAC AAG CTG TAC CAG CAG ATC AAG GCT GGG GCC TAT GAC TTC CCA TCC CCC GAG TGG GAC ACC
 211  Tyr Pro Pro Phe Trp Asp Glu Asp Gln His Lys Leu Tyr Gln Gln Ile Lys Ala Gly Ala Tyr Asp Phe Pro Ser Pro Glu Trp Asp Thr

 721  GTT ACC CCT GAA GCC AAA AAC CTC ATC AAC CAG ATG TTG ACC ATC AAC CCC GCC AAG CGC ATC ACG GCC CAC GAG GCC CTG AAG CAC CCA
 241  Val Thr Pro Glu Ala Lys Asn Leu Ile Asn Gln Met Leu Thr Ile Asn Pro Ala Lys Arg Ile Thr Ala His Glu Ala Leu Lys His Pro

 811  TGG GTC TGC CAA CGA TCC ACG GTG GCC TCC ATG ATG CAC AGA CAG GAG ACT GTG GAA TGT CTG AAG AAG TTC AAT GCA AGG AGG AAG CTC
 271  Trp Val Cys Gln Arg Ser Thr Val Ala Ser Met Met His Arg Gln Glu Thr Val Glu Cys Leu Lys Lys Phe Asn Ala Arg Arg Lys Leu

 901  AAG GGA GCC ACT CTC ACC ACT ATG CTG GCC ACA CGG AAT TTC TCA GTG GGC AGA CAG ACC ACC GCT CCG GCC ACA ATG TCC ACC GCG GCC
 301  Lys Gly Ala Ile Leu Thr Thr Met Leu Ala Thr Arg Asn Phe Ser Val Gly Arg Gln Thr Thr Ala Pro Ala Thr Met Ser Thr Ala Ala

 991  TCC GGC ACC ACC ATG GGG CTG GTG GAA CAA GCC AAG AGT TTA CTC AAC AAG AAA GCA GAC GGA GTC AAG CCC CAG ACA AAC AGC ACC AAA
 331  Ser Gly Thr Thr Met Gly Leu Val Glu Gln Ala Lys Ser Leu Leu Asn Lys Lys Ala Asp Gly Val Lys Pro Gln Thr Asn Ser Thr Lys

1081  AAC AGC TCG GCC ATC ACC AGC CCC AAA GGA TCC CTC CCT CCT GCC GCC CTG [GAG CCT CAA ACC ACC GTT ATC CAT AAC CCA GTG GAC GGC]
 361  Asn Ser Ser Ala Ile Thr Ser Pro Lys Gly Ser Leu Pro Pro Ala Ala Leu Glu Pro Gln Thr Thr Val Ile His Asn Pro Val Asp Gly

1171 [ATT AAG] GAA TCT TCC GAC AGC ACC AAC ACA ACC ATA GAG GAC GAA GAT GCC AAA GCC CGG AAG CAG GAA ATC ATC AAG ACC ACA GAG CAG
 391  Ile Lys Glu Ser Ser Asp Ser Thr Asn Thr Thr Ile Glu Asp Glu Asp Ala Lys Ala Arg Lys Gln Glu Ile Ile Lys Thr Thr Glu Gln

1261  CTC ATC GAG GCC GTC AAC AAC GGC GAC TTT GAG GCC TAT GCG AAA ATC TGT GAC CCA GGC CTG ACC TCA TTT GAG CCT GAA GCT CTG GGC
 421  Leu Ile Glu Ala Val Asn Asn Gly Asp Phe Glu Ala Tyr Ala Lys Ile Cys Asp Pro Gly Leu Thr Ser Phe Glu Pro Glu Ala Leu Gly

1351  AAC CTG GTC GAA GGG ATG GAT TTC CAC AGA TTC TAC TTT GAG AAC CTG CTG GCC AAG AAC AGC AAG CCG ATC CAC ACC ACT ATC CTG AAC
 451  Asn Leu Val Glu Gly Met Asp Phe His Arg Phe Tyr Phe Glu Asn Leu Leu Ala Lys Asn Ser Lys Pro Ile His Thr Thr Ile Leu Asn

