A Novel Calmodulin-like Protein Gene in Rice Which Has an Unusual Prolonged C-terminal Sequence Carrying a Putative Prenylation Site

Chuan Xiao, Hua Xin, Aiwu Dong, Chongrong Sun, and Kaiming Cao*

Department of Biochemistry, Fudan University, Shanghai 200433, P.R.China

(Received 27 April 1999)

Abstract

A rice cDNA encoding a novel calmodulin-like protein was identified. It has 38 additional amino acids at the C-terminus of a complete, typical calmodulin (CaM) sequence of 149 amino acids. The four C-terminal amino acid residues form a CAAL motif which could be a site for protein prenylation and may subsequently cause the protein to become membrane associated. RT-PCR analysis confirmed that such a combined protein gene truly exists in rice. Sequence analysis of its genomic counterpart showed that there is an intron located at junction of the normal CaM sequence and the 38 C-terminal amino acids. This introduces a potential stop codon for normal CaM if an alternative splicing mechanism is involved. Southern blot analysis of rice genomic DNA revealed that there is only one locus for this gene. The northern blot analysis showed that this gene is highly expressed in rice roots, shoots and flowers. The distribution of this protein demonstrates the functional importance of this novel CaM-like protein in rice.

Key words: Calmodulin-like protein; cDNA; genomic DNA; protein prenylation; RT-PCR; Southern blot; northern blot

Calmodulin has been implicated in a variety of cellular activities related to signal transduction pathways. However, the functions of plant CaMs are not as well known as their animal counterparts. In addition to having functions similar to animal CaMs, plant CaMs have been shown to participate in some processes unique to plants such as pollen germination and tube growth, cell wall regeneration, and environmental stimuli response. Consistent with its multifunctional characteristics, CaMs were found to be widely distributed in plant subcellular compartments including in the plasma membrane. Some were also shown to be located outside the plasma membrane. A CAAL motif (C is cystenine, A is an aliphatic amino acid and L is leucine) located at the C-terminus of a protein can be a target for genanylgenanyltransferase I (GGTase I) which adds a genanylgenanyl isoprenoid to the cystenine residue. This causes, the prenylated protein to become anchored to the cell membrane. Thus far, a number of proteins have been identified that require prenylation for proper function.

A randomly sequenced cDNA clone from a rice cDNA library was shown to be 80–90% homologous to known CaM sequences. In addition to the 149 amino acid CaM sequence, this predicted protein has an unusually long C-terminal sequence with an additional 38 amino acid residues. The four amino acids at the C-terminus comprise a CAAL motif, a putative recognition site for GGTase I. Thus, this cDNA clone was defined as a novel CaM-like protein gene. To rule out any possible mistake introduced by a point mutation or chimeric event during cDNA library construction, reverse transcriptase polymerase chain reaction (RT-PCR) was performed using two primers located immediately upstream of the putative stop site for normal CaM and downstream of the actual stop codon. Sequence analysis of the RT-PCR product confirmed our original finding.

We also obtained its genomic counterpart by PCR. Two introns within the coding region were identified which obey the GU-AG rule for mRNA splicing and are AT-rich. The second intron consists of 126 bp which interrupts the protein sequence between the N-terminal CaM counterpart and C-terminal extended sequence. As shown in Fig. 2, a comparison of the cDNA and genomic sequences of this novel CaM-like protein gene with those of several other plant CaMs reveals a consensus sequence just upstream of the stop codon. Replacement of the stop
Novel Calmodulin-like Protein Gene in Rice

Figure 1. Amino acid sequence of a novel CaM-like protein encoded by a rice cDNA clone. The underlined part is the 38 additional amino acids at the C-terminus of CaM.

CaM-like cDNA

TCGTCAAGTG CATGATGGCC AAGAAGAGGA

CaM-like genomic sequence

TCGTCAAGTG CATGATGGCC AAGTGAGGAG

Reported CaM (Oryza sativa)

TCGTCAAGTG CATGATGGCC AAGTGAGGAG

CaM (Triticum aestivum)

TCGTCAAGTG CATGATGGCC AAGTGAGTAA

CaM (Hordeum vulgare)

TTGTCAAGGT GATGATGGCC AAGTCATACT

CaM (Zea mays)

GCGTCAAGGT TATGATGGCC AAGTGAGAAC

CaM (Halus Domestica)

TCGTTAAGGT CATGATGGCC AAGTGATCCC

CaM (Solarium tuberosum)

TCGTCAAGGT CATGATGGCC AAATGAGCGC

CaM (Arabidopsis thaliana)

TCGTCAAGGT CATGATGGCC AAAATGAGCGC

CaM (Medicago sativa)

TTGTCAAGGT GATGATGGCC AAGTCATACT

**...**...**#**.......**#**....

Figure 2. Comparison of the novel CaM-like protein cDNA and its genomic sequence with several other plant CaM sequences at the normal CaM terminus. Asterisks indicate highly conserved nucleotides and dots indicate relatively conserved nucleotides. Stop codons are underlined.

codon with AAG is novel to our protein. Interestingly, the position of this TGA codon is the same in the genomic sequence as it is in other CaM sequences. If there is an alternative 5' splicing site, a normal CaM derived from the same genomic sequence can be generated.

Southern hybridization of rice genomic DNA was performed using the probe of normal CaM sequence and the probe of the second intron alone. As shown in Fig. 3, hybridization with the exon probe showed several major bands in addition to several minor signals. However, only one distinct band appeared upon hybridization with the intron probe, and their good correspondence to one of the major bands in exon hybridization provides evidence that there is only one locus for this special CaM-like protein in the rice genome. To test the level of transcription of this CaM-like protein gene in different rice tissues, northern blot analysis of RNA from rice roots, etiolated shoots and flowers were carried out using two DNA probes. One corresponds to the coding region of normal CaM, while the other is a gene-specific probe composed of the 3' UTR of the cDNA and a short sequence (about 50 bp) of the 3' terminal coding sequence. Figure 4 shows that there are high levels of transcription in all tissues examined, and hybridization with the two probes exhibited the same localization of positive signals.

Up to now, a wide range of subcellular compartmentalization of plant CaMs has been observed. The existence of a putative prenylation motif on this novel CaM-like protein indicates that it may be anchored to cell membrane via the C-terminus and Trewavas. Collinge detected a pea CaM bound to the plasma membrane, and its N-terminal region may project from the inner face of the membrane. However, it is hard to assume that a substantial amount of rice CaM attaches to plasma membrane. It is likely that the mRNA or the protein turns over rapidly, or that some portion of the protein is anchored to the other intracellular membrane structures which serve as a stage either for protein trafficking...
or storage. Also, there might be a regulatory pathway governing the alternation between membrane association and soluble localization of this protein. Future studies addressing these possibilities should prove to be very intriguing.

References
