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PROTEINASES INVOLVED IN THE DEGRADATION OF TRYPsin INHIBITOR IN GERMINATING MUNG BEANS**

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The mung bean (Vigna radiata (L.) Wilczek) trypsin inhibitor (MBTI) is rapidly modified by limited proteolysis during the early stages of seedling growth. Using an electrophoretic assay that separates the unmodified inhibitor (MBTI-F) and the first two modified species (MBTI-E and -C), a pH optimum of approximately 4 was found for the modification reaction. The inhibitor modifying activity is initially low in ungerminated seeds, with the reaction F→E being the primary reaction catalyzed. Activity catalyzing the production of MBTI-C appears on the first day of germination. This activity (F→E→C) increases up to 6 days after imbibition, at which time the cotyledons begin to abscede. The activity converting MBTI-F and -E to MBTI-C was strongly inhibited by phenylmethylsulfonyl fluoride (3.3 mM) but only weakly by iodoacetate (9 mM) and not at all by pepstatin A (9 μM), leupeptin (18 μM), or EDTA (5 mM). These results suggest the involvement of proteinases other than the major endopeptidase of the germinating seed, vicilin peptidohydrolase. This conclusion is further supported by gel filtration of the extracts of cotyledons on Sephadryl S-200. At least three proteinases are present in germinated cotyledons capable of modifying MBTI-F to MBTI-C and/or -E. All are distinguishable from vicilin peptidohydrolase on the basis of their molecular weight and inhibition by low molecular weight organic reagents.

During the germination and early growth of seedlings, there is a mobilization of the various storage forms, e.g. polysaccharides, proteins, and lipids, present in the seed. These macromolecules are hydrolyzed to lower molecular weight forms which are in turn exported either directly or in modified forms to the growing seedling to support its growth (Chrispeels et al., 1976; Mayer & Marbach, 1981). In seeds containing large amounts of storage proteins, as the Leguminosae, the processes of proteolysis are especially prominent. The legumes are also known for the high levels of protein proteinase inhibitors contained in their cotyledons, especially

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those active against the mammalian pancreatic enzymes (Laskowski, Jr. & Kato, 1980; Wilson, 1981). We have recently demonstrated that the Bowman-Birk type proteinase inhibitors, a prominent class of proteins in many of the Leguminosae, are also subject to proteolysis during germination (Lorenzen et al., 1981; Tan-Wilson et al., 1982).

The events of this degradation have been most completely described in the mung bean (Vigna radiata) (Wilson & Chen, 1982, 1983). The mung bean trypsin inhibitor is a typical Bowman-Birk type inhibitor with 80 amino acid residues. Its degradation is initiated by the removal of the carboxyl-terminal peptide -Lys-Asp-Asp-Asp. This is followed by the loss of the amino-terminal octapeptide Ser-Ser-His-His-His-Asp-Ser-Ser-, the loss of an additional two residues (Met-Asp) from the carboxyl-terminal, and a cleavage at the internal peptide bond Ala\(^{35}\)-Asp\(^{36}\). The majority of these cleavages involve aspartyl residues as either the carboxyl or amino group donor to the peptide bond involved. In this paper we describe some of the properties of the proteinases involved in these initial stages of Bowman-Birk inhibitor degradation in the germinating mung bean.

**MATERIALS AND METHODS**

*Plant materials and reagents.* Mung bean (Vigna radiata (L.) Wilczek) seeds, cv. Jumbo, were obtained from Johnny’s Selected Seeds, Albion, Maine, and had a germination greater than 95%. Agarose (Seakem LE) was from FMC Marine Colloids. Azocasein, bovine trypsin (thrice crystallized, EC 3.4.21.4), Z-\(\alpha\)-phenylalanyl-L-leucine\(^1\), BAPA, PMSF, Na-iodoacetate, DTT, pepstatin A and leupeptin were obtained from Sigma Chemical Company. Sephacryl S-200 was from Pharmacia Fine Chemicals, while acrylamide was from Serva. \(N,N'\)-Methylenebisacrylamide and \(N,N,N',N'\)-tetramethylethylenediamine were from BioRad. MBTI-F and E were prepared as previously described (Lorenzen et al., 1981). All other chemicals were reagent grade or better. All pH adjustments were made at room temperature (20 ± 1°C), and twice distilled H\(_2\)O used throughout.

