SERINE PROTEASE INHIBITORS ISOLATED FROM CARRIBEAN MARINE INVERTEBRATES: STRUCTURE-FUNCTION.

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Proteolytic processing is involved, directly or indirectly, in practically all biochemical and physiological processes in living organisms. Their regulatory counterparts, protease inhibitors (PI), have become essential tools for structure-function studies and the development of novel biotechnological and therapeutic applications. Herein we will present our results on proteic protease inhibitors isolated from marine invertebrates. ShPI-1 (6.1 kDa), isolated from the anemone *Stichodactyla helianthus*, is structurally a BPTI Kunitz inhibitor, interestingly it does not inhibit just serine proteinases, but also some cysteine and aspartic proteases. Based on bioinformatic models, we hypothesized on the structural determinants into enzyme-inhibitor interactions, especially against pepsin. Among serine PI, elastase and subtilisin-like inhibitors are very attractive candidates for biomedical applications, due to the involvement of these serine proteases on inflammatory and parasitic diseases. Recently, we have isolated from the mollusc *Cenchritis muricatus,* three PI (5.6, 5.5 and 20 kDa). The main inhibitor CmPI-II is a canonical non-classical Kazal-type PI which differs functionally and structurally from other Kazal-type inhibitors. CmPI-II is a tight-binding inhibitor of pancreatic trypsin, human neutrophil elastase (HNE) and subtilisin A with Ki values of 10^{-8} to 10^{-9}M, respectively. From the analysis of the 3D structure model of CmPI-II/HNE complex, we hypothesize that the main interactions of this inhibitor with HNE and subtilisin A involve the primary inhibitor binding sites P6-P3´. On the other hand, metallo carboxypeptidases (CP) represent potential drug targets in different diseases, due to their diversity of functions. Additionally, we have isolated a bifunctional inhibitor of 19.7 kDa, active against some serine proteases and metallo CP (SmPI), mainly located in the tentacle crown of the annelid *Sabellastarte magnifica.* It was possible to establish in SmPI the presence of three Kunitz domains, responsible for trypsin-like protease inhibition. Finally, the production of recombinant SmPI and each of its domains will let the study of the inhibition mechanism of metallo CP by SmPI.