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New isoforms of multifunctional calcium/calmodulin-dependent protein kinase II

Peter Mayer, Matthias Möhlig, Helmut Schatz, Andreas Pfeiffer*

Medizinische Universitätsklinik Bergmannsheil, Ruhr-Universität, Gilsingstr. 14, 44789 Bochum, Germany

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Calcium/calmodulin dependent protein kinase II (CaM kinase II) seems to act as an important regulator of intracellular signal transmission. Four subtypes, termed α to δ, have been cloned; some of them can exist as different splicing variants. All these isoforms share a great overall homology, and they contain 3 areas of low homology. We have identified 5 new variants of subtype delta so that the total number of different isoforms now adds up to 12. These variants are probably a result of different splicing and show several deletions in regard to subtype delta. The deletion sites do exactly match regions of low homology between the subtypes. This suggests a functional division of the CaM kinase II molecule into homologous and variable domains. The homologous domains are highly conserved. Therefore, it might be the case that the constitution of the variable domains is more significant for a certain isoform than its belonging to one of the 4 subtypes α to δ.

Calmodulin; Protein kinase; Isoform

1. INTRODUCTION

Calcium/calmodulin dependent protein kinase II (CaM kinase II) was originally described in rat brain tissue. Its complex autoregulation and its broad substrate range make it a candidate for an important regulator of intracellular signal transmission [1,2]. Important functions of CaM kinase II include the control of neurotransmitter release [3] and the mediation of stimulus induced gene expression [4].

From rat brain, five different subtypes were identified and cloned, termed α, β, β′, γ and δ [5]. Subtype β′ is probably a splicing variant of subtype β [6]. CaM kinase II-like enzyme activities were also found in non-neuronal tissues [1]. In our previous work we described such an enzyme activity in the insulin secreting cell line RINm5F [7].

Northern blot analysis and PCR amplification of cDNA revealed that subtypes γ and δ, but not α and β, were present in non-neuronal tissues (ref. 5 and our unpublished observations). The calculated molecular weights of subtypes γ and δ are 59.0 kDa and 60.1 kDa, respectively. On the other hand, CaM kinase II type enzymes that were purified from several tissues except brain tissue often displayed molecular masses much smaller than these values (for example, 50 kDa in intestinal brush border, [8], and 51 kDa in rat pancreas [9]. This suggests that there could be further subtypes of CaM kinase II or, alternatively, splicing variants of the established isoforms. Nghiem et al. [10] recently described two putative splicing variants of human CaM kinase II γ. We searched for variants of subtype δ.

*Corresponding author. Fax: (49) (234) 302 6403.

2. MATERIALS AND METHODS

Total RNA was extracted from rat brain and RINm5F rat insulinoma cells by the guanidinium isocyanate method [11]. For reverse transcription, M-MLV reverse transcriptase from Gibco was used. The reaction buffer was also supplied by Gibco.

3 pairs of PCR primers were used to amplify the whole coding region of CaM kinase II δ cDNA. The first upstream primer (sequence, 5′-GGA GGA GGA GGA AGA AGG AT) started at base position −200 (base position 1, start of translation). The first downstream primer (sequence, 5′-CAC CAG CAA GAT GTA GAG GA) started at base position 627 of the complementary strand. The next primer pair started at base position 502 and 1,417, respectively. Its sequence was 5′-CAG CAG GCG TGG TTT GGT TT (upstream) and 5′-CCA TGT ACT GTG TGA GCC GA (downstream). The third primer pair started at base position 917 and 1,647, respectively. Its sequence was 5′-CAA CTA TGC TGG CTA CGA GA (upstream) and 5′-GAC GTG GCA CTG TTG ACA AT (downstream). The PCR conditions were as follows: denaturation at 95°C for 50 s; annealing at 55°C for the first primer pair and at 57°C for the other pairs for 2 min 30 s; elongation at 72°C for 5 min. *Taq* polymerase and reaction buffer were from Perkin Elmer or from Boehringer Mannheim. The concentration of $MgCl_2$ was adjusted to 1.5 mM for the left primer pair and to 3.5 mM for the other primer pairs.

The amplificates were blunt-end ligated into the plasmid pBluescript from Stratagene or, alternatively, cloned into the plasmid pAMP (CloneAmp System from Gibco) according to manufacturer's instructions. Nucleotide sequencing was performed by the dideoxynucleotide chain termination method [12] using Sequenase 2.0 kit from USB.