*Germination of mung bean seeds.* Seeds were imbibed for 24 h at room temperature in distilled H\(_2\)O. The seeds were then rinsed twice in distilled H\(_2\)O, and planted in moist vermiculite. Growth was continued in a growth chamber with a 16 h light period (24°C) and 8 h dark period (18°C). After the desired period of growth the cotyledons were harvested, rinsed with distilled H\(_2\)O, and blotted dry. The cotyledons were stored at −80°C until needed. Germination time was reckoned from the beginning of imbibition.

*Preparation of cotyledon extracts.* For initial studies cotyledons were partially thawed and homogenized with 5.5 ml of ice cold 50 mm-Tris/Cl, pH 8.0, per gram seed.

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\(^{1}\) Abbreviations used (in order of appearance): Z-, benzoxycarbonyl-; BAPA, \(\alpha\)-\(\text{N-benzoyl-}\)-arginine \(\beta\)-nitroanilide; PMSF, phenylmethylsulfonyl fluoride; DTT, DL-dithiothreitol; MBTI, mung bean trypsin inhibitor; BAPA’ase, amidase activity hydrolyzing BAPA; MES, 2\((\text{N-morpholino})\)ethane sulfonic acid; PIPES, 1,4-piperazinediethanesulfonic acid.
(fresh weight) of cotyledons. Dry ungerminated cotyledons were first ground to a fine meal and extracted with 11 ml of the same buffer per gram of meal. After holding at 0°C for 45 min the homogenates were centrifuged at 23 400 g for 45 min. The clarified extracts were divided into small aliquots and frozen at −80°C for later use. Extracts for chromatographic studies were prepared in a similar manner with the exception that the extraction buffer contained 2 mM-DTT. The extracts were chromatographed immediately without freezing.

Trypsin inhibitor assays. Trypsin inhibitor activity was assayed with bovine trypsin and α-N-benzoyl-L-arginine ethyl ester as previously described (Lorensen et al., 1981). One unit of inhibitor activity inhibits 1 mg of active bovine trypsin under the conditions of the assay. Mung bean trypsin inhibitor protein was determined by radial immunodiffusion (Mancini et al., 1965). Antiserum specific for MBTI were raised in New Zealand White rabbits with glutaraldehyde polymerized MBTI-F using the techniques and immunization schedule previously used for the soybean Bowman-Birk trypsin inhibitor (Tan-Wilson & Wilson, 1982). Concentrations of standard MBTI-F solutions were determined using an extinction coefficient E<sub>1%<sub>em</sub></sub> of 2.76 (as determined by amino acid analysis).

Protein determination. The protein content of extracts were initially determined by both the methods of Lowry et al. (1951) and Zamenhof (1957). Bovine serum albumin was used as the standard in each case. Similar results were obtained with either method, indicating there was no significant interference by seed phenolics in the Lowry method. The Lowry assay was therefore subsequently used in all later studies.

Enzymatic assays. Carboxypeptidase activity was determined spectrophotometrically with Z-L-phenylalanyl-L-leucine by the method of Hayashi (1977) with the exception that 50 mM-Na-phosphate buffer, pH 6.0, was used as the assay buffer. One unit of carboxypeptidase activity was defined as the amount of enzyme hydrolyzing 1 μmole of substrate per min at 25°C. Activity hydrolyzing BAPA, henceforth termed amidase (BAPA’ase), was assayed by a modification of the method of Erlanger et al. (1961). Assay mixtures contained 3 ml of 1 mM-BAPA in 50 mM-Tris/Cl, pH 8.0, containing 1% (v/v) dimethylsulfoxide, and 0.1 ml of sample. One unit of amidase was defined as producing a change in absorbance at 410 nm of 1.0 per minute at 25°C in the above system.

