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COMPARISON OF TRYPSIN INHIBITORS FROM SEVERAL TYPES OF CORN

RICK JOHNSON,* HOWARD L. MITCHELL,* CLYDE E. WASSOM† and GERALD R. REECK*‡
Departments of Biochemistry* and Agronomy;† Kansas State University, Manhattan, Kansas 66506, U.S.A.

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Abstract—Trypsin inhibitors were isolated from seeds of floury-2 corn, dent corn, and popcorn and found to be similar in physicochemical and immunological properties to the inhibitor that was previously isolated from seeds of opaque-2 corn. Thus, very similar and perhaps identical trypsin inhibitors occur in genetically diverse types of corn. Our results therefore suggest that the differences between the opaque-3 inhibitor and the amino acid sequence reported by Hochstrasser *et al.* for a trypsin inhibitor from an unspecified type of corn, do not stem from variations in source material.

INTRODUCTION

We have previously reported the isolation and characterization of a trypsin inhibitor from seeds of opaque-2 corn [1]. The physicochemical properties we determined for the opaque-2 inhibitor are rather different from the properties Hochstrasser *et al.* [2, 3] reported for a trypsin inhibitor they isolated from an unspecified type of corn (maize). Different source material is one possible explanation for the reported differences in the properties of the two trypsin inhibitor preparations. In fact, opaque-2 corn seeds are known to contain more trypsin inhibitor activity than seeds of other types of corn [4]; so, *a priori*, a qualitative difference in types of inhibitor in opaque-2 corn and in the corn used by Hochstrasser *et al.* is not out of the question. But the results we describe here indicate that the trypsin inhibitors from genetically diverse types of corn are very similar, and perhaps identical, to the inhibitor from opaque-2 corn.

RESULTS AND DISCUSSION

Activities extracted per gram of defatted, milled seeds of dent corn, popcorn, and floury-2 were 43, 35, and 44% respectively, of the amount extracted from opaque-2 corn seeds. This agrees with earlier observation of increased levels of trypsin inhibitor activity in opaque-2 corn seeds [4].

Polyacrylamide gel electrophoresis of inhibitors isolated from the four types of corn by chromatography on trypsin–agarose showed that the four inhibitor preparations are indistinguishable. We know from our earlier work on the opaque-2 inhibitor [1] that the slower major band represents a single-chain inhibitor and that the less intense band of somewhat higher mobility is a two-chain form that apparently results from exposing the inhibitor to trypsin–agarose.

We have examined immunological cross-reactivities among the inhibitor preparations by Ouchterlony double immunodiffusion in which the four inhibitor preparations were allowed to react with antibodies elicited in rabbits against the opaque-2 inhibitor. The test did not reveal any unique antigenic determinant sites on the various inhibitors. That is, by this assay, the four inhibitors were indistinguishable.

In Table 1, we present amino acid compositions of the peptide inhibitors from dent corn and popcorn along with our previously determined composition for the opaque-2 inhibitor [1] and a composition calculated from the amino acid sequence reported by Hochstrasser *et al.* [3] for the trypsin inhibitor from seeds of an unspecified type of corn. The compositions of the inhibitors from dent corn and popcorn clearly resemble the composition of the opaque-2 inhibitor more closely than the composition calculated from the sequence proposed by Hochstrasser *et al.* [3].

A striking difference between the properties we reported for the opaque-2 inhibitor and the amino acid sequence reported by Hochstrasser *et al.* [3] lies in the tryptophan content. We reported that the opaque-2 inhibitor contains several tryptophan residues (probably 4), but the sequence proposed by Hochstrasser *et al.* [3] is totally devoid of tryptophan. The distinctive and strong absorption spectrum of tryptophan in the near ultra-violet makes it quite easy to establish the presence of this amino acid residue [5]. The near UV absorption spectrum of the trypsin inhibitor from opaque-2 corn in 6 M guanidine hydrochloride–20 mM sodium phosphate at pH 6.7 is dominated by the indole side chain of tryptophan, which has a maximum absorption at 280 nm and a distinctive shoulder at 289 nm [5]. A protein having the sequence proposed by Hochstrasser *et al.* [3] would have its near UV spectrum dominated by tyrosine, with a maximum at 275 nm and no shoulder at 290 nm [5]. The spectra of the inhibitor isolated from the three other sources all displayed a maximum at 280 nm and a shoulder at 290 nm. On this basis, we infer that the inhibitors from dent corn, popcorn, and floury-2 seeds all contain tryptophan.

