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A new enzyme-assay for PLA_2 activity in jellyfish venom based on phosphorus detection using HPLC-CC-ICP-MS

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Although their function in the marine ecosystem is not yet fully understood, jellyfish seem to become important key species in the environment due to the limited number of natural enemies, global warming effects and environmental pollution especially with micro nutrients. Mass occurrences in some regions indicate a strong impact on the local food webs with negative effects on all trophic levels due to their continuous ingestion of different prey species. Fishing tentacles and mesenteric tentacles, which are equipped with specialized cells harbouring venom containing capsules (nematocysts) are used for prey capture and self defence.

Up to now only little is known about the chemical composition of the jellyfish toxin cocktail, the chemical structures and the biochemical effects. To understand their role in the environment it is essential to gather more information about the mechanisms and the compounds especially, which are mainly responsible for the toxic effects. The complexity of the venom cocktail requires an effect orientated fractionation strategy in order to distinguish toxicologically active and inactive fractions and to reduce the complexity of the sample.
In several reptile and insect venoms phospholipase A_2 (PLA_2) is known as a major compound. The phospholipase A_2 family is an enzyme class, which catalyze the hydrolysis of phospholipids at the *sn*-2 position to release the corresponding fatty acid and lysophospholipid. PLA_2-activity of the jellyfish venom may be responsible for some typical inflammation reactions like pain or oedema and toxic effects such as myo- or neurotoxicity in the affected organism. Additionally PLA_2 can destabilize the cell membranes and alter the cell permeability. The responses caused by a fatty acid activated cascade reaction whose products are responsible for the inflammation reactions.

This contribution will present first results on the development of an assay for the investigation of the venom of *Lion's Mane jellyfish* with respect to PLA_2-activity. The main goal is to separate and simultaneously determine the phosphorus containing enzyme substrate and the reaction products caused by the PLA_2 activity by using high performance liquid chromatography (HPLC) on-line hyphenated to collision cell inductively coupled plasma mass spectrometry (CC-ICP-MS). Changes of the enzyme substrate and the product quantity directly indicate an PLA_2-activity of the sample fraction under investigation. Sonicated vesicles consisting of phospholipids are applied as enzyme substrate. Reversed phase liquid chromatography (RP-HPLC) has been used to separate the lipid and the lysophospholipid. Molecule ions formed and interfered with the sensitive phosphorus specific detection, are minimized by using helium as cell gas. The established assay should allow effective indication of PLA_2-active compounds in different fractions of nematocyst extracts to guide their final identification during a multidimensional separation process.