General proteolytic activity was measured using azocasein (Charney & Tomarelli, 1947) as substrate. The sample (0.7 ml) and 0.3 ml buffer (0.2 mM-Na-citrate + 10 mM-2-mercaptoethanol, pH 5.7) were incubated at 37°C for 15 min. Azocasein, 1% (w/v) in 25 mM-Na-citrate+10 mM-2-mercaptoethanol, pH 5.7, was then added. After 1 h at 37°C, 1 ml of 15% (w/v) trichloroacetic acid was added, and the reaction mixture held on ice for 20 min. After centrifugation at room temperature, the absorption of the supernatant at 390 nm was determined. Blanks were run in the same manner with the exception that the sample was added after the addition of the trichloroacetic acid. One unit of proteinase was defined as producing a change in absorbance at 390 nm of 1.0 in the above assay system.
Electrophoresis. Polyacrylamide disc gel electrophoresis was performed in 1.5 mm thick vertical slab gels. The system of Davis (1964) was used except that the stacking gel was omitted. Gels containing 10% acrylamide were used throughout. The gels were stained overnight (a minimum of 18 h) with 0.5% (w/v) Coomassie Blue R-250 in 45.5% (v/v) methanol and 9.2% (v/v) acetic acid. Staining for shorter periods of time resulted in poor staining of the inhibitors. The gels were destained by diffusion in 12.5% (v/v) 2-propanol + 10% (v/v) acetic acid. The gels were photographed as soon as they were sufficiently destained as prolonged destaining resulted in broadening and eventual loss of the inhibitor bands.

Assay of inhibitor proteolysis. The assay of proteinases active in the conversion of MBTI-F (the unmodified mung bean trypsin inhibitor) to its modified forms was based on the electrophoretic separation of the inhibitor forms as previously described (Lorensen et al., 1981). To determine the pH optima for these reactions, MBTI-F or MBTI-E, approximately 15 μg (in 15 μl of 0.01% (v/v) triethylamine) and 15 μl of the sample of interest were incubated at 25°C with 40 μl of buffer. The following buffers were used: pH 3.0 and 4.0, Na-formate; pH 5.0, Na-acetate; pH 6.0, Na-MES; pH 7.0, Na-PIPES; pH 8.0, Tris/Cl; and pH 9.0, Na-borate. All buffers were 0.1 m. Blanks were also incubated containing only the extract and buffer or only the inhibitor and buffer. After 25 h, 30 μl of sample buffer (0.38 m-glycine + 50 mm-Tris + 10% (w/v) sucrose, pH 8.3) was added and electrophoresis performed as above. Both MBTI-F and MBTI-E are stable for at least 48 h under the incubation conditions in the absence of the extracts.

For the routine assay of modifying proteinase, reaction mixtures consisted of 15 μl of MBTI-F as above, 20 μl of sample and 15 μl of 1 m-Na-formate, pH 4.0, with the incubation at 37°C for 20 h. The reaction was stopped and electrophoresis performed as above. The presence of inhibitor modifying activity was assessed qualitatively and semi-quantitatively on the basis of the appearance of the modified forms MBTI-E and MBTI-C and the disappearance of MBTI-F. Similar assays were also performed as needed using MBTI-E as substrate. While the assays reported here were not performed under aseptic conditions, similar experiments performed under asepsis have yielded identical results.

Inhibition of proteinases by organic reagents. Extracts with activity converting MBTI-F to the modified forms were incubated with the organic reagents at 5°C for 20 h. All reagents were added as aqueous solutions with the exception of PMSF, which was dissolved in 2-propanol. An incubation with 2-propanol alone was included as a control for any effect of this solvent on the enzymes. The pH of all incubation mixtures was between pH 7.2 and 8.0. The inhibitor modifying activity and proteinase activity remaining in the reaction mixtures were then determined as described above.

Gel filtration chromatography. Aliquots (20 ml) of the extracts from ungerminated and 5 day germinated cotyledons were chromatographed at 5°C on a 2.5 × 95 cm column of Sephacryl S-200 in 50 mm-Tris/Cl + 2 mm-DTT, pH 8.0. The column was eluted at 30 ml/h with 150 drop (5.05 ml) fractions collected.
RESULTS AND DISCUSSION

pH optimum for the conversion of MBTI to its modified forms. A distinctly acidic pH optimum was found for the proteolysis of either MBTI-F or MBTI-E by the extracts of cotyledons from beans germinated for 6 days. The conversion of MBTI-F to the further modified form, MBTI-E, occurred between pH 3 and 6 with the optimum at pH 4 (Fig. 1). The conversion E→C appears to have a somewhat narrower pH dependence, with little activity noted at pH 3 or 6 under the conditions used in our assay. A similar pH dependence was found for the MBTI-F modifying activity found in the extracts of ungerminated cotyledons. Activity modifying MBTI-E was not detected in ungerminated mung beans.

