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Additional isoforms of multifunctional calcium/calmodulin-dependent protein kinase II in rat heart tissue

Recently, Schworer et al. [1] reported the discovery of three new isoforms of Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II, also known as multifunctional Ca^{2+}/calmodulin-dependent protein kinase) in rat heart and vascular smooth-muscle tissue. Each isoform was a splicing variant of subtype δ, and all these isoforms differed from each other exclusively in one distinct variable domain which was up to 102 bases (34 amino acids) long; it was located between the regulation and the association domain (for a recent review about CaM kinase II and its domain organization, see [2]). We have detected all but one (δ_3, according to Schworer et al. [1]) of these isoforms in rat insulinoma cells and brain, along with three additional subtypes [3]. The latter displayed a deletion of the C-terminus (the last 21 amino acids), with respect to the originally described subtype δ_1 [4]. Thus the δ isoforms of CaM kinase II possess two independent variable domains: the first is located between the regulation and the association domain, and the second represents the C-terminus. These variable domains will be referred to as 1 and 2 respectively. A schematic drawing is shown in Figure 1(a).

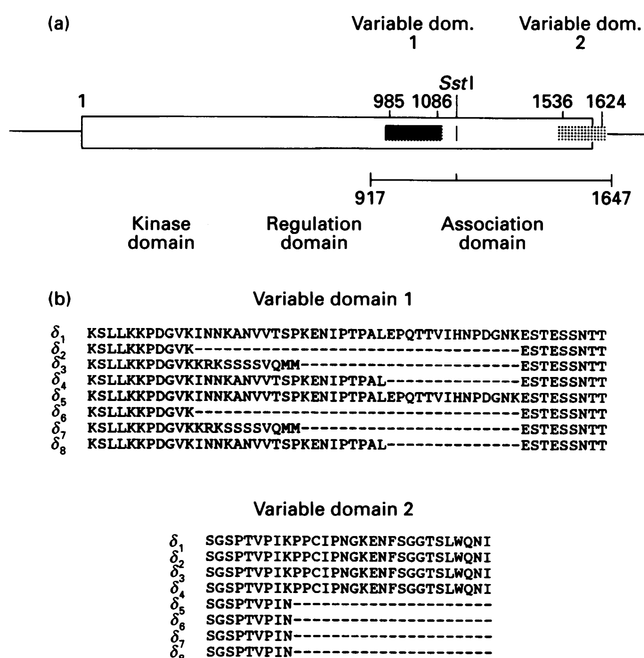

Figure 1 (a) Schematic view of CaM kinase II cDNA (coding region), and (b) alignment of deduced amino acid sequences from all CaM kinase II δ isoforms spanning the two variable domains (single-letter code)

In (**a**), the numbers refer to base positions (position 1, start of translation). Shaded boxes represent the variable domains. The horizontal line (917–1647) indicates the product of our PCR amplification procedure; the *Sst*I site used to cut it is also shown. In (**b**), dashes indicate gaps.

Schworer et al. [1] described four different versions of variable domain 1. These four versions, together with a deleted or undeleted variable domain 2, raise the possibility of eight different δ isoforms. Schworer et al. [1] were not able to detect a deletion of variable domain 2, because one of their PCR primers was placed into the potentially deleted domain. We were therefore interested to see whether all predicted isoforms, δ_1–δ_8, may indeed exist. At this point it seems advisable to suggest a common classification for the δ isoforms, since Schworer et al. [1] introduced a different nomenclature from ours. We shall join Schworer et al. [1] in terming the four versions of variable domain 1 δ_1–δ_4 when variable domain 2 is present. The isoforms that lack the second variable domain will be termed δ_5–δ_8, in the

