Phytochemistry 70 (2009) 1374–1381

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Characterization of a highly stable trypsin-like proteinase inhibitor from the seeds of *Opuntia streptacantha* (*O. streptacantha* Lemaire)

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ARTICLE INFO

Article history:
Received 28 January 2009
Received in revised form 29 May 2009
Available online 16 September 2009

Keywords:
Opuntia streptacantha
Cactaceae
Prickly pear
Proteinase inhibitor
Seed protein
Trypsin inhibitor
Insect resistance

ABSTRACT

A trypsin inhibitor from *Opuntia streptacantha* Lemaire (Prickly pear) seeds was purified and characterized. Of several proteases tested, this inhibitor showed specificity to trypsin-like enzymes. The major inhibitor present in these seeds showed distinctive characteristics, most notably a low molecular weight of 4.19 kDa, as determined by MALDI TOF, and an unusually high thermal stability, retaining most of the activity after heating the sample 1 h to 120 °C with a pressure of 1 kg/cm^2. Its complete amino acid sequence was obtained through mass spectrometry, this establishing presence a blocked N-terminal region. When comparing its sequence in the MEROPS database for peptidases and peptidase inhibitors, it showed 34.48% identity with a serine-proteinase inhibitor from the I15 family.

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1. Introduction

Plant proteinase inhibitors (PI's) constitute a complex group of proteins. It is well documented that they have different biological functions, such as storage proteins, and as part of defense mechanisms against insects and pathogens (Shewry, 1999; Pernas et al., 2000; Lawrence and Koundal, 2002; Konarev et al., 2004; Valueva and Mosolov, 2004; Jones, 2005).

Interest in PIs has increased due to their potential as tools in different research fields. Due to their regulatory effects in different processes related to proteinase–proteinase inhibitor interactions, these proteins are thought to be useful for possible applications in development of antiviral agents, as well as for treatments of disorders involving proteinase defects. For instance, in the case of cancer progression, proteinases have long been associated mainly with their ability to degrade extracellular matrices, a step required for the process of invasion and metastasis. In this regard PIs have been found to be highly effective suppressors of carcinogenesis for both in vitro and in vivo systems (Kennedy, 1998; García-Gasca et al., 2002; Sakamoto et al., 2005). PI's have also showed potential applications for several medical treatments, among them: peri-anal dermatitis was treated with a potato inhibitor (Ruseler-Van et al., 2004), and also as antibacterial agents, like the potide G, a small serine-proteinase inhibitor that was able to prevent growth of three different human pathogen bacteria (Kim et al., 2006), among others.

Recently, new inhibitors have been described and classified according to the classification proposed in MEROPS database (Rawlings et al., 2008), with new families being established when an inhibitor shows novel sequences and biochemical properties. For example, a new family of serine-proteinase inhibitors present in *Veronica heredifolia* here an unusual motif that could be used for designing new inhibitors with potential applications (Conners et al., 2007). As for variegin, a PI isolated from the bont tick (*Amblyomma variegatum*) showed a potent inhibitory effect on thrombin (Koh et al., 2007), which suggests that new inhibitors could have new applications.

Proteinase inhibitors are proteins widely distributed in the seeds of diverse groups of plants including Angiosperms (monocotyledons and dicotyledons) and Gymnosperms (Cycadales, Coniferales and Gynkoales) (Konarev et al., 2002a,b, 2004, 2008; Mello et al., 2003; Sawano et al., 2008). Although PI's have been detected in the seeds of many plant species, there are many taxa where they still have not been studied, and thus these could represent a source of novel information on PI's structures and properties, which could in turn open new possibilities for their applications in crop protection and in biotechnology (Konarev et al., 2004, 2008).

Considering that the protein concentrations remain constant in the seed of some plants through different environmental conditions, seed proteins and also PI's have been suggested to be useful

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doi:10.1016/j.phytochem.2009.08.009

as markers in studies of evolution and systematics (Tiffin and Gaut, 2001; Konarev et al., 2002a,b, 2004; Qi et al., 2005).

Cactaceae, which include almost 850 species on the American continent, where Mexico presents 78% endemism (Gómez-Hinostrosa and Hernández, 2000; Ortega-Baes and Godínez-Alvarez, 2006) is one of the botanical families that still lack studies in this field. Among the Cacti family, one of the most important genera considering its biodiversity as well as its cultural and economic use, is *Opuntia* (Prickly pear). Different reports describing the physiology, morphology, reproduction, culture and increased fruit yields, have been published (Mondragon, 2001; Mohamed-Yasseen et al., 1996; Nerd and Nobel, 2000; Nobel and Zutta, 2007); however, information at the molecular level is still limited. Previous literature on *Opuntia* spp. has focused mainly on the protein contents present in the vegetative parts of the plant and their use as livestock feed and as food for human populations in arid zones (López-García et al., 2001; Mohamed-Yasseen et al., 1996). Different studies have, however, focused on seed proteins, rendering valuable information. Uchoa et al. in (1998) reported a protein present in the seeds of *Opuntia ficus-indica* (L.) Miller, that appeared to be the first seed protein described from a cacti source which was catalogued as a storage protein of the 2S albumin group.

