Programmed Cell Death (PCD) and their Relationship with Protease Activities in Legume Seed Coats

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INTRODUCTION: PCD is a regulated process that occurs in multicellular organisms causing the suicide of a cell. The present work had as its main objective the diagnosis of such phenomenon in quiescent seed coats of Glycine max, mainly by the search of proteases with caspase-like activity. MATERIAL AND METHODS: Protein profiles and enzymatic activities from seed coat extracts were analyzed by electrophoresis. Caspase-like activities were investigated by in vitro assays with caspase substrates and inhibitors. Occurrence of PCD events were further confirmed by the DNA fragmentation pattern analysis (kit Cell Death Detection ELISA - Roche - version 10.0). The nature of activity bands was analyzed by mass spectrometry. RESULTS AND DISCUSSION: In gel protease activities were observed under favorable conditions to cysteine proteases catalysis. One band exhibited thermo-stability, even under 15 min of boiling. The two dimensional electrophoresis studies show that these activities patterns can be part of an autocatalytic enzyme activation system or of a multiprotein complex. The in vitro assays were able to detect caspase-like activities against Ac-DEVD-pNa, Ac-VEID-pNa and Ac-LHED-pNa, being the first two inhibited when incubated in the presence of corresponding inhibitors. The monitoring of developmental events before quiescence indicated a decreasing detection of fragmented DNA along the seed coat developmental course, reinforcing the idea that the detected active proteases are involved in PCD events prior to quiescence. The spectrometric analysis did not reveal protease sequence from the bands obtained by electrophoresis, although a predicted protein with a BAG domain, which may be involved in PCD regulation, was detected. CONCLUSIONS: PCD events seem to be part of the development of soybean seed coat considering both the detection of caspase-like activities in quiescent stage and the DNA fragmentation along the developmental stages. A protein with a PCD-related BAG domain co-migrated with protease band extracted from polyacrylamide gels.