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SHORT REPORTS

N-TERMINAL AMINO ACID SEQUENCE OF TRYPSIN INHIBITOR 3 FROM WINGED BEAN

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Key Word Index—*Psophocarpus tetragonolobus*; Leguminosae; winged bean; trypsin inhibitor homology; *N*-terminal amino acid sequence; Kunitz-type inhibitors; winged bean inhibitors.

Abstract—The *N*-terminal amino acid sequence of winged bean trypsin inhibitor 3 showed extensive homology with the soybean trypsin inhibitor (Kunitz) and other legume Kunitz-type inhibitors. The other inhibitors of winged bean, winged bean trypsin inhibitor 2 and the specific winged bean chymotrypsin inhibitor, have blocked amino terminals and do not appear to be homologous with the other Kunitz-type inhibitors discussed.

INTRODUCTION

Winged bean [*Psophocarpus tetragonolobus* (L.) DC.] seed contains two major trypsin inhibitors [1] and a specific chymotrypsin inhibitor [2]. These inhibitors have MWs of *ca* 20000 and a low cystine content (*ca* 2%) and on this basis may be grouped with the well-known Kunitz soybean trypsin inhibitor. Recently the trypsin inhibitors from *Albizia* [3] and *Acacia* [4] seed were characterized and found to resemble the Kunitz soybean trypsin inhibitor in size and cystine content. Even though *Albizia* and *Acacia*, subfamily Mimosoideae, are phylogenetically distant from soybean, their trypsin inhibitors were found to be homologues of the Kunitz soybean trypsin inhibitor. To establish the relationship of the winged bean inhibitors to other legume Kunitz-type inhibitors, amino terminal sequence analyses were performed and the results are reported here.

RESULTS AND DISCUSSION

Winged bean trypsin inhibitor 3 showed a single *N*-terminal glutamic acid in excellent yield. The sequence of the first 18 residues of this inhibitor is shown in Table 1 where it is compared with the reported *N*-terminal sequences of soybean [5], acacia [4] and silk tree [3] trypsin inhibitors. Winged bean trypsin inhibitor 2 and the winged bean chymotrypsin inhibitor failed to yield amino terminal sequences indicating that the *N*-terminal residues, are blocked. Cleavage of trypsin inhibitor 2 with cyanogen bromide (3 methionine residues) resulted in the release of three amino terminal residues (Ser, Val, Tyr) in good yield consistent with the parent molecule being blocked.

It is clear that winged bean trypsin inhibitor 3 is homologous to the Kunitz soybean inhibitor and the other related inhibitors (Table 1) with over half the residues the same and four conservative replacements. Recently, proteinase inhibitors from *Erythrina latissima* [6] and *Peltophorum africanum* [7], of the subfamily Caesalpinioideae, have been shown to resemble the Kunitz-type inhibitors. They also show a high degree of homology with the soybean trypsin inhibitor [7]. These findings demonstrate that the gene for the Kunitz-type of inhibitor occurs in more legume species than was previously recognized and that the various legume inhibitors probably evolved from a common ancestral gene. Furthermore, the similarity of the *N*-terminal sequence of the rice plant subtilisin inhibitor [8] to that of the legume inhibitors suggests an even wider distribution of Kunitz inhibitors among plant families. It is of interest that the partial *N*-terminal sequences of the acacia and silk tree trypsin inhibitors are identical and that some residues, which are not identical in winged bean and soybean trypsin inhibitors, show identity with residues in the Mimosoideae inhibitors (e.g. compare residues 3 and 11). This suggests that the trypsin inhibitors of winged bean and soybean may have evolved from or after the Mimosoideae inhibitors. These results may have some significance from an evolutionary point of view as the Mimosoideae is often regarded as the most primitive subfamily within the legumes [9].

On the other hand the absence of a free amino terminus in winged bean trypsin inhibitor 2 and the winged bean chymotrypsin inhibitor, and the earlier findings that tryptic peptide maps failed to show any similarity between these inhibitors and the Kunitz soybean trypsin inhibitor [2], suggests that they are not related structurally and may have evolved separately from a different ancestral gene(s), even though they share similar physicochemical properties with the homologous Kunitz-type inhibitors. Thus there may be several families of plant proteinase inhibitors characterized by molecular weights of *ca* 20000 and low cystine content.