3. RESULTS AND DISCUSSION

We amplified the coding region of CaM kinase II δ cDNA from RINm5F rat insulinoma cells and from rat brain using 3 pairs of PCR primers to obtain 3 overlap-

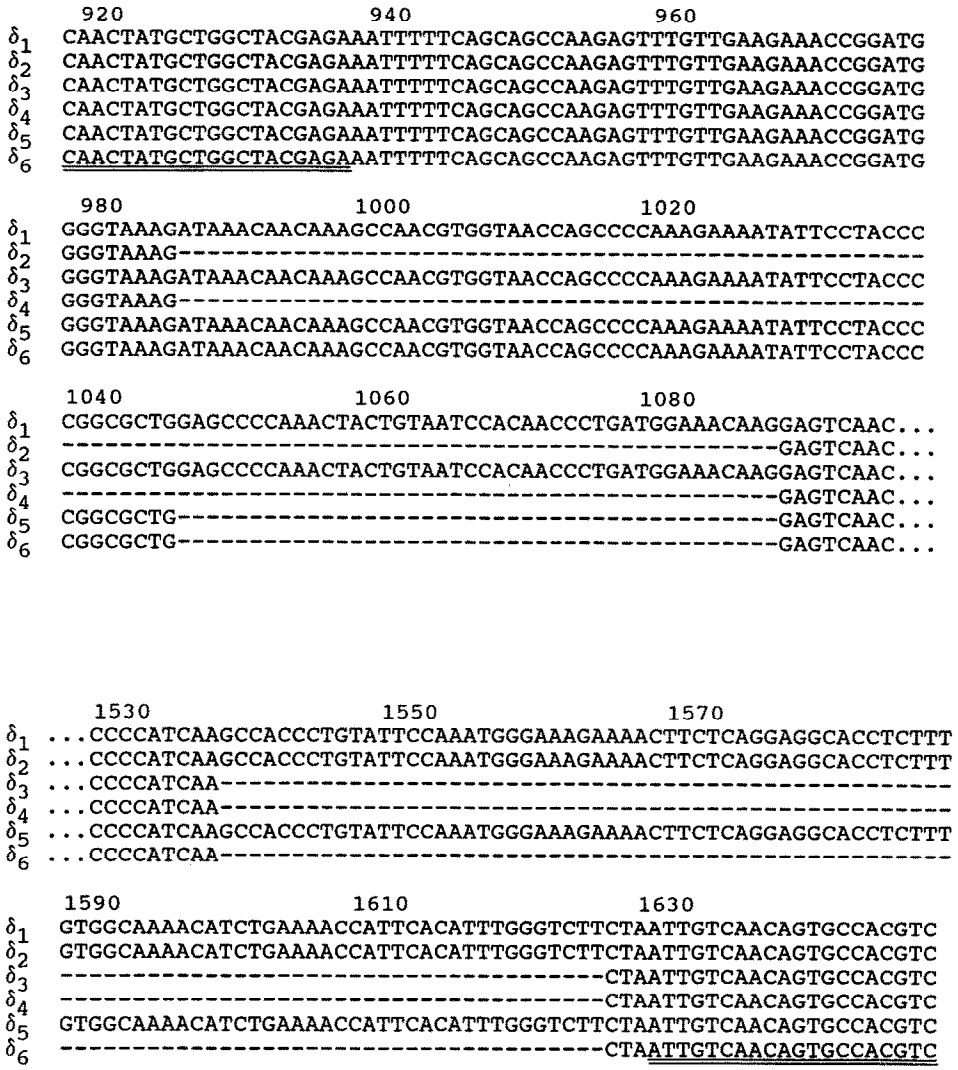

Fig. 1. Nucleotide sequence alignment of delta isoforms covering the potential deletion sites. Nucleotides are numbered beginning with the first nucleotide of the translation initiation codon. PCR primers are indicated by underlining. Gaps are indicated by dashes.

ping fragments (see section 2 and Fig. 3). Within the coding region of CaM kinase II δ cDNA, we detected 2 distinct sites of possible deletions (Fig. 1, Fig. 3). These sites will be referred to as variable delta-domain #1 and #2, respectively. Variable domain #1 was 102 bases long, coded for 34 amino acids, and spanned base positions 985–1,086. This domain could be completely deleted, or, alternatively, only the right part of the domain could be absent. This part was 42 bases or 14 amino acids in length and spanned base positions 1,045–1,086.