Changes in proteinase and inhibitor levels during germination. The content of total extractable protein, as well as MBTI (as measured by both trypsin inhibitory activity and radial immunodiffusion) remains essentially constant during the first three days of germination in the mung bean (Fig. 2). However, we have previously demonstrated that during this same time there is a rapid conversion of MBTI-F

![Graph showing changes in trypsin inhibitor, protein, and proteinase in the cotyledons of mung bean during germination and early seedling development.](image)

Fig. 2. Changes in trypsin inhibitor, protein, and proteinase in the cotyledons of mung bean during germination and early seedling development. Mung beans were grown, extracts of the cotyledons prepared, and assays performed as described in Materials and Methods. Top frame: △, total extractable protein (mg/g); ●, trypsin inhibitor (TI) protein (μg/g); ○, trypsin inhibitory (TI) activity (U/g). Bottom frame; ●, proteinase, as measured by caseinolysis (U/g); △, amidase (BAPA'ase, U/g).

All values are expressed in terms of the original dry weight of the ungerminated cotyledons.
Fig. 3. Changes in trypsin inhibitor modifying activity in germinating mung beans. Extracts from cotyledons of seeds germinated for the indicated times were incubated with MBTI-F at pH 4 as described in the text. The amount of extract used in each incubation was chosen to be equivalent to 1 mg (initial dry weight) of cotyledons. S, standard MBTI-F and -E as in Fig. 1; 0 - 6, reactions with extracts from ungerminated cotyledons and those from seeds germinated 1, 2, 3, 4, 5, and 6 days. No protein bands with the same mobilities as MBTI-F. -E or -C were noted in the blank reactions.
Fig. 1. pH optimum for the modification of MBTI-F and MBTI-E by the enzymatic activity in extracts of cotyledons from beans germinated for 6 days. The extract and inhibitors were incubated together at the indicated pH values as described in the text. The reaction mixtures were subjected to slab polyacrylamide disc gel electrophoresis. Frame A (MBTI-F as the initial substrate); S, standard containing (from top to bottom) MBTI-E and MBTI-F; 3 - 8, reaction mixtures at pH values of 3, 4, 5, 6, 7, and 8 respectively. The protein band appearing immediately above MBTI-E in the pH 4 and 5 reaction mixtures is MBTI-C. Frame B (MBTI-E as the initial substrate); E, standard MBTI-E; 3 - 8, reaction mixtures at pH 3, 4, 5, 6, 7, and 8 respectively. Blank reactions (e.g. with no added MBTI) exhibited no protein bands at the positions of MBTI-F, E and C. Migration (here and in all other gels shown in this paper) was from cathode to anode (top of gel to bottom).
to the proteolytically modified forms MBTI-E, -C, and -A (Lorensen et al., 1981). After 3 days, there is a decline in both inhibitor and total protein in the cotyledons.

Activity catalyzing the reaction F→E is present in ungerminated seeds as well as seeds imbibed 24 h (Fig. 3). Only traces of activity catalyzing the reaction E→C are found in extracts from these stages of development. However, after 24 h of germination, there is an increasing level of activity producing MBTI-C from MBTI-F with MBTI-E as an intermediate. This activity reaches a maximum at 5 or 6 days of germination, i.e. at about the time that the cotyledons begin to abscise under the growth conditions used here.

In an attempt to correlate the inhibitor modifying enzyme(s) with other proteolytic activities, we also determined the levels of those enzymes hydrolyzing BAPA, Z-L-phenylalanyl-L-leucine, and azocasein in the same extracts. As previously noted by Chrispeels & Boulter (1975) there is an increase in caseinolytic activity during germination (Fig. 2). This activity appears to be due primarily to the sulfhydryl-dependent enzyme vicilin peptidohydrolase (Baumgartner & Chrispeels, 1977), as omission of mercaptoethanol from the assay results in 50% lower activity. Amidase cleaving BAPA (Fig. 2) and carboxypeptidase (not shown) activities are similar to each other, showing an increase during early development followed by a later decline. Thus of the three types of proteolytic activity examined, only the caseinolytic activity (vicilin peptidohydrolase) appears to change in a manner similar to that of the inhibitor modifying enzyme(s) during germination.

Effect of organic reagents on inhibitor modifying activities. Of the five reagents tested here (Table 1 and Fig. 4), only PMSF was found to inhibit MBTI modification, specifically at the E→C reaction(s). This reaction could be totally inhibited by 3.3 mm-PMSF, while the reaction F→E was unaffected by the same treatment.