EXPERIMENTAL

The inhibitors (trypsin inhibitors 2 and 3, and the chymotrypsin inhibitor) were isolated from winged bean seed (variety Tpt-1)

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Table 1. *N*-Terminal sequence of winged bean trypsin inhibitor 3 compared with soybean, acacia (*Acacia elata*) and silk tree (*Albizia julibrissin*) trypsin inhibitors

Winged bean	Glu – Pro	Leu – Leu – Asp	Ser	Glu	Gly	Glu – Leu – Val – Arg	Asn – Gly – Gly – Thr – Tyr – Tyr
Soybean	Asp – Phe – Val	Leu – Asp	Asn	Glu	Gly	Asn – Pro – Leu – Glu	Asn – Gly – Gly – Thr – Tyr – Tyr
Acacia	Lys – Glu	Leu – Leu – Asp	Ala	Asp	Gly	Asp – Ile – Leu –	
Silk tree	Lys – Glu	Leu – Leu – Asp	Ala	Asp	Gly	Asp – Ile – Leu – Leu	Asn – Gly – Gly – * – Tyr – Tyr

Identical residues are in the boxes.
*Residue not determined.

and purified as described [1, 2]. The inhibitors were reduced with dithioerythritol (0.1 M) and alkylated with iodoacetic acid [10]. The alkylated inhibitors were desalted on Sephadex G-25 (100 × 1 cm) in 0.1 M ammonium bicarbonate buffer, pH 8, and recovered by freeze-drying.

The alkylated inhibitors (0.1 μmol) were subject to sequence analysis in a protein sequenator as described elsewhere [11]. The thiazolinone derivatives were converted to phenylthiohydantoin derivatives and were identified by TLC on Si gel plates [12] and by HPLC [13].

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REFERENCES

1. Kortt, A. A. (1979) *Biochim. Biophys. Acta* **577**, 371.
2. Kortt, A. A. (1980) *Biochim. Biophys. Acta* **624**, 237.
3. Odani, S., Odani, S., Ono, T. and Ikenaka, T. (1979) *J. Biochem.* **86**, 1795.
4. Kortt, A. A. and Jermyn, M. A. (1981) *Eur. J. Biochem.* **115**, 551.
5. Koide, T. and Ikenaka, T. (1973) *Eur. J. Biochem.* **32**, 417.
6. Joubert, F. J., Carlsson, F. C. and Haylett, T. (1981) *Hoppe-Seyler's Z. Physiol. Chem.* **362**, 531.
7. Joubert, F. J. (1981) *Hoppe-Seyler's Z. Physiol. Chem.* **362**, 1515.
8. Kato, I., Tominaga, N. and Kihara, F. (1972) in *Proceedings of the 23rd Conference on Protein Structure* (Iwai, K., ed.) p. 53.
9. Heywood, V. H. (1971) in *Chemotaxonomy of the Leguminosae* (Harborne, J. B., Boulter, D. and Turner, B. L., eds.) p.1. Academic Press, London.
10. Crestfield, A. M., Moore, S. and Stein, W. H. (1963) *J. Biol. Chem.* **238**, 622.
11. Inglis, A. S., Strike, P. M., Osborne, W. C. and Burley, R. W. (1979) *FEBS Letters* **97**, 179.
12. Inglis, A. S. and Nicholls, P. W. (1973) *J. Chromatogr.* **79**, 344.
13. Dugaiczyk, A., Inglis, A. S., Strike, P. M., Burley, R. W., Beattie, W. G. and Chan, L. (1981) *Gene* **14**, 175.

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GLUCOSIDASE AND TREHALASE INHIBITION BY 1,5-DIDEOXY-1,5-IMINO-D-MANNITOL, A CYCLIC AMINO ALDITOL FROM *LONCHOCARPUS SERICEUS*

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Key Word Index—*Lonchocarpus sericeus*; Leguminosae; glucosidase; trehalase; 1,5-dideoxy-1,5-imino-D-mannitol.

Abstract—1,5-Dideoxy-1,5-imino-D-mannitol, a cyclic amino alditol isolated from *Lonchocarpus sericeus* has been found to be a potent inhibitor of certain α- and β-glucosidases and insect-derived trehalase. In structure and biological activity it resembles nojirimycin (5-amino-5-deoxy-D-glucopyranose) and deoxynojirimycin (1,5-dideoxy-1,5-imino-D-glucitol), two glucosidase inhibitors previously isolated from bacteria.

INTRODUCTION

Nojirimycin, (5-amino-5-deoxy-D-glucopyranose, **1**) an antibiotic isolated from species of *Streptomyces* was the first naturally occurring 5-amino sugar to be discovered. This compound differs structurally from glucose only in the replacement of oxygen in the ring by nitrogen, and is a