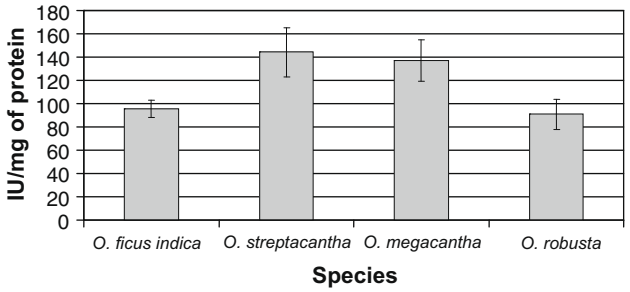

Fig. 1. Comparison of *Opuntia* species screening for trypsin inhibitor levels. IU, trypsin inhibitory units. Standard deviation is indicated by error bars.

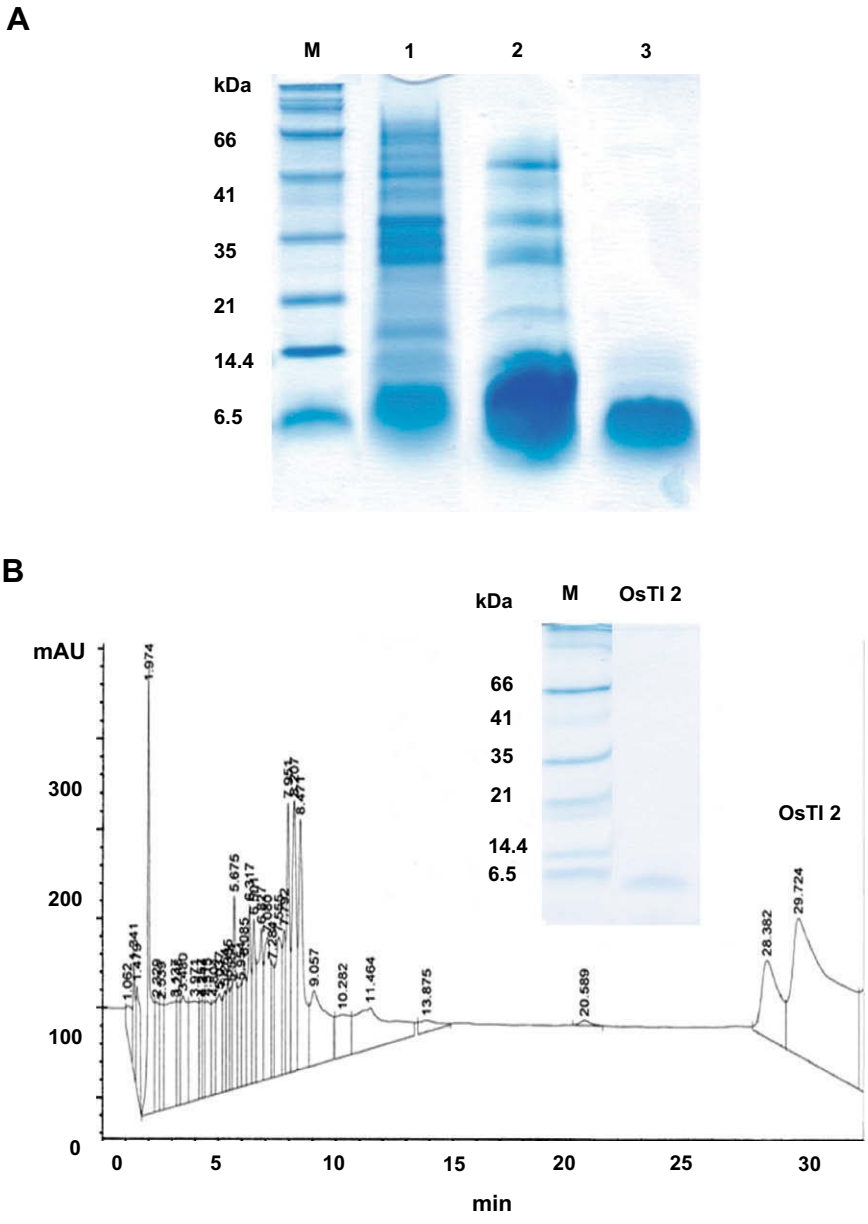

Fig. 2. OsTI purification. (A) SDS–PAGE monitoring for the OsTI purification process. (1) Crude protein extract; (2) Protein sample after heating; (3) Enriched fraction after ionic exchange chromatography. (B) HPLC profile and SDS–PAGE of the purified OsTI 2. M, molecular weight marker.

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