PURIFICATION AND ANALYSIS OF THE *SCHIZOLOBIUM PARAHYBA* CHYMOTRYPSIN INHIBITOR (SPCI) IN COMPLEX WITH CHYMOTRYPSIN

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Protease inhibitors are widely distributed in plant seeds acting as anti-nutritional agents in insects, nematodes and fungi, where they inhibit specific proteases. *Shizolobium parahyba* chymotrypsin inhibitor (SPCI) is a Kunitz type inhibitor with two disulfide bonds, suppressing the chymotrypsin proteolytic activity through the formation of a complex with a 1:1 stoichiometry. The SPCI tends to form oligomeric molecular arrangements and have been recently crystallized in a dimeric form. In the present work, the SPCI and its complex with chymotrypsin were purified and analyzed concerning the stability and pH effects. The SPCI was purified from crude extract from *S. parahyba* seeds by precipitation with 0.3% TCA followed by ion exchange chromatography in SP Sephadex. The complex SPCI-chymotrypsin was purified by molecular exclusion in Sephadex G-75, in 50 mM Tris-HCl pH 7.6. The purity and inhibitory activity of the complex were analyzed by SDS-PAGE and chymotrypsin assay, respectively. The intrinsic fluorescence band of the SPCI-chymotrypsin complex was decreased in comparison with emission bands of the chymotrypsin and SPCI molecules, suggesting conformational changes after the complex formation. The crystallization assays of the complex were done in order to identify the structural and functional features charactering the SPCI inhibition activity.

Keywords: SPCI, Chymotrypsin, serine protease.
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