1441  CCG CAC GTG CAC GTC ATC GGC GAG GAT GCA GCC TGC ATC GCT TAC ATC CGC CTC ACA CAG TAC ATC GAC GGC CAG GGC AGA CCC CGC ACC
 481  Pro His Val His Val Ile Gly Glu Asp Ala Ala Cys Ile Ala Tyr Ile Arg Leu Thr Gln Tyr Ile Asp Gly Gln Gly Arg Pro Arg Thr

1531  AGC CAG TCC GAA GAG ACC CGT GTG TGG CAC CGC CCC GAC GGC AAG TGG CAG AAT GTC CAT TTC CAC TGC TCG GGC GCT CCA GTG GCC CCA
 511  Ser Gln Ser Glu Glu Thr Arg Val Trp His Arg Pro Asp Gly Lys Trp Gln Asn Val His Phe His Cys Ser Gly Ala Pro Val Ala Pro

1621  CTG CAG TGA GAGCTGCGCC TGGTTTCACC GGACAGAGTT GGTGTTTGGA GCCCAGCCGC CCTCGGGCGC ACGGCCTGCC TGTCGCATGT TTGTGTCTGC
 541  Leu Gln  *

1720   CTCGTCCCCT CCCCTGGTGC CTGTGTCTGC AGAAAAACAA GACCAGATGT GATTTGTTT
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FIG. 3. Nucleotide sequence and predicted amino acid sequence of the β subunit of type II CaM kinase. The nucleotide sequence, covering the region illustrated in Fig. 1, was compiled from analysis of seven overlapping β-subunit cDNA clones. The predicted amino acid sequence for the longest open reading frame is shown below the DNA sequence. The sequence enclosed in a box (nucleotides 1194–1238; nucleotides 1132–1176 in the coding sequence) is the region deleted from two independent clones. The region of sequence included in the comparison with other protein kinases in Fig. 4 is underlined. The putative ATP-binding residue (Lys-43) is indicated by an asterisk. The entire region was sequenced on both strands with each position having been determined an average of five times. Details of the sequencing strategy are presented elsewhere (27).

