CHARACTERIZATION AND 3D MODEL OF A NEW PROTEINASE INHIBITOR ISOLATED FROM Stichodactyla helianthus

Vivian Huerta,[1] Vivian Morera,[1] Nelia López,[1] Lázaro Betancourt,[1] Vladimir Besada,[1] Gabriel Padrón,[1] Gipsi Lima,[2] María de los A Chávez,[2] Julieta Delfín[2] and Joaquín Díaz[2]

[1]*División de Química-Física, Centro de Ingeniería Genética y Biotecnología. Apartado postal 6162, Ciudad de La Habana, CP 10600, Cuba. E-mail: vivian.morera@cigb.edu.cu*

[2]*Departamento de Bioquímica, Facultad de Biología, Universidad de La Habana, Calle 25 No. 455, Plaza, Ciudad de La Habana, Cuba.*

Introduction

Protein proteinase inhibitors are widely distributed in living organisms and classified upon their similarities according to sequence, topology, active site localization and the binding mechanism. Proteinase inhibitors from sea anemones are specially interesting as they represent the phylogenetically oldest aprotinin type inhibitors known until now. They can be easily isolated in large quantities. Isoinhibitors with different inhibitory specificity may provide desirable properties for therapeutic uses and for the study of structure-functions relationships.

In 1996 we reported the isolation and characterization of the ShPI-I protease inhibitor from the sea anemone *Stichodactyla helianthus* (1). Here we report the primary structure and the dissociation constant against trypsin of a new proteinase inhibitor ShPI-2, isolated from the same source. We also discuss a 3D model of the ShPI-2/Trypsin complex.

Results and Discussion

ShPI-2 was isolated from the whole body of the anemone as described (1). A fraction with protease inhibitory activity was subjected to a final purification step by rp-HPLC using a C8 column. ShPI-2 constituted the main fraction and was obtained with high purity. An average molecular mass of 6196.0 Da. was obtained by FAB-MS on a Jeol JMS-HX110 mass spectrometer. 52 N-terminal residues were determined by automatic sequencing of S-carboxamidomethylated protein using a Knauer 810 dual-phase sequencer. The remaining sequence including the C-terminus was established by digestion of the protein with endoproteinase Glu-C and combining automatic sequencing and FAB-MS data. FAB-MS analysis of peptides obtained by successive digestions with endoproteinase Glu-C and Lys-C corroborated the sequence (Figure 1).

ShPI-2 has 92 % of sequence identity with ShPI-I from *S. helianthus*. Disulfide bond between Cys3-Cys53 was determined by partial acid hydrolysis followed by successive digestions with pepsin and trypsin. Sequence similarity searches revealed that ShPI-2 has the sequence pattern common to the Kunitz family of BPTI (Figure 1). Due to this fact disulfide bridges between Cys12-Cys36 and Cys28-Cys49 were assigned by homology with the inhibitor protein family.

ShPI-2 has a strong inhibitory capacity against trypsin (Ki 3.8 x 10^{-10}) determined according to Bieth (2).

The 3D model of the ShPI-2/Trypsin complex was build using the WHATIF program (3). The structure of the BPTI/Trypsin complex (2PTC PDB code) was used as template for the regions that interact with trypsin and the structure of ShPI-I solved by RMN (1SPH PDB code), for the rest of the inhibitor. The most important interactions for the trypsin inhibition are present in our model as well as in several structures of enzymes/inhibitors complexes. There are specific residues in the contact region with trypsin that could explain the difference in the inhibition constant between ShPI-2 and BPTI. The fact that the differences between ShPI-I and ShPI-2 sequences are in regions that are not in contact with trypsin explains the similarity in their inhibition constants (ShPI-I, Ki 1.1 x 10^{-10}).

1. Delfín J, Martínez I, Antuch W, Morera V, González Y, Rodríguez R. Toxicon 1996;34:1367-1376.

2. Bieth JG. Methods in enzymology 1995;248:59.

3. Vriend G. J Mol Graph 1990;8:52-56.

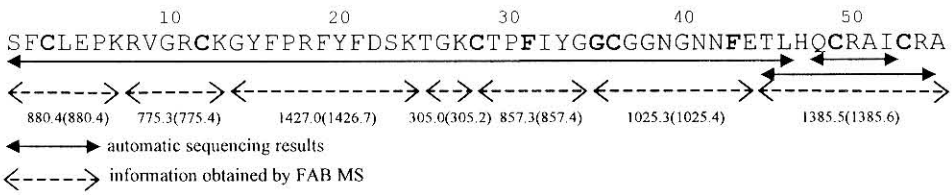

Figure 1. ShPI-2 amino acid sequence. In bold faces are denoted residues that belong to the sequence common pattern of the BPTI Kunitz family. The observed and expected (in parentheses) mass values are shown for each peptide.