Variable delta-domain #2 spanned base positions 1,536–1,624. This domain was 89 bases in length and included the stop codon of subtype δ. Deletion of domain #2 led to a shift of the reading frame for mRNA translation. By this means, a new stop codon (TAA) appeared immediately behind the deletion (Fig. 2). The result was a protein which lacked 21 amino acids at the C-terminus of CaM kinase II δ. Simultaneously, Lys^{512}, which is located immediately in front of the deleted region, changed to Asn. The tail of 21 amino acids which constitutes the variable delta-domain #2 is not present in the subtypes α to γ.

The potential deletions described above make, by combination, 6 different variants of CaM kinase II δ possible. Indeed, all these isoforms could be detected in rat tissues. The variants will be referred to as follows: The originally described subtype δ will be termed δ_1 for clarity.

In δ_2, variable domain #1 is deleted completely.

In δ_3, variable domain #2 is deleted completely.

In δ_4, both variable domains are completely deleted.

In δ_5, the right part variable domain #1 (base positions 1,045–1,086) is deleted.

In δ_6, the right part variable domain #1 (base positions 1,045–1,086) is deleted as well as variable domain #2.

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δ1   511  Ile Lys Pro Pro Cys Ile Pro Asn Gly Lys Glu Asn Phe Ser
    1531  ATC AAG CCA CCC TGT ATT CCA AAT GGG AAA GAA AAC TTC TCA

δ3   511  Ile Asn  .   .   .   .   .   .   .   .   .   .   .   .
    1531  ATC AA- --- --- --- --- --- --- --- --- --- --- --- ---

δ1   525  Gly Gly Thr Ser Leu Trp Gln Asn Ile ***
    1573  GGA GGC ACC TCT TTG TGG CAA AAC ATC TGA AAA CCA TTC ACA

δ3        .   .   .   .   .   .   .   .   .   .   .   .   .   .
          --- --- --- --- --- --- --- --- --- --- --- --- --- ---

δ1
    1615  TTT GGG TCT TCT AAT TGT ...

