IN-SILICO STUDIES ABOUT CA\textsuperscript{2+} BINDING ON RGS-CAM, A REGULATOR OF GENE SILENCING FROM NICOTIANA TABACUM

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Introduction: RNA silencing is a potent host defense mechanism against plant viruses. This mechanism is generally counteracted by viral proteins that possess RNA silencing suppression activities. Host suppressor proteins were also identified and a tobacco calmodulin-like, named rgs-CaM, was the first to have silencing suppression activity demonstrated. The structure of CaMs is generally well conserved and presents N- and C-terminal globular lobes linked by a central alpha helix. Each lobe possesses two EF-hand motifs containing a Ca\textsuperscript{2+}-binding loop. In this work, we employed some in-silico techniques to know more about the Ca\textsuperscript{2+} binding properties and the conformation of rgs-CaM in presence of Ca\textsuperscript{2+}.

Material and Methods: The model of rgs-CaM was built using the HHpred server and Modeller employing a calmodulin from \textit{Paramecium tetraurelia} (PDB ID 1EXR) as a template. Next, nine additional models were generated with calcium ions occupying, in combination, the four binding sites found in the rgs-CaM model. To minimize and improve the models, a molecular dynamics simulation was executed by the program GROMACS v.4.5.3 through these steps: Water box generation, neutralization, energy minimization, and simulation with position restrained and unrestrained.

Results and Conclusions: According to the obtained results, only three binding loops of rgs-CaM retained the calcium ions after the MD simulation. These results are in agreement with previous ITC experiments and amino acid sequence analysis. Furthermore, the Ca\textsuperscript{2+}-binding on rgs-CaM trigger an expansion of its tertiary structure with reorientation of alpha-helices in the EF-hands. This conformational change leads to the exposure of a large negatively charged region that may be implicated in the electrostatic interactions between rgs-CaM and viral suppressors.

Key Words: Molecular Dynamics, RNA silencing, Calmodulin

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