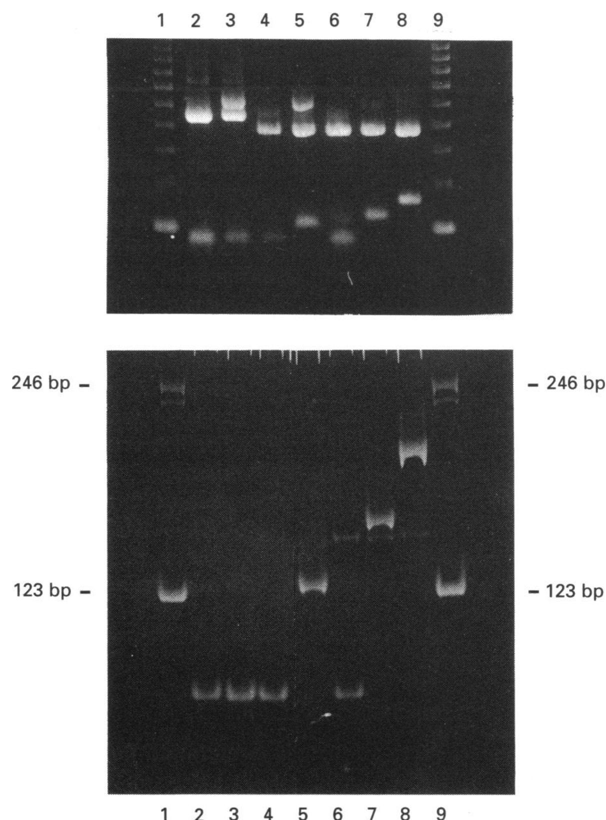

Figure 2 cDNA clones of CaM kinase II δ from rat heart tissue

Total RNA was extracted from rat heart, reverse-transcribed, and subjected to PCR amplification. The primers were 5′-CAA CTA TGC TGG CTA CGA GA (upstream) and 5′-GAC GTG GCA CTG TTG ACA AT (downstream) [3]. The products of amplification covered base positions 917–1647 (base position 1, start of translation). The PCR product was cloned, and inserts from single clones were PCR-amplified again and digested with *Sst*I. The agarose gel (above) shows both restriction fragments of each subtype detected in heart tissue, and the polyacrylamide gel (below) further resolves the small fragments (which contain variable domain 1). Subtypes from other tissues are shown for comparison. In some lanes, the undigested amplification product is also visible. Lanes: 1 and 9, marker for fragment length (123-base-pair ladder); 2, δ_2 from rat insulinoma cells; lanes 3–5, amplification products from heart tissue; lane 6, δ_6 from rat insulinoma cells; lane 7, δ_8 from rat brain; lane 8, δ_5 from rat brain.

same order. This means that the subtype originally termed δ_3 by our group will change to δ_5, δ_4 will change to δ_6, and δ_6 will become δ_8. This classification is illustrated in Figure 1.

In our recent study, we had already observed three out of four versions of variable domain 1. The missing version belongs to the heart- and blood-vessel-specific subtype δ_3. To find out whether a subtype δ_7 (i.e. δ_3 without variable domain 2) exists, we subjected total RNA from rat heart to reverse transcription, and amplified the cDNA with our primer pair covering both variable domains (see Figure 1). The experimental procedures were the same as previously described [3]. As a result, we obtained a broad band on an ethidium bromide-stained agarose gel, implying the presence of more than one isoform. The PCR product was subsequently cloned into the pAMP1 plasmid (Gibco) by using the CloneAMP System (Gibco). The inserts were digested with the restriction endonuclease *Sst*I (Gibco) to obtain two fragments of different length. The short fragment contained variable domain 1, and the long fragment contained variable domain 2 (see Figure 1). The short fragments from different clones were resolved via native PAGE, and were compared with all already identified δ isoforms as well as with molecular-mass markers. We detected short fragments which corresponded to subtype δ_2 and to a band of approx. 123 base-pairs which was not apparent in our previously identified δ subtypes. The short fragment of the heart subtype δ_3 is expected to be 126 base-pairs long, and is represented by the 123-base-pair band mentioned above, as confirmed by nucleic acid sequencing. The long fragments, which contained the second variable domain, were resolved on a 1 %-agarose gel. Surprisingly, all clones that contained the heart-/blood-vessel-specific version of variable domain 1 displayed a deletion of variable domain 2 (see Figure 2, lane 5). Therefore, this form represented the new subtype δ_7. None of 26 positive clones showed the combination of a heart-type variable domain 1 with an undeleted variable domain 2 (this combination would represent subtype δ_3). Our findings strongly argue for a much higher expression level of subtype δ_7 in rat heart tissue compared with subtype δ_3. In contrast, when the first variable domain was completely deleted, both versions (present or deleted) of variable domain 2 were observed (i.e. subtypes δ_2 and δ_6; see Figure 2, lanes 3 and 4).

Taken together, our data demonstrate that each version of variable domain 1 is indeed combined with each version of variable domain 2, resulting in 8 different δ isoforms of CaM kinase II. The latest subtype, δ_7, was hereby identified in rat heart tissue.

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