CHYMOTRYPTIC INHIBITOR I FROM POTATOES: THE AMINO ACID SEQUENCES OF SUBUNITS B, C AND D

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Received 11 May 1974

1. Introduction

Chymotryptic Inhibitor I from potato tubers is a tetrameric protein composed of a heterogeneous mixture of protomers (subunits). Melville and Ryan [1] have shown that the protomers could be separated into four main types (A, B, C and D) by ion-exchange chromatography on sulphoethyl cellulose in 8 M urea. The amino acid compositions of A, B, C and D thus obtained, were significantly different and also indicated that the protomers as isolated might be groups of variants that eluted together as a result of similarities in their overall charge.

Investigation in this laboratory has shown that the subunit A isolated by this method gives a single band when examined by polyacrylamide gel disc electrophoresis and a unique amino acid sequence exhibiting only a very low level of micro-heterogeneity [2]. On the other hand, the subunits B, C and D did not give single bands on electrophoresis, even after their peaks were separately re-chromatographed on SE-cellulose or SP-Sephadex in 8 M urea (M. Richardson, unpublished results).

This paper reports the amino acid sequences of the variants isolated as subunits B, C and D; and the results of preliminary experiments which indicate that the reactive site (anti-chymotrypsin) of this inhibitor is the Met^{60}–Asp^{61} sequence.

2. Materials and methods

Inhibitor I was purified from potato tubers (*Solanum tuberosum* L. var. 'Ulster Prince'), and the subunits purified as described previously [1,2].

Reduced and *S*-carboxymethylated samples of the subunits were cleaved with cyanogen bromide and *N*-bromosuccinimide, and by dilute acid hydrolysis as described previously [2]. In addition, some samples were also cleaved by treatment with 2 *N*-hydroxylamine [3]. Peptides were obtained from the intact subunits and fragments by digestion with trypsin, thermolysin, pepsin and papain, as described previously [2]. Certain fragments and peptides were also further digested with chymotrypsin (Worthington Biochemical Corp; enzyme/substrate ratio 1:50, in 0.1 M ammonium carbonate pH 8.0, at 37°C for 2 hr).

Mixtures of fragments and peptides were fractionated by gel-filtration on Sephadex G-50 and G-25, by high-voltage paper electrophoresis at pH 6.5, 4.7, and 1.9, and by paper chromatography using butan-1-ol–acetic acid–water–pyridine (15:3:12:10, by volume) and isoamyl alcohol–pyridine–water (70:70:60, by volume) as the solvents [2].

The amino acid sequences of peptides were determined by the dansyl–Edman procedure and by digestion with carboxypeptidase A, as described previously [2]. Amide positions were assigned where possible by using the electrophoretic mobilities of the peptides at pH 6.5, by the release of certain residues with carboxypeptidase A and by direct dansylation without subsequent acid hydrolysis at suitable stages of the Edman degradation.

Amino acid analyses of the subunits, fragments and peptides were obtained using a Locarte amino acid analyzer.

Preliminary attempts were made to determine the identity of the reactive (anti-chymotrypsin) site by

incubation of Inhibitor I and the purified subunits with catalytic amounts of chymotrypsin at pH 3.0 for 24 hr, as described by Kiyohara et al. [4].

3. Results and discussion

The complete amino acid sequences of the variants isolated as subunits B, C and D are shown in fig. 1, with the previously reported [2] sequence of subunit A for comparison. Subunit B is similar to A in having Glu as its N-terminus, but subunits C and D both have an additional Lys residue at their N-termini. In contrast, previous workers who have investigated this protein from other varieties of potato have always found only Glu as the N-terminal residue [1,4,5]. A similar situation has been reported previously for the N-terminus of bovine pancreatic RNase which normally has the N-terminal sequence Lys–Glu–[6], but has also been isolated in forms in which the terminal LYS is missing [7].

