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Biochimica et Biophysica Acta 1298 (1996) 95–101

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Primary structure and specificity of a serine proteinase inhibitor from paprika (*Capsicum annuum*) seeds

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Received 2 May 1996; revised 17 July 1996; accepted 23 July 1996

Abstract

Several fractions demonstrating trypsin inhibitory activity were isolated from the seeds of the paprika plant (*Capsicum annuum*). One of the inhibitors, PSI-1.1, was purified to homogeneity and characterised. The mature form of PSI-1.1 has a molecular mass of 6053 Da and consists of 55 amino acids in a sequence showing over 80% identity with members of the inhibitors of potato-2 family. PSI-1.1 is a potent inhibitor of trypsin ($K_i = 4.8 \cdot 10^{-10}$ M) and a somewhat weaker inhibitor of chymotrypsin ($K_i = 4.7 \cdot 10^{-8}$ M) and pronase E ($K_i = 5.9 \cdot 10^{-8}$ M). PSI-1.1 is resistant to heat up to 85°C, to acidic conditions (down to pH 2.0) and to pepsin digestion, presumably due to its four disulfide bridges.

Keywords: Proteinase inhibitor; Primary structure; (*Capsicum annuum*)

1. Introduction

Enzyme inhibitors are important tools of nature for regulating the activity of enzymes in cases of emergency, like pathogenic attack or germination. Plant seeds are known to produce a variety of proteinase inhibitors that are thought to protect the seed against insects and microbial pathogens. Inhibitors of serine proteinases are the best studied of this group [1,2]. Expression of inhibitor genes in transgenic plants seems to provide protection against pathogens [for a review see Ryan [3]].

The first member of the potato-2 inhibitor family to have its amino acid sequence determined was the small trypsin inhibitor from aubergine (*Solanum melongena*) [4]. Homologous pancreatic serine protease inhibitors were isolated from potato tubers [5,6]. Wounded tomato leaves [7] and potato tubers [8,9] were found to contain the so-called inhibitor II molecules that contain domains homologous to the potato-2 proteinase inhibitors.

It is interesting to note that although the members of the potato-2 inhibitor family and the related carboxypeptidase inhibitors are believed to have a storage role [2], no examples have been found so far in seeds.

Here we report on the purification, characterisation and the primary sequence of PSI-1.1, a trypsin/chymotrypsin inhibitor isolated from the seeds of the paprika plant (*Capsicum annuum*), a member of the

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PII S0167-4838(96)00121-5