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CHARACTERISATION OF THE ENDOSPERMAL TRYPSIN INHIBITOR OF BARLEY

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Abstract—A trypsin inhibitor was isolated from grains of two row barley (cv. Proctor). The purified protein was identical with the corresponding inhibitor of a six row barley (cv. Pirkka); both proteins showed a *Pi* of 7.4. The *N*-terminal amino acid was phenylalanine and an arginine residue was involved in the active site. Effects of substrate concentration showed that the inhibition was noncompetitive with a K_i of about 0.9×10^{-7}M. An enzyme-inhibitor complex was demonstrated by disc electrophoresis.

INTRODUCTION

A trypsin inhibitor was characterized earlier from whole grains of Pirkka barley, a six row cultivar [1]. This inhibitor is localized in the endosperm of the grain whereas another trypsin inhibitor is present in the embryo [2]. A corresponding inhibitor has now been characterized from a two row barley. This is very similar and probably identical with the endospermal inhibitor from Pirkka barley. The results are reported in this paper.

RESULTS AND DISCUSSION

The trypsin inhibitor was purified from Proctor barley by the method described for the inhibitor from Pirkka barley [1]. The preparation showed a single protein zone in disc electrophoresis.

A single zone (*Pi* 7.4) was also obtained by gel electrophoretic focusing. The same *Pi* was obtained for a sample of Pirkka trypsin inhibitor. The amino acid composition of the Proctor inhibitor was within experimental error the same as that reported for the Pirkka inhibitor (1) and the two inhibitors are most probably identical. The *N*-terminal amino acid determined with the Dansyl method [3] was phenylalanine. No traces of other Dansyl amino acids were detected.

The nature of the active site was studied by modifying either the lysine residues or the arginine residues, using the methods described by Fritz *et al.* [4]. No decrease in activity was shown with up to 4 hr of incubation with the lysine reagent (103% of the control). However, activity decreased after incubation with the arginine reagent (13% of the control after 3 hr, and 6% of the control after 4 hr) and the basic amino acid residue at the active inhibitor site appears to be arginine. This amino acid residue is also involved in the active site of the embryonal trypsin inhibitors of wheat and rye [4].

The nature of the inhibition was studied with L-BAPA as substrate (substrate concentrations from 0.13×10^{-3}M to 0.8×10^{-3}M, inhibitor concentrations: 0, 0.21×10^{-7}M and 0.84×10^{-7}M, trypsin concentration: 1.67×10^{-7}M, pH 8.2, temp. 25°). The inhibition is noncompetitive with a K_i of about 0.9×10^{-7}M ($V_{max} = 1.14 \times 10^{-7}$ sec^{-1}, K_m of L-BAPA = 0.95×10^{-3}M). Naturally occurring trypsin inhibitors are generally thought to be competitive inhibitors, and this is supported by the observation that the barley inhibitor loses activity after modification of the arginine residues. However, the results obtained may be due to a very small dissociation constant of the enzyme-inhibitor complex [5].

The possible presence of a stable enzyme-inhibitor complex was studied with disc electrophoresis. When samples containing both trypsin and inhibitor were run at pH 8 a new intermediate zone appeared on the gels. This zone is probably due to the complex between trypsin and the barley inhibitor.

EXPERIMENTAL

Plant material. Samples of barley (*Hordeum vulgare* L. cv. Proctor) were obtained from National Seed Development Organisation LTD Newton Hall, Newton, Cambridge.

Reagents. Trypsin was obtained from the Sigma Chemical Company and L-BAPA from E. Merck AG. A sample of trypsin inhibitor prepared from Pirkka barley was provided by Dr. J. Mikola, Department of Biology, University of Jyväskylä, Finland. The 2,3-butanedione reagent was prepared as described in ref. [6].

Trypsin inhibitor assay was made as described in ref. [1] with one modification: the inhibitor was not preincubated with the enzyme but added to the substrate soln. In the kinetic experiments L-BAPA was used as substrate instead of DL-BAPA.

Disc electrophoresis was carried out using the method described in ref. [7] (gel system 6).

Gel isoelectric focusing was performed as described in ref. [8].

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