The activity and location of cathepsin D inhibitor in seeds of common vetch (*Vicia sativa* L.)

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Abstract

The activity of cathepsin D inhibitor is markedly higher in common vetch seed coat than in embryo cotyledons. The occurrence of considerable amounts of the inhibitor in the seed coat of vetch was confirmed by the fluorescent microscopic technique, with the use of fluorescein-marked cathepsin D.

Key words: cathepsin D inhibitor, fluorescein-marked cathepsin D, seed coat, common vetch (*Vicia sativa* L.).

Introduction

A peptide inhibitor of cathepsin D is present in common vetch seeds [1, 2]. However, it does not occur in the stem, roots, leaves, flowers, and pods of this plant. The vetch seeds are endosperm-free, they consist of an embryo and multilayer seed coat [3, 4].

The aim of the study was to compare the activity of cathepsin D inhibitor in seed coat and embryo cotyledons of common vetch.

Material and methods

The location of the inhibitor in cells of the seed coat and cotyledons was analysed by the fluorescent microscopic technique, using fluorescein-marked cathepsin D, capable of selective binding with the inhibitor.

Fluorescein isothiocyanate (FITC), cathepsin D, Bradford reagent and Folina and Ciocalteau reagent, Sigma, USA; haemoglobin, Difco, Laboratories, USA.

Seed coats of common vetch (*Vicia sativa* L.), cv. Szelejewska, were separated from the cotyledons, using the MF1 machine and shred in a mechanical mill. The seed coats and cotyledons were extracted. using distilled water in the 1:4 ratio of w/v. The extraction was carried out for 2 hours in laboratory temperature, while continuously stirring. In the supernatant, obtained in result of centrifugation (2700x g, 30 min, 4^{0}C), the activity of the inhibitor was determined, using cathepsin D and haemoglobin as substrates [5], as well as protein contents with the use of the Bradford method [6].

Fluorescent microscopic technique was incorporated to examine the location of the inhibitor at the level of the cells in seed coats and cotyledons, using fluorescein-marked cathepsin D [7]. Fragments of seed coats and cotyledons (7µm thick), obtained by a freezing microtome, were placed on microscope slides, covered with polylysine, stippled with marked cathepsin D (unmarked cathepsin D in the control) and incubated for 30 min. at 37^{0}C. Then, the slides were washed with phosphatic buffer, stippled with buffered glycerine, and closed with a cover glass. The preparations were evaluated, using a Nikon Eclipse E600 fluorescent microscope, at the excitation wave length of 450-490 nm, at which, fluorescein emits radiation of 520-550 wave length (green light).

Results and discussion

Approximately 70% of cathepsin D inhibitor activity occurs in the seed coat and the rest is present in the embryo cotyledons Table 1. The activity of cathepsin D inhibitor in seed coat is 1360.0 U/g of tissue and, in the cotyledons - 421.5 U/g. The protein content in the seed coat is 9.5 mg/g of tissue and that in the cotyledons - 53.5 mg/g. The inhibitor activity, expressed in pro-

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Table 1. The activity of cathepsin D inhibitor and protein contents in extracts of seed coats and cotyledons of common vetch.

Extract	Protein, mg/g tissue	Activity of inhibitor		Inhibition, %
		u/g tissue	u/g protein	
Seed coat	**9.5**	**1360.0**	**143.1**	69
Cotyledons	**53.5**	**421.5**	**7.9**	22

Figure 1. Fragment of seed coat of common vetch, incubated with fluorescein-marked cathepsin D. L-light line, A-epidermis (palisade layer), B-osteosclereid layer, C-parenchymal cells. The arrows point to the sites of cathepsin D inhibitor occurrence. x 200.

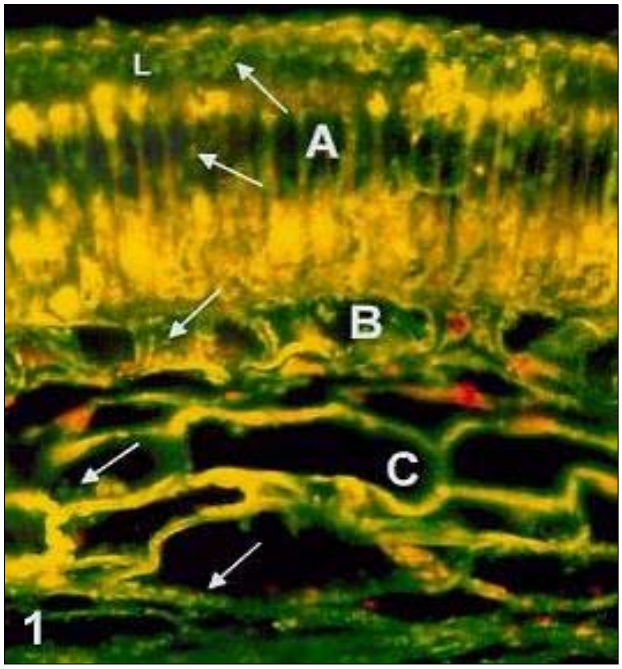


Figure 2. Fragment of seed coat of common vetch, incubated with cathepsin D, which was not marked with fluorescein. x 200.