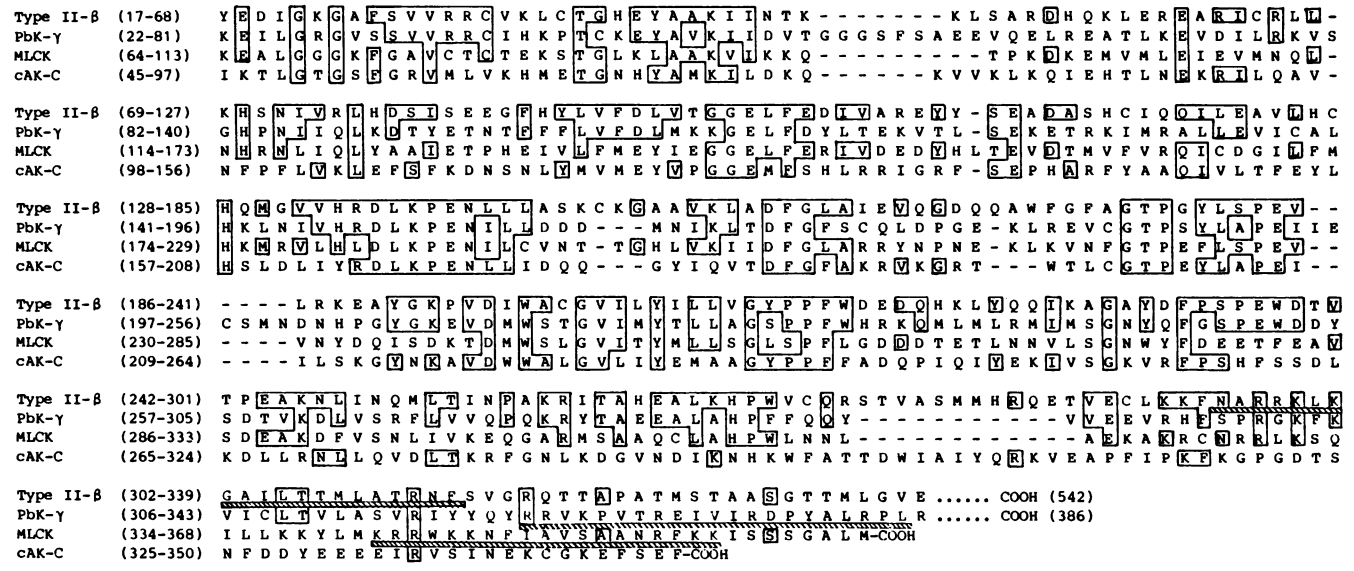

FIG. 4. Amino acid sequence alignment of the β subunit of type II CaM kinase (Type II-β), the γ subunit of phosphorylase *b* kinase (Pbk-γ), skeletal muscle myosin light chain kinase (MLCK), and the catalytic subunit of cAMP-dependent protein kinase (cAK-C). The sequences of Pbk-γ, MLCK, and cAK-C are from Reimann *et al.* (28), Takio *et al.* (29), and Shoji *et al.* (30), respectively. Residue numbers for each kinase are indicated in parentheses at the beginning of each line. Sites of amino acid identity between the β-subunit sequence and the other kinases are boxed, and gaps inserted to optimize alignment are indicated by dashes. The putative ATP-binding residue of the β subunit (Lys-43) is marked with an asterisk. The putative calmodulin-binding domains for the β subunit of type II CaM kinase (amino acids 295–315), the γ subunit of phosphorylase *b* kinase (amino acids 322–342), and skeletal muscle myosin light chain kinase (amino acids 342–360) are underlined by hatched bars. Standard one-letter amino acid symbols are used.

amino acids are included in the comparison (31), the homologies are 58%, 52%, and 48% for the conserved domain of the three kinases. Near the amino-terminal of the homologous domain is a conserved lysine residue (asterisk in Fig. 4) that is labeled in several protein kinases by photoaffinity analogues of ATP (32–34). Lys-43 in the β-subunit sequence is in a position that corresponds to this lysine, and thus it may be a component of the ATP binding site.

This region of the β subunit is also highly homologous to two recently published serine kinase sequences. It is identical to the mammalian C-kinase (residues 343–595) at 30% of its residues (35, 36) and to the yeast *snf1* gene product (residues 59–306) at 38% of its residues (37). There are no homologies to either of these proteins outside the conserved kinase domain.

Calmodulin-Binding Domain. Calmodulin binding sites from a number of calmodulin-dependent protein kinases have been identified (38, 39). Although no consensus primary sequence has emerged from these studies, there is a secondary structural feature, a basic amphiphilic α-helix, that is common to many calmodulin-binding peptides (40). One strongly basic region exists within the type II CaM kinase β-subunit sequence (amino acids 295–315) which could form an α-helix (41). Two-dimensional projections of this α-helix (42) reveal that it has a basic surface (including three arginine residues and one lysine residue) and a nonpolar surface (including three alanines, one leucine, and one isoleucine) that is similar in its amphiphilic nature to the calmodulin-binding domains identified in other calmodulin-dependent protein kinases (38, 39). This potential calmodulin-binding domain (underlined sequence in Fig. 4) has only limited homology with other protein kinases. However, it is positioned in the primary sequence near the putative calmodulin-binding domains in skeletal muscle myosin light chain kinase (38) and in phosphorylase *b* kinase (39) (also underlined in Fig. 4).

