

SERINE AND METALLO PROTEASE INHIBITORS ISOLATED FROM MARINE INVERTEBRATES: STRUCTURE-FUNCTION AND SOME POTENTIAL APPLICATIONS

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Proteolytic activity 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. Marine invertebrates constitute an important natural source of inhibitors against proteases from different mechanistic classes. ShPI-1 (6.1 kDa), ShPI-2 (6.2 kDa) and ShPI-3 (13, 4 kDa), the first PI isolated by our laboratory from the sea anemone *Stichodactyla helianthus* (Coelenterata) are structurally BPTI Kunitz inhibitors, but functionally they have the peculiarity of inhibiting, not only serine proteinases, such as trypsin, chymotrypsin, kallikrein and plasmin, but also some cysteine and aspartic proteases, such as papain and pepsin, although the latter with higher K_i values. Taking into account their wider specificity, an affinity product containing all these three inhibitors is successfully being used in different biotechnological processes. Among serine PI, elastase inhibitors are very attractive candidates for biomedical applications, due to the physiological role of this serine protease on inflammatory diseases. Recently, we have isolated from the mollusk *Cenchritis muricatus*, three PI (5.6, 5.5 and 20 kDa) strongly active toward trypsin and human neutrophil elastase with K_i values in the nanomolar range, while chymotrypsin, plasma kallikrein, plasmin and thrombin are not inhibited, neither proteases from other mechanistic classes. The main inhibitor CmPI-2 is composed by a single polypeptidic chain of 5.5 kDa with 3 intrachain disulphide bridges. Its sequence alignment showed homology with Kazal inhibitors, exhibiting the typical consensus sequence of this family. On the other hand, metallo carboxypeptidases (CP) represent potential drug targets in different diseases, due to their diversity of functions. We have isolated an interesting bifunctional inhibitor of 19.7 kDa, active against some serine proteases and metallo CP, 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 inhibition, with 18 cysteine residues involved in the formation of 9 disulphide bonds. Although SmCI C-terminus is longer than that of other CP inhibitors, the presence of some conserved residues (Val, Tyr, Gly) could indicate that it displays the same mechanism of action as CP inhibitors described so far. Functionally, SmPI inhibits trypsin and pancreatic elastase and CPA with K_i values in the nanomolar range, while chymotrypsin is slightly inhibited with a K_i in the micromolar region. A second metallo inhibitor, but in this case of non protein nature, HcPI, was isolated from *Hermodice carunculata*, belonging also to the phylum *Annelide*. HcPI (580 Da) efficiently and specifically inhibits pyroglutamyl aminopeptidase II (PPII), the TRH-degrading ectoenzyme. As TRH has been found to have potential use in the treatment of brain and spinal injury and CNS disorders, potent and selective inhibitors of PPII may be used to enhance the therapeutic actions of TRH in CNS.