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Purification of phospholipases A_2 from marine coelenterates: Industrial applications

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Phospholipases A_2 (PLA_2) are enzymes that participate in numerous important physiologic and metabolic processes in plants and animals. The phospholipase A_2 activity is also associated to different pathologies, that's why the purification and further characterization of these enzymes result from big interest, both for basic and applied studies with a leading role in medicine, physiology, nutrition, immunology, microbiology and toxicology. Their usefulness is growing in different biotechnological, pharmaceutical and general industries, such as food industry where is not always needed a highly purified enzyme. Numerous investigations have demonstrated similarities between marine coelenterate venom compositions with that from snakes, based on pharmacological properties they possess. They constitute complex mixtures of several components, mainly of protein nature and phospholipase A_2 activity has been described. Affinity chromatography constitutes one of the most effective methods for protein purification due to the specificity of interactions established in this method, hence the importance of selecting supports adapted to achieve this purpose. The aim of the present research study is to test a two-step purification protocol for phospholipases A_2 (PLA_2) from the sea anemone *Condylactis gigantea* that allows for the purification of those enzymes for their later characterization through a chromatographic affinity support MANA-Sepharose CL 4B with covalently immobilized egg phosphatidylcholine. The above mentioned support allows the purification of three protein components with molecular weights between 18000 and 14000, in a two-step protocol with at least one component possessing phospholipase A2 activity. These enzymes may provoke edemas and myotoxic activity. Marine invertebrates constitute a remarkable source of bioactive compounds and the isolation of these compounds through bio-guided chromatographic techniques could be of great value for drug discovery and pharmacological studies.

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Anti-angiogenic stigmasterol derivatives

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Angiogenesis plays a critical role in initiating and promoting several diseases such as cancer and herpes stromal keratitis (HSK). Herein, we studied the inhibitory effect of two synthetic stigmasterol derivatives (22S,23S)-22,23-dihydroxystigmast-4-en-3-one (compound 1) and (22S,23S) 3β-bromo-5α,22,23-trihidroxistigmastan-6-ona (compound 2) on capillary tube-like structures and cell migration in human umbilical vein endothelial cells (HUVEC). We also studied their effect on VEGF expression in IL-6 stimulated macrophages and in LMM3 breast cancer cells. Furthermore, we investigated the anti-angiogenic activity of the compounds on corneal neovascularization in the murine model of HSK and in an experimental model of tumor induced angiogenesis in mice. Both compounds were able to inhibit capillary tube-like formation but only compound 1 restrained cell migration. Also only compound 1 reduced the incidence and severity of corneal neovascularization when it was administered at the onset of HSK and was able to inhibit the development of neovascular response induced by tumor cells in mice skin. Our results show that compound 1 inhibits angiogenesis *in vitro* and *in vivo*. This property would not be a consequence of its anti-inflammatory activity already reported since other synthetic molecules belonging to the same family exhibited anti-inflammatory activity but did not behave as anti-angiogenic compounds. Hence, it would be a promising drug for the treatment of those diseases in which angiogenesis represents one of the main pathogenic events.

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