Table 1

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
<th>Caseinolytic activity</th>
<th>Inhibition of MBTI modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>—</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>PMSF</td>
<td>1.2 mm*</td>
<td>96</td>
<td>+</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>1.2% (v/v)</td>
<td>102</td>
<td>—</td>
</tr>
<tr>
<td>Na-iodoacetate</td>
<td>9.1 mm</td>
<td>31</td>
<td>±</td>
</tr>
<tr>
<td>Peptatin A</td>
<td>9.1 μM</td>
<td>70</td>
<td>—</td>
</tr>
<tr>
<td>Leupeptin</td>
<td>18.2 μM</td>
<td>27</td>
<td>—</td>
</tr>
<tr>
<td>EDTA</td>
<td>4.9 mm</td>
<td>132</td>
<td>—</td>
</tr>
</tbody>
</table>

* Also contained 1.2% (v/v) 2-propanol.
A slight inhibition of the E→C reaction was also noted with iodoacetate. PMSF had essentially no effect on the caseinolytic activity of extracts from germinated seeds. In contrast, the caseinolytic activity was strongly inhibited by iodoacetate and leupeptin, and to a lesser extent by pepstatin A. This is consistent with the majority of the caseinolytic activity of the extract being due to the mung bean vicilin peptidohydrolase (Alpi & Beevers, 1981; Baumgartner & Chrispeels, 1977), while the majority of the trypsin inhibitor modifying activity is due to one or more other proteinases.

**Chromatographic separation of proteolytic activities in germinated and dry beans.** Gel filtration of the extract from cotyledons germinated 5 days yielded three distinct peaks of caseinolytic activity (Fig. 5). The first of these, peak A, eluted near the

![Graph of gel filtration results](image)

Fig. 5. Gel filtration of the extract of cotyledons from seedlings germinated for 5 days. See text for details. Frame A: —, A_280; ———, carboxypeptidase (CP'ase, U/ml). Frame B: o, proteinase (caseinase, U/ml); ———, amidase (BAPA'ase, U/ml) of same chromatography. Proteinase peaks A, B, and C are indicated in frame B.

void volume of the column with the amidase and carboxypeptidase activities. The second and third caseinolytic peaks (B and C), with apparent molecular weights of approximately 60 000 and 26 000, respectively, were devoid of amidase and carboxypeptidase-activity. Peak C was identified as mung bean vicilin peptidohydrolase on the basis of its molecular weight (Baumgartner & Chrispeels, 1977) and susceptibility to inhibition (87 and 85% inhibition by 9.1 mm-iodoacetate and 18.2 μM-leupeptin, respectively). In contrast, the caseinolytic activity in peak B was affected much less by these same inhibitors (24 and 19% inhibition by the same respective concentrations of iodoacetate and leupeptin). The low caseinolytic
Fig. 6. Assays for trypsin inhibitor modifying activity in the fractions from the gel filtration column in Fig. 5. The leftmost lane is standard, from top to bottom, MBTI-E and MBTI-F. Remaining lanes, assays of fractions as indicated. Assay blanks (not shown) had no protein bands corresponding to MBTI-F, -E, or -C.
Fig. 4. The effect of low molecular weight organic reagents on the trypsin inhibitor modifying activity in extracts from 5 day germinated cotyledons. Cotyledons were extracted with DTT-containing buffer as described in Materials and Methods, and the extract reacted with the indicated reagents at the concentrations listed in Table 1. The extract/reagent mixtures were then assayed for remaining MBTI-F inhibitor modifying activity as described. S, standard from top to bottom, MBTI-E and -F; lanes 1 - 6, reactions with PMSF, 2-propanol, Na-iodoacetate, pepstatin A, leupeptin, and EDTA, respectively, and lane 7, control reaction (with extract and MBTI-F only). Reaction blanks (not shown) had no protein bands corresponding to MBTI-F, -E or -C.
activity in peak A made analogous measurements difficult. However, its response to the two proteinase inhibitors appeared to be similar to that of peak B.