The results reported here indicate that the inhibitors from floury-2, dent corn, and from popcorn are very similar to the inhibitor from opaque-2 corn in physicochemical and immunological properties. The inhibitors from the four genetically diverse sources may well be identical. In

‡ To whom correspondence should be addressed.

Table 1. Amino acid compositions of corn trypsin inhibitors*

	Dent corn (K41 × K55)	Popcorn†	Opaque-2 corn‡	Unspecified type of corn§
Lys	1.8	2.1	1.1	1.7
His	1.1	1.0	1.0	1.7
Arg	9.8	9.0	8.8	13.6
Asp	6.8	7.0	5.7	5.1
Thr	6.5	6.4	6.7	5.1
Ser	5.0	5.3	5.3	5.6
Glu	10	10	9.8	8.5
Pro	13.8	14	12.7	16.9
Gly	11	11	12	11.9
Ala	9.3	9.1	11.0	6.8
Val	5.9	6.2	4.8	3.4
Met	1.2	0.6	1.2	1.7
Ile	5.5	5.8	6.2	6.9
Leu	9.1	9.3	11	10
Tyr	1.6	1.8	1.6	1.7
Phe	1.8	1.2	1.0	0

* All compositions are given in mol percent, excluding tryptophan and half-cystine.
† Single analysis after 24-hr hydrolysis. No corrections for hydrolytic losses.
‡ Taken from Swartz *et al.* [1].
§ Calculated from the sequence proposed by Hochstrasser *et al.* [3].

any event, they are clearly much more similar to each other than to the protein represented by the amino acid sequence reported by Hochstrasser *et al.* [3]. This suggests to us that the differences between the opaque-2 inhibitor and the sequence reported by Hochstrasser *et al.* [3] do not stem from differences in source material.

EXPERIMENTAL

Trypsin inhibitor activity was assayed as described previously [1] with bovine trypsin (Sigma, Type III). Trypsin inhibitors were purified from seeds of four sources of corn: opaque-2 and floury-2 (both soft endosperm); popcorn (corneous endosperm); and dent corn, represented by K41 × K55, a single-cross hybrid between 2 inbred lines of hard endosperm corn. All of the corn was grown in the agronomy fields at Kansas State University.

Inhibitors were isolated from 5 g of floury-2 seeds and 10 g of each of the other types of corn seeds. Dry seeds were ground in a burr mill, defatted with acetone, and extracted with 0.15 M NaCl as described previously [1]. Trypsin inhibitor in the salt extract was purified by affinity chromatography on trypsin–agarose. As described previously for opaque-2 corn, this allows a single-step purification of inhibitor [1]. From such a procedure, one obtains a mixture of single-chain inhibitor and a two-chain inhibitor that results from the action of the immobilized trypsin [1].

Amino acid analyses were performed on a Beckman Model 120C amino acid analyzer after hydrolysis of purified inhibitors for 24 hr in 6 N HCl at 100°. Disc gel electrophoresis was carried out as described by Davis in gels polymerized from 7.5% acrylamide [6].

Antibodies were elicited in rabbits against trypsin inhibitor that had been isolated on a large scale from opaque-2 corn seeds [1]. Inhibitor was eluted from trypsin–agarose with 1 M acetic acid, dialyzed against 10 mM 2-amino-2-methyl propane-diol (pH 9.0), and applied to a DEAE-cellulose column (DE-32, Whatman) that had been equilibrated with the same buffer. Inhibitor was eluted in concentrated form by application of 0.2 M NaCl/10 mM 2-amino-2-methylpropanediol (pH 9.0). The concentrated inhibitor (5 mg/ml) was emulsified with an equal volume of Freund's complete adjuvant, and 0.25 ml portions of the emulsion were injected subcutaneously in each of four sites on the backs of 3 rabbits. 3 weeks later, each rabbit was injected similarly with trypsin inhibitor emulsified with Freund's incomplete adjuvant. Starting 2 weeks later, the rabbits were bled from the ear, and antiserum was obtained by centrifugation after blood had been allowed to clot.

Double immunodiffusion was carried out on plates covered with 2% agarose (Ionagar No. 2; Colab, Inc.) in 0.025 M NaCl/0.025 M sodium borate (pH 8.0). After immune precipitates formed, the plates were soaked for 36 hr in the saline/borate buffer, dried, and stained with 0.5% Light Green SF Yellowish in 70% MeOH /10% HOAc.

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