Studies of the rgs-CaM, an RNA silencing suppressor from tobacco
(*Nicotiana tabacum*)

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RNA silencing is a conserved mechanism activated by double-stranded RNA molecules (dsRNA) present in eukaryotes. It plays basic roles in the regulation of gene expression and in host resistance to viruses and transposons. It is known that proteins of different origins, viral or endogenous, are able to suppress RNA silencing, but the mechanism of action of most of them are not fully understood. One of these endogenous suppressor is a protein called rgs-CaM (regulator of gene silencing CaM), which is a calmodulin-like protein (CaM) identified in tobacco plants. Calmodulins are proteins that play important roles in calcium signaling in eukaryotic cells and regulate the activity of numerous proteins with diverse cellular functions. Considering this point, the primary objective of this work was to obtain pure recombinant rgs-CaM for future functional studies. For such, the coding region of the rgs-CaM protein was cloned into the expression vector pGEX-5x-1 for heterologous protein expression in *Escherichia coli*. After induction, the resulting recombinant protein was subjected to different buffers conditions to improve protein solubility. The only condition found to promote protein solubilization was in the presence of 100 mM NaCl and 1% SDS. Protein refolding was promoted by the addition of 2-methyl-2,4-pentanediol (MPD) 2M, after a 24 h incubation period. Addition of MPD was crucial for protein purification, since in its absence, the recombinant GST: rgs-CaM fusion was unable to bind to the glutathione resin. After finding the appropriate conditions, a yield of ~4 mg of pure recombinant protein per liter of induced culture was obtained. Overall, our data demonstrate that appropriate amounts of soluble GST:rgs-CaM fusion protein could be obtained and purified, and also emphasize that MPD is essential for protein purification in the presence of SDS.

Word Keys: RNA silencing suppression, rgs-CaM, protein expression, Calmodulins, MPD

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