

OC 2. FUNCTIONAL AND STRUCTURAL CHARACTERIZATION OF THE RECOMBINANT THIRD BPTI-KUNITZ DOMAIN FROM SmCI, A BIFUNCTIONAL INHIBITOR ISOLATED FROM *Sabellastarte magnifica*.

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The role of natural inhibitors in the control of proteolytic processes has converted these molecules in valuable tools with multiple biotechnological, pharmaceutical (1) and basic biochemistry research applications. Our group previously reported the isolation of a novel bifunctional inhibitor from the tentacle crown of the annelid *Sabellastarte magnifica* (SmCI). This molecule is active against the serine proteases: trypsin, pancreatic elastase and chymotrypsin, and the metallocarboxypeptidase A (MCPA). SmCI is the first multidomain inhibitor composed by three BPTI-Kunitz domains able to inhibit CPA. Thus, the structure-function relationship of SmCI domains will contribute to the knowledge of the enzyme inhibition mechanisms. This work describes the characterization of SmCI-D3, which has been previously cloned and expressed in *P. pastoris*. rSmCI-D3 was purified from culture supernatant using a combination of reverse phase chromatography on Sep-Pack C8 column and anion exchange chromatography on DEAE-Sepharose. The purified inhibitor was analyzed by N-terminal sequencing using automatic Edman degradation and the molecular mass was determined by MALDI-TOF mass spectrometry. Cysteine residues were also determined by MALDI-TOF mass spectrometry after reduction and carbamidomethylation of the purified inhibitor. The specificity was evaluated with pancreatic elastase, trypsin, chymotrypsin, papain, plasmepsin II and MCPA and B. Enzyme inhibition was determined by measuring the residual enzyme activity after pre-incubation

with the inhibitor for 10-30 min, before the substrate addition.

Purification on DEAE-cellulose revealed only one peak which also showed trypsin activity (2). Functional characterization demonstrated that rSmCI-D3 is a highly specific tight binding inhibitor (3) against trypsin (K_i 10^{-8} M) and it is not able to inhibit other serine proteases. Inhibition against MCPA was not detected, even after increasing lo/Eo relationships and pre-incubation time to attain equilibrium, indicating that the C-terminal of SmCI is not involved in CPA inhibition. This result suggests a different inhibition mechanism to that previously reported for other MCPs inhibitors.

In order to study the interface interaction between rSmCI-D3 and trypsin, comparative molecular modelling of rSmCI-D3 (figure 1) and rSmCI-D3/trypsin complex was performed. Detailed analysis of enzyme-inhibitor contacts, showed a lower amounts of contacts in rSmCI-D3/trypsin complex when compared with BPTI/trypsin complex. This result is in agreement with the higher K_i value against trypsin observed in rSmCI-D3.

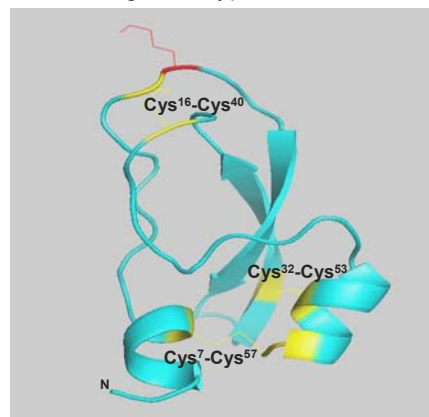


Fig 1. Comparative 3D structure model of rSmCI-D3. The model shows the typical folding of BPTI-Kunitz domains. Cys residues are in yellow and the disulphide bonds numbers are in agreement with the topology of these bonds in BPTI-Kunitz family.

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REFERENCES

- 1) Abbenante, G. and Fairlie, D. P. (2005) Medicinal Chemistry 1: 71-104.
- 2) Erlanger, B. F., Kokowsky, N., Cohen, E. (1961) Arch. Biochem. Biophys. 95: 271-278.
- 3) Bieth, J. G. (1995) Meth. Enzymol. 248: 59-84.