Activity modifying MBTI-F was found extending across all three caseinolytic peaks (Fig. 6). Fractions 35 to 45 (corresponding to caseinolytic peak A) catalyzed the reaction F→E, but not E→C, even with prolonged incubation. This activity was partially inhibited by 3.3 mM-PMSF. Activity converting F→E→C was found in fractions 50-56, i.e. corresponding to caseinolytic peak B. Additional activity converting MBTI-F to MBTI-E was also found in fractions 57 to 62, i.e. in the first half of caseinolytic peak C. Treatment of these fractions with 3.3 mM-PMSF abolished the activity catalyzing the reaction E→C in fractions 50 to 56. However, activity converting MBTI-F to MBTI-E remained, especially in fractions 50 to 62. These results suggest the possibility of two inhibitor modifying enzymes of similar molecular weight in the region of caseinolytic peaks B and C. The first, capable of converting MBTI-F to MBTI-C, is approximately coincident with caseinolytic peak B (i.e. peaking at fraction 53). The second, catalyzing the conversion of MBTI-F to MBTI-E (but not MBTI-C), peaks at approximately fraction 56 under these chromatographic conditions.

Gel filtration of an extract from ungerminated seeds (not shown) yielded a protein elution profile similar to that from germinated seeds. The caseinolytic activity was in general much lower, eluting as two peaks with molecular weights similar to those of caseinolytic peaks A and B described above. The trypsin inhibitor modifying activity was likewise much lower, with only a single peak of activity eluting at approximately the position of caseinolytic peak B. This modifying activity appears equivalent to the F→E converting activity peak noted above in the germinated seed extract.

The results presented here indicate the presence of at least two and possibly three enzymes in the germinating mung bean involved in catalyzing the initial stages of trypsin inhibitor degradation. We have recently demonstrated that MBTI-E differs from MBTI-F by the loss of the carboxyl-terminal tetrapeptide -Lys-Asp-Asp-Asp (Wilson & Chen, 1982, 1983). It is therefore tempting to speculate that one of the inhibitor modifying enzymes is the same as the carboxypeptidase, especially since mung bean carboxypeptidase is inhibited by PMSF (Chrispeels & Boulter, 1975). However, while the carboxypeptidase activity peak is present in the chromatograms of both germinated and ungerminated cotyledon extracts, inhibitor modifying activity is found at this location only in germinated cotyledons. It therefore seems unlikely that this carboxypeptidase contributes significantly to the F→E conversion.

The remainder of the inhibitor modifying activity in germinated cotyledons likewise does not appear to correspond to any previously described proteinase from germinating or ungerminated legume seeds. We are presently isolating the inhibitor modifying proteinases from germinating mung beans in the hope of further understanding their involvement in proteolysis during seed germination.
REFERENCES


ROLA PROTEINAZY W ROZKŁADZIE INHIBITORA TRYPSYNY W KIEŁKUJĄCYCH NASIONACH VIGNA RADIATA

Streszczenie

wczesnych etapach wzrostu siewki, wykazano za pomocą analizy elektroforetycznej, że optimum reakcji modyfikującej natywny inhibitor (MPTI-F) w dwie formy (MBTI-E i MBTI-C) przypada na pH 4.0. Czynność modyfikująca inhibitor jest niska w nasionach nieskłękowanych, przy czym pierwszą reakcję jest modyfikacja F→E. Powstawanie MBTI-C obserwowane jest już w pierwszym dniu kiełkowania. Reakcja F→E→C narasta do 6-go dnia po spęcznieniu nasion, kiedy liście zaczynają odpadać. Przekształcenie MBTI-F i MBTI-E do MBTI-C hamowane jest silnie przez fluorek fenylometylosulfonowy (3.3 mm), w niewielkim stopniu przez jodoocutan (9 mm) i nie jest hamowane przez pepstatynę A (9 µm), leupeptynę (18 µm) i EDTA (5 mm). Wyniki te sugerują, że jest to proteinaza odmienna od peptydohydrolazy wycyliny, zasadniczej endopeptydazy kiełkujących nasion. Sugestia ta poparta jest wynikami sączenia na żelu Sephacylu S-200 ekstraktów z liści. W liściach skłękowanych nasion występują co najmniej trzy proteinazy zdolne do przekształcania MBTI-F w MBTI-C i/lub w MBTI-E. Wszystkie trzy wykazują odmienną masę cząsteczkową oraz inną wrażliwość na niskocząsteczkowe odczynniki organiczne niż peptydohydrolaza wycyliny.

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