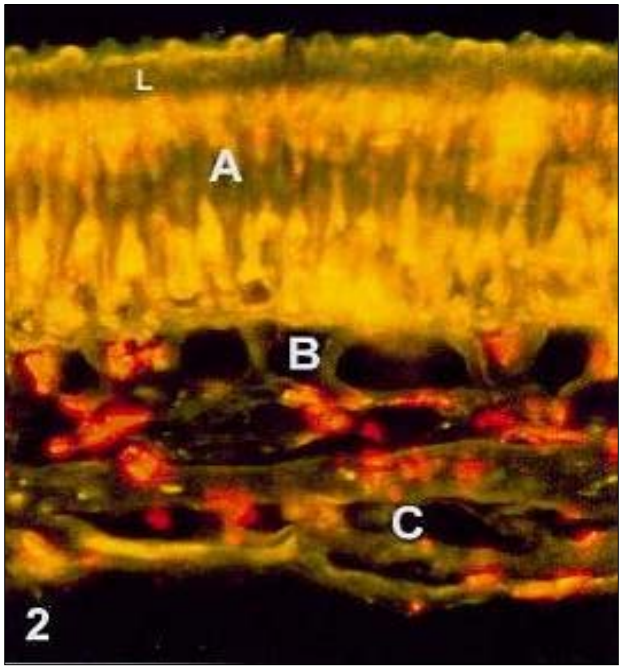


Figure 3. Fragment of embryo cotyledon tissue of common vetch, incubated with fluorescein-marked cathepsin D. S - starch grain. x 200.

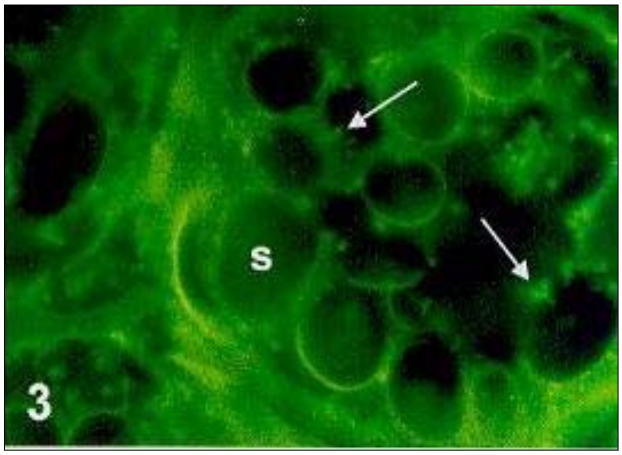
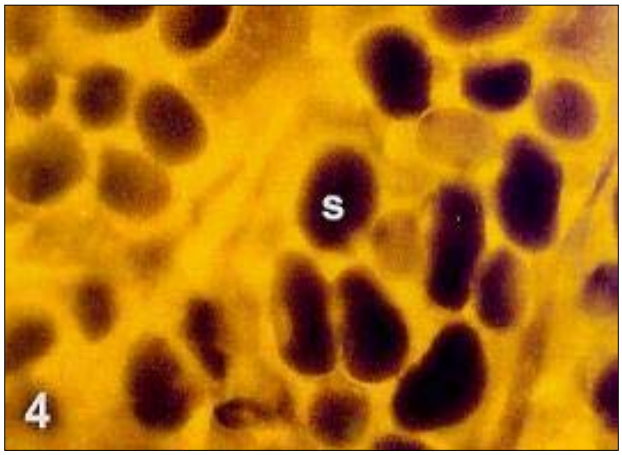


Figure 4. Fragment of embryo cotyledon tissue of common vetch, incubated with cathepsin D, which was not marked with fluorescein. x 200.



tein g, is 143.1 U/g in seed coat and 7.9 U/g of protein in cotyledons.

A similar distribution of the inhibitor was determined by the fluorescent microscopy technique. As it was observed (Fig. 1), the fragments, taken from the seed coat, incubated with fluorescein-marked cathepsin D, revealed a green signal, specific for this fluorochrome in the shape of small granules, occurring specifically in palisade cells of seed coat epidermis (A). The signal is markedly intensified in elongate protoplasts of these cells, as well as in the area of light line of the cell wall (L). The granules, emitting green light, occur close to cell walls in the cells, lying deeper in the seed coat, specifically in the parenchyma (B

and C). In the control preparations of the seed coat, there were no green light emitting granules (Fig. 2). The preparations of cotyledons, incubated with fluorescein-marked cathepsin D, revealed only few small clusters of granules of fluorochrome, emitting green light and located between starch grains in the cells of cotyledon (Fig. 3). However, the control cotyledons did not show any such granules (Fig. 4).

The study showed that the distribution inhibitor of cathepsin D in the seeds of common vetch is differentiated in particular types of its tissues. Proteolytic enzyme inhibitors, occurring in the seeds, protect reserve proteins during long (many months) storage against the activities of own proteases and the proteases of bacteria, mould, and insects that are present in the environment [8, 9]. Such a role is probably fulfilled by the cathepsin D inhibitor, which occurs mainly in the cells of the outer layer of seed coat in common vetch seeds.

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