Study of complexes between a snake venom or human secreted phospholipase A_2 and calmodulin by high-resolution NMR

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Secreted phospholipases A_2 ($sPLA_2s$) are phospholipid-hydrolyzing enzymes, which enzymatic activity is implicated in numerous pathophysiological settings, for example innate immunity, atherosclerosis, cancer, pain, ischemia and Alzheimer's disease. Ammodytoxin (Atx), a neurotoxic $sPLA_2$ from the *Vipera a. ammodytes* venom, belongs to the group IIA of $sPLA_2s$ and as such presents a model for studying the above motioned pathophysiological processes. Atx forms a high-affinity complex with cytosolic regulatory protein calmodulin (CaM), which results in substantial increase of the enzymatic activity of Atx[1]. To understand the role of CaM in the regulation of intra-cytosolic enzyme activity of $sPLA_2s$, we are currently studying the structure of the complexes formed between Atx or the human group X (hGX) $sPLA_2$ and CaM using high-resolution NMR spectroscopy. To this end we prepared fully functional recombinant unlabelled and $^{13}C/^{15}N$-labelled samples of CaM, Atx and the hGX $sPLA_2$. ^{15}N-HSQC spectra of CaM alone and in the complex with unlabelled Atx or hGX $sPLA_2$ were well resolved, while the ^{15}N-HSQC spectra of both $sPLA_2$ alone and in the complex with unlabelled CaM were not, therefore optimization of NMR conditions are still required. The comparison of the spectra of CaM alone and in the complex with either of $sPLA_2s$ revealed several significant changes of chemical shifts in CaM. This can be explained by conformational change of CaM upon its binding to $sPLA_2$, which seems to be different as reported in cases of CaM binding to other binding proteins. Therefore it seems that $sPLA_2s$ bind to CaM in a unique binding fashion.

Reference:
1. L. Kovačič, M. Novinec, T. Petan, A. Baici, I. Križaj, *Biochemistry* **2009**, 48, 11319–11328.

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