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CHARACTERIZATION OF A BOWMAN-BIRK INHIBITOR FROM LENTIL: EXPRESSION AND ANTITUMORAL PROPERTIES

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Proteinase inhibitors are widely diffused in plants. In legumes, mainly two types of these inhibitors have been identified: the larger Kunitz inhibitor (16-21 kDa) generally with two disulfide bonds and one reactive site for trypsin or chymotrypsin, and the smaller (6-9 kDa), double-headed Bowman-Birk one (BBI), generally with seven disulfide bridges, a high cysteine content and two reactive sites, one for trypsin and the other for trypsin or chymotrypsin. These two reactive sites are separately distributed in two homologous active loops on the same polypeptide chain and can interact simultaneously and independently with two target proteases.

In plants, the trypsin inhibitor site has the ability to inhibit animal digestive enzymes, thus representing an ideal candidate to protect plants against insect predation through genetic engineering, but may also be involved in the regulation of endogenous plant proteases.

On the other hand, the chymotrypsin inhibitor site seems to be involved in the prevention or suppression of carcinogen-induced transformation *in vitro* and of carcinogenesis in animal model systems. Moreover, BBIs have also displayed anti-inflammatory activity and have been tested in the treatment of experimental autoimmune encephalomyelitis, an animal model disease for human multiple sclerosis.

Two BBI gene classes have been reported in lentil, one coding a trypsin/trypsin inhibitor, the other encoding a trypsin/chymotrypsin inhibitor, even though the sequence of the latter was not complete at the 3' end. We isolated a complete cDNA sequence coding for lentil trypsin/chymotrypsin BBI. The inhibitor was expressed in the methylotrophic yeast *Pichia pastoris*. After purification, recombinant molecules were analysed by MALDI-TOF mass spectrometry, and the inhibitory activity evaluated, by means of enzymatic assays using specific substrates for trypsin or chymotrypsin. The expressed lentil BBI showed an inhibitory activity similar to BBIs from other plants. The ability of lentil BBI to modulate the viability of human colorectal adenocarcinoma HT29 cells *in vitro* was also assessed.