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SBTX, a new toxic protein distinct from soyatoxin and other toxic soybean [*Glycine max*] proteins, and its inhibitory effect on *Cercospora sojina* growth

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Abstract

SBTX, a novel toxin from soybean, was purified by ammonium sulfate fractionation followed by chromatographic steps DEAE-Cellulose, CM-Sepharose and Superdex 200 HR fast-protein liquid chromatography (FPLC). Lethality of SBTX to mice (LD_{50} 5.6 mg/kg) was used as parameter in the purification steps. SBTX is a 44-kDa basic glycoprotein composed of two polypeptide chains (27 and 17 kDa) linked by a disulfide bond. The N-terminal sequences of the 44 and 27 kDa chains were identical (ADPTFGFTPLGLSEKANLQIMKAYD), differing from that of 17 kDa (PNPKVFFDMTIGGQSA-GRIVMEEYA). SBTX contains high levels of Glx, Ala, Asx, Gly and Lys and showed maximum absorption at 280 nm, $\varepsilon^{1\%}_{1\,cm}$ of 6.3, and fluorescence emission in the 290–450 nm range upon excitation at 280 nm. The secondary structure content was 35% α-helix, 13% β-strand and β-sheet, 27% β-turn, 25% unordered, and 1% aromatic residues. Immunological assays showed that SBTX was related to other toxic proteins, such as soyatoxin and canatoxin, and cross-reacted weekly with soybean trypsin inhibitor and agglutinin, but it was devoid of protease-inhibitory and hemagglutinating activities. The inhibitory effect of SBTX on growth of *Cercospora sojina*, fungus causing frogeye leaf spot in soybeans, was observed at 50 μg/ml, concentration 112 times lesser than that found to be lethal to mice. This effect on phytopathogenic fungus is a potential attribute for the development of transgenic plants with enhanced resistance to pathogens.

Keywords: *Glycine max*; Soybean; Plant toxin; Toxicity; Antifungal protein

1. Introduction

The presence of toxicity in the crude extract of soybeans injected intraperitoneally (i.p.) to experimental animals was first reported in 1951 (Liener, 1951). This toxic effect was initially attributed to

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soybean trypsin inhibitors (SBTI) and/or to the soybean agglutinin (SBA) present in these seeds. However, in 1952, Liener and Pallansch (1952) observed that the toxic fraction was virtually free of antitryptic activity and possessed marked hemagglutinating properties. Although these results strongly suggested that toxicity could be related to hemagglutinating activity, no firm correlation was found between these two biological effects. Pallansch and Liener (1953) determined some physical characteristics of this toxic fraction, denominated soyin, which, besides having toxicity and hemagglutinating activity, differentiated it from other soybean proteins such as SBTI, urease and lipoxidase. Sambeth et al. (1967) confirmed the above findings, although the toxic protein had not been purified to homogeneity on account of its instability and low yield during the purification steps. Nevertheless, none of these authors reported the symptoms preceding death of the injected animals. Several years later, Carlini et al. (1988) observed that soybean aqueous extract was able to produce acute neurotoxic effects in mice, with convulsions preceding death, similar to canatoxin (CNTX), a toxic protein isolated from *Canavalia ensiformis* (Carlini and Guimarães, 1981). Again, the toxic principle was not comprehensively studied. Later, Vasconcelos et al. (1994) clearly showed that this toxicity was, in part, associated to the presence of a novel protein, nominated as soyatoxin (SYTX), distinct from SBTI and SBA. Moreover, during the course of SYTX purification, the presence of other unknown convulsant, lethal protein in soybean aqueous extract was observed, which was not further studied. Actually, based on proteomic studies, more than 100 proteins have been mapped from whole soybean seeds (Herman et al., 2003); however, many remain to be purified and characterized (Mooney and Thelen, 2004; Hadjuch et al., 2005).

Therefore, the present paper describes the purification and physicochemical characterization of a novel toxic protein from soybeans, designated soybean toxin (SBTX), distinct from SYTX, SBA and SBTI. Additionally, in order to shed light on the physiological role of this protein and make it available as a new biotechnological tool, its inhibitory activity against the conidial growth of the phytopathogenic fungi *Cercospora sojina*, *Aspergillus niger* and *Fusarium solani* was evaluated.

2. Materials and methods

2.1. General

Mature seeds of soybean [*Glycine max* (L.) Merr.], cv. BR-10, adapted to Brazilian low latitudes were developed and supplied by Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA-Meio Norte, Piauí, Brazil). Three-month-old New Zealand rabbits were provided by the Departamento de Zootecnia, Universidade Federal do Ceará (UFC), Fortaleza, Brazil, and were used for production of anti-SBTX antibodies and as donors of blood cells used in the hemagglutinating assay. Swiss mice (*Mus musculus*), 20–25 g, were from the animal house at UFC. The filamentous fungus *Cercospora sojina* was from the Departamento de Fitopatologia of the Universidade Federal de Lavras, Minas Gerais, Brazil, whereas *Aspergillus niger* (URM 3292) and *Fusarium solani* (URM 3708) were provided by the Departamento de Micologia, Universidade Rural de Pernambuco, Recife, Brazil.

2.2. Protein determination

The method described by Bradford (1976) was used with bovine serum albumin (BSA) as standard. Absorbance at 280 nm was also used to determine the protein content of column eluates.

2.3. Toxicity assay

These studies were reviewed and approved by the Animal Ethics Committee (CEPA) of UFC, Brazil.

Toxic activity was defined as mortality observed in Swiss mice within 24 h after i.p. injections of the samples. One LD_{50} unit is taken as the amount of protein (in mg protein/kg mouse body weight) producing convulsions and death of 50% tested animals (six doses; six mice per dose) (Vasconcelos et al., 1994).

2.4. Purification of the toxin

Mature seeds were ground in a coffee grinder fitted with a 1-mm-mesh screen and the resulting flour treated with petroleum ether (1:10, w/v). Defatted flour was extracted with 0.025 M Tris-HCl/0.005 M dithiothreitol (DTT), pH 7.5, containing 1.5×10^{-6} M leupeptin, 1.0×10^{-7} M pepstatin and 1.2×10^{-5} M phenylmethylsulfonyl fluoride, in