δ3        .   .   .    ***
    1536  --- --- --- -CT AAT TGT ...
```

Fig. 2. Illustration of the C-terminal deletion which includes the stop codon of subtype δ_1. Nucleotide sequences and deduced amino acid sequences are shown for subtype δ_1, which possesses no deletion, and for subtype δ_3 in which variable domain #2 (see text) is deleted. 3 asterisks mark a stop codon. The new stopcodon in δ_3 resulting from a frame-shift is indicated by underlining.

Subtypes δ_3, δ_5, δ_6 were amplified and sequenced from rat brain, subtypes δ_2 and δ_4 were amplified and sequenced from RINm5F rat insulinoma cells.

All isoforms of rat CaM kinase II which have been identified (including the 2 variants of human CaM kinase II γ described recently [10]) show a high overall homology to each other. Three areas of low homology are apparent [5]. The variable domains #1 and #2 of the delta subtypes exactly match 2 of these areas of low homology (Fig. 4). In subtypes β and γ there is another area (#3) of low homology which is located N-terminal with respect to the variable delta domains focussed upon. This area is deleted in all delta isoforms. Note that the 'variable domain' defined by Nghiem et al. [10] is composed of low homology area #3, variable domain #1 and a short stretch of high conservation lying between these variable areas. Interestingly, the 15 amino acid deletion which discriminates the subtypes β and β' is homologous to the 14 amino acid deletion which we detected in the isoforms δ_5 and δ_6.

The variable delta-domain #1 is located between the regulation and the association domain of CaM kinase II. The variable domain #2 is placed behind the association domain at the C-terminus of the protein. These locations suggest an involvement of the variable domains in regulatory events as well as in the association behavior of CaM kinase II towards other proteins. If so, the conserved regions (including the kinase domain and the calmodulin binding site) would serve to recognize the calcium signal and to perform phosphorylation whereas the variable regions would direct the signal to its destination. The different isoforms could then be responsible for different and compartmentalized actions of calcium within the cell.

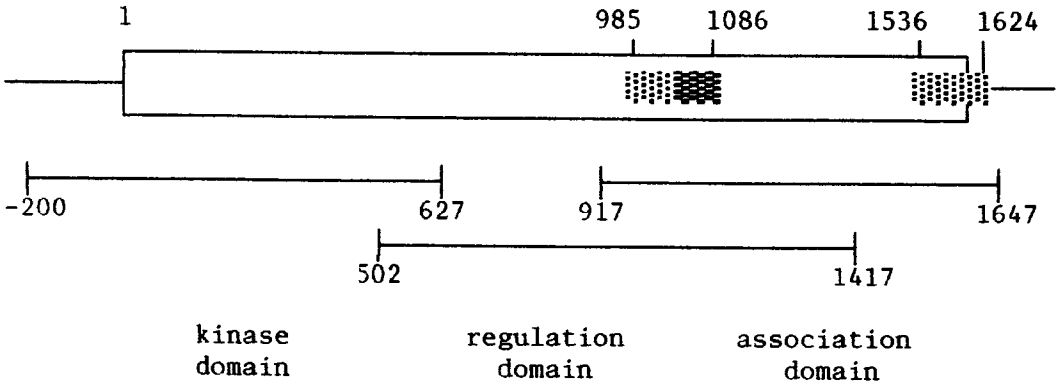

Fig. 3. Schematic view of CaM kinase II δ mRNA. The coding region is indicated by a box. The areas of potential deletion are shaded (left, variable domain #1; right, variable domain #2). Note that region #1 can be completely deleted or, alternatively, only in the right part (nucleotide positions 1,045 to 1,086, dark shading). The numbers refer to nucleotide base positions. The positions of the PCR amplificates are indicated by horizontal lines.

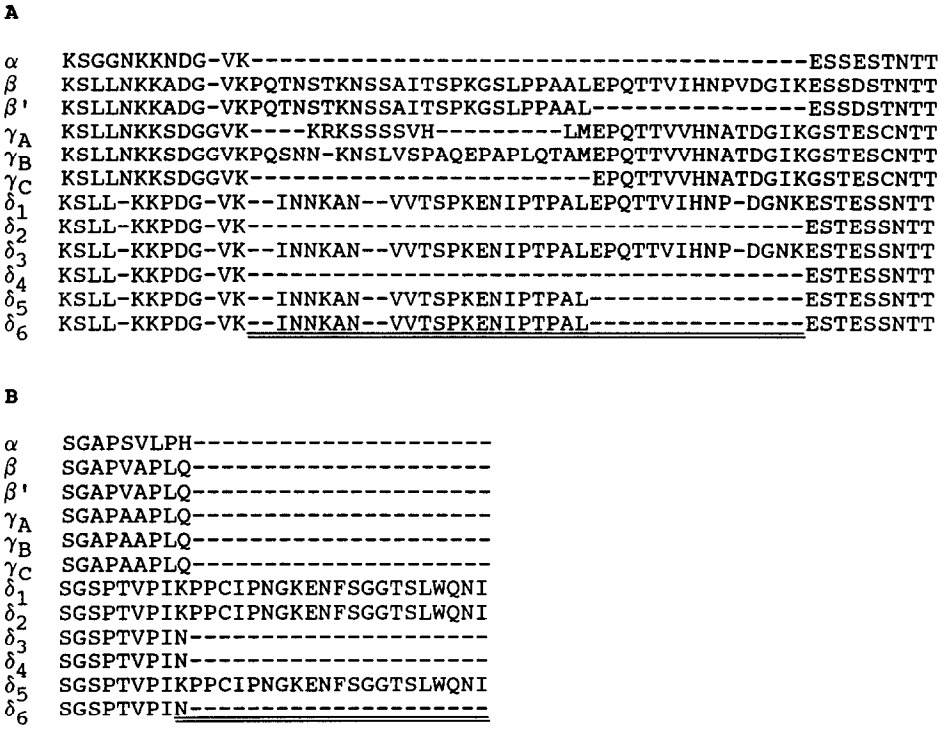

Fig. 4. Alignment of the deduced amino acid sequences of all identified CaM kinase II isoforms. The alignment only surrounds the potential deletion sites. Amino acids are represented by single letter code. Gaps are indicated by dashes. Note that the deletion sites in subtype δ match the regions of low homology between the subtypes α to δ (underlined). A, alignment surrounding variable domain #1 (see text). B, alignment surrounding variable domain #2 (see text).

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