DISCUSSION

We have used antibodies against the brain type II CaM kinase to isolate cDNA clones coding for the β subunit. The coding specificity of the clones was determined from the identity of the protein synthesized from transcripts of the longest cDNA (Fig. 2*C*) and from the differential distribution of the messages recognized by the clones in different brain regions (Fig. 2 *A* and *B*). The molecular weight (60,333) of the protein coded for by the open reading frame (Fig. 3) is in close agreement with that estimated for the β subunit from its mobility in $NaDodSO_4$/polyacrylamide gels (1). Primer-extension analysis indicates that the β-subunit message extends an additional 134 nucleotides beyond the 62 nucleotides of 5′ untranslated sequence contained in the clones (data not shown). Because the amino terminus of the β subunit is blocked, we have not been able to directly confirm the translation initiation site from the amino acid sequence. However, the neighboring sequences of the first ATG codon in the open reading frame are consistent with it being the true start codon (43). Moreover, the λ10β5-2 cDNA insert appears to encode a full-length β-subunit protein (Fig. 2*C*).

The deduced amino acid sequence of the β subunit contains a region of high homology to a number of other protein kinases (Fig. 4). The homology is strongest to serine/threonine-specific kinases. It is weaker to tyrosine-specific kinases such as the oncogene kinases and cell surface receptor kinases. Within this domain most of the kinases contain a consensus sequence, Gly-Xaa-Gly-Xaa-Xaa-Gly, 16–28 residues amino-terminal to the ATP-binding lysine residue. It has been proposed that this sequence is involved in nucleotide binding (34). Within the β-subunit sequence, Lys-43 is in a position that corresponds to the ATP-binding residue in other protein kinases; the sequence Gly-Xaa-Gly-Xaa-Xaa-Ser begins at residue 21. The γ subunit of phosphorylase kinase also contains a serine residue in place of the third glycine in the consensus sequence. Therefore, this third glycine may not be an absolute requirement for nucleotide binding. The functions of the other regions of high homology among protein kinases (Fig. 4) are not known.

Amino acid sequences outside the putative catalytic domain are not homologous among the various protein kinases and are probably important for functions that are specific to particular kinases, including regulatory interactions (35, 36,

38, 44). Within the β-subunit sequence, we have identified one potential regulatory domain, a calmodulin binding site (Fig. 4). This domain shares the sequence Ala-Arg-Arg-Lys with the calmodulin-binding peptide from smooth muscle myosin light chain kinase (39) and the sequence Asn-Ala-Arg-Arg-Lys-Leu-Lys with an unidentified brain calmodulin-binding protein (47). The autophosphorylation sites that control the generation of calcium-independent kinase activity will also be important to identify (12). Of the 29 serine and 40 threonine residues within the β subunit, approximately 10 occur within the consensus sequence Arg/Lys-Xaa-Xaa-Ser/Thr characteristic of phosphorylation sites on several substrates of the type II CaM kinase (45) and thus are potential autophosphorylation sites. Since peptide mapping has shown that there are only three major calcium-dependent autophosphorylation sites on the β subunit (S. G. Miller and M.B.K., unpublished observations), peptide sequencing will be necessary to identify the relevant sites unambiguously.

Most purified preparations of brain type II CaM kinase contain a minor subunit of molecular mass 58 kDa, which has been termed the β′ subunit (1, 16, 26). Peptide maps suggest that it is closely related to the β subunit (26, 46). Several laboratories have suspected that this protein is generated by artifactual proteolytic cleavage of the β subunit, although it appears to be present in different amounts in different brain regions (16, 26). Our results suggest an alternative hypothesis. Two independent clones coding for the β subunit contain a 45-nucleotide deletion in the region coding for the carboxyl half of the subunit. The open reading frame present in these clones codes for a 58-kDa protein that could be the β′ subunit. If these clones represent the sequence of a separate β′ message, that message must result either from expression of a β′-specific gene or from posttranscriptional processing of the β-subunit message (alternative splicing). The sequence at each end of the deletion is consistent with alternative splicing of a single exon.

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