

CHICKEN MARCKS N-TERMINAL PEPTIDE: STRUCTURAL ANALYSIS OF THE PHOSPHORYLATION INFLUENCE IN SER25 AND ANTIBODY BINDING

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The epitope recognized by monoclonal antibody 3C3 (mAb3C3) on myristoylated alanine-rich protein kinase C substrate (MARCKS) was identified in the N-terminal domain and should to be phosphorylated at Ser25 and includes Lys28 and Ala29 for antibody binding. We investigated the structural differences between phosphorylated (pS25) and non phosphorylated (npS25) peptides (EKPGEAVAA**p**SPSKANGQENG) and the conformational changes of pS25 in the presence of mAb3C3 using CD and NMR spectroscopy. 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. The ¹H NMR spectra for both peptides show a line broadening in the amidic region and significant chemical shift differences in the serine H β region for npS25 when compared to pS25. ¹H chemical shifts for the pS25 with mAb3C3 are very similar to the free peptide, but line broadening was observed at serine H β , proline H δ and asparagine H β regions, indicating that these regions are the most affected by the binding. The NMR results indicate that the phosphorylation at Ser25 induces a light structural difference between the two peptides forms. There are not conformational changes when pS25 binds to mAb3C3.

CNPq, FAPERJ, FINEP, IMBEBB

Key Words: protein phosphorylation, neuronal differentiation, circular dichroism, NMR.