COLOCALIZATION STUDY OF AMMODYTOXIN AND ITS BINDING PROTEINS IN PC12 CELL LINE

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Multiple secreted phospholipases A_2 ($sPLA_2$) are present in various tissues where they participate in several physiological and pathological processes. The molecular basis of one such process, presynaptic neurotoxicity of certain snake venom $sPLA_2$s, is not known in detail yet, but it is obvious that beside their enzymatic action, these $sPLA_2$s must bind to specific receptors in the nerve ending. Identification of these targets and characterization of their interactions with Atx within cells is therefore of crucial importance for the understanding of this process.

To gain further insight into its intracellular action, we prepared a fluorescently labelled AtxA and observed its localization inside the cells and its colocalization with some of the previously identified intracellular binding proteins using laser scanning confocal microscopy. PC12, a cell line used in these experiments, exhibits neuronal-like properties after differentiation with nerve growth factor and has thus proved to be a suitable model for studying various processes that occur in neurons. The toxin was shown to enter these cells already within a few minutes after the addition to the cell culture medium. The observed staining was granular and suggestive of vesicular mode of internalization. Within the cells, the toxin colocalized with mitochondrial cytochrome c oxidase, cytosolic 14-3-3 proteins and protein disulfide isomerase.