# Structural Studies of Phosphorylated *N*-Terminal Peptide of MARCKS Protein and the Monoclonal Antibody 3C3

Fraga J. L.[1], Anobom, C. D.[1]; Zolessi F. R.[2], Arruti C.[2], Tinoco L. W.[1]

[1]LADIE - Núcleo de Pesquisa de Produtos Naturais –UFRJ; [2]Facultad de Ciências – Universidad de la República –Uruguay

The Myristoylated Alanine-Rich C Kinase Substrate (MARCKS) is a protein that plays an important role as “connecting devices” between different intracellular signaling pathways. In the neuronal development stage MARCKS can be identified by the Monoclonal Antibody 3C3 (mAb3C3). For antibody binding the Ser25 in the MARCKS must be phosphorylated. The *N*-terminal domain of MARCKS has a peptide (EKPGEAVAApSPSKANGQENG) that binds to mAb3C3. We investigated the structural differences between phosphorylated (pS25) and non phosphorylated (npS25) peptides and the conformational changes of pS25 in the presence of mAb3C3 using CD and NMR spectroscopy at pH 7.2 and 5.0. The CD spectra show that both peptides are in random coil conformation and the addition of mAb3C3 does not cause conformational changes in the pS25. Analysis of the amidic region of the $^{1}$H NMR spectra of the peptides npS25 and pS25 shows that the phosphorylation increases the spectrum resolution at the amidic region between 8.3 and 8.5 ppm. Comparison between $^{1}$H NMR spectra of pS25 and npS25 peptides in the presence of mAb3C3 showed line broadening and chemical shifts changes only for pS25, confirming that phosphorylation is crucial for interaction with the antibody. Comparative analysis of the TOCSY and NOESY spectra at 5 $^{o}$C and 25 $^{o}$C indicate that the peptide acquires a more stable conformation at lower temperature. In this condition the NOESY spectrum showed several inter-residue NOE cross peaks, which will be used to get us information about the structural conformation of the free and antibody bounded peptide.

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Key words: Antibody, CD spectra, MARCKS and Neuronal desorder.