Cloning, Expression and Purification of rgs-CaM, a Calmodulin-like Protein that Suppress RNA Silencing

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RNA silencing is a conserved mechanism activated by double-stranded RNA molecules (dsRNA) present in all eukaryotes. It plays basic roles in the regulation of the gene expression and in host resistance to viruses and transposons. Although different viral and host endogenous proteins are able to suppress RNA silencing, their mechanism of action stills not completely understood. One such a suppressor protein, named rgs-CaM (regulator of gene silencing CaM), is a calmodulin-like protein (CaM) that was identified in tobacco plants. CaMs are proteins that play important roles in calcium signaling in eukaryotic cells and regulate the activity of numerous proteins with diverse cellular functions. Until today, however, very little is known about the structural features and regulatory properties of these proteins. In this study, the tobacco rgs-CaM cDNA was amplified by RT-PCR and cloned into the expression vector pMal-c2E. This vector was then transferred to cells of *E. coli* BL21 DE3 Rosetta, which were then used to express large amounts of soluble protein. The expressed protein was purified by affinity chromatography using affinity column chromatography (maltose) and then analyzed by DLS. The purified protein formed aggregates of high molecular weight with elevated polydispersity at different conditions (temperature, pH and salt concentration). However, in the presence of detergents such as Tween 20, protein aggregates were solubilized and the polydispersity reduced to acceptable levels for crystallization purposes. By varying the temperature in the presence of Tween 20, we observed significant changes in hydrodynamic radius of the protein in solution. At 18°C the value was consistent with a monomer, and at 25°C equivalent to a dimer.

Key words: RNA silencing supressor, rgs-CaM, protein expression, DLS