It is clear from the amino acid sequences that there is a remarkable homology exhibited between the variants which constitute the tetrameric Inhibitor I. Some 72 out of the 84–85 residues are completely invariant amongst the subunits. Major substitutions of amino acids occur only at positions 5, 23, 33, 36, 39, 54, 55, 62 and 76. Other differences also occur at positions 51, 65 and 72, but as these involve Asp/Asn alterations it is not possible to ascertain whether these are true substitutions or merely reflect varying degrees of deamidation amongst the subunits. Minor examples

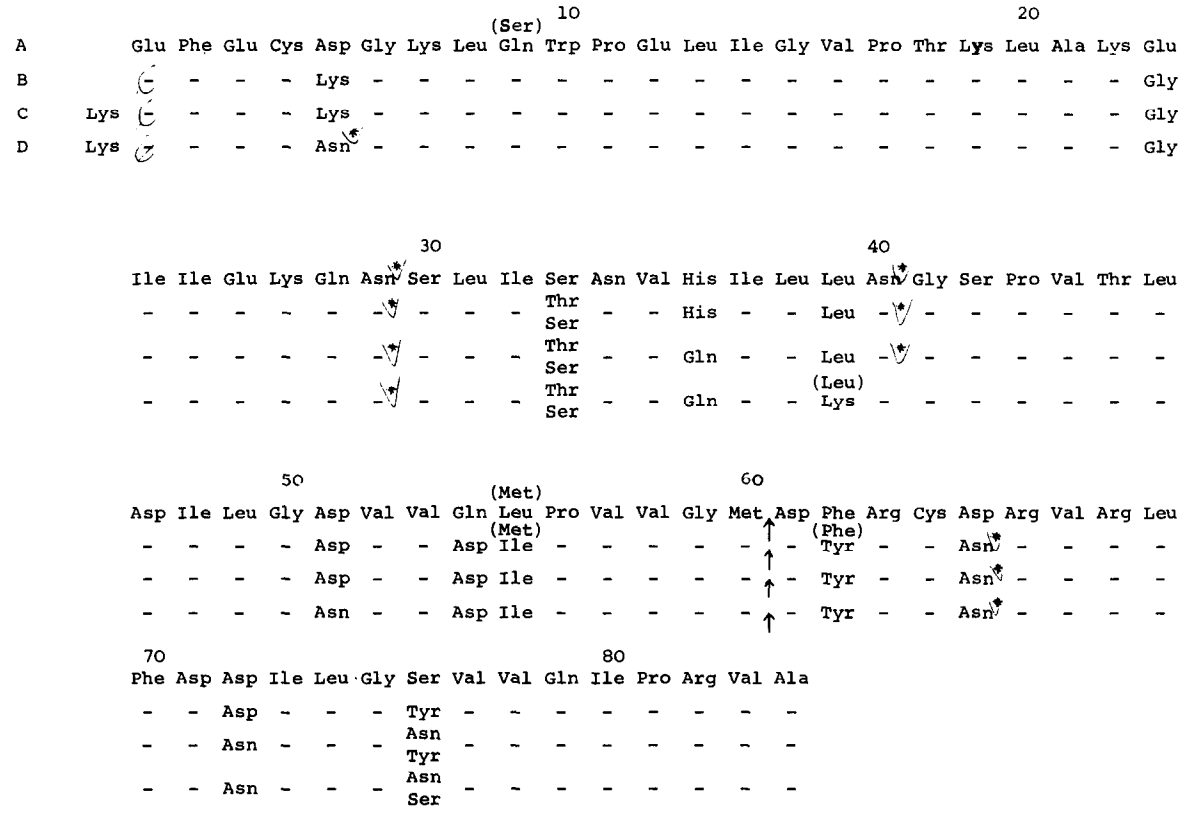

Fig. 1. The amino acid sequences of the variants isolated as the subunits B, C and D of potato Chymotryptic Inhibitor I. The sequence of subunit A [2] is also shown for comparison. Residues shown by a dash (–) are the same as in subunit A; residues in brackets are mino micro-heterogeneous (< 10%) forms observed in some preparations; residues marked with an * indicate amides for which there was evidence of deamidation, ↑ indicates site of partial hydrolysis by catalytic amounts of chymotrypsin at pH 3.0.

of micro-heterogeneity were also found at position 9 in all four subunits, and at position 55 in subunits A and B.

In the previously reported primary structure of subunit A there was a striking repetition or similarity of the sequence between residues 47 and 58 with that between residues 72 and 84 [2]. This occurrence of homologous repetitive regions which has also been reported in the sequences of other plant proteinase inhibitors [8,9], was confirmed in the subunits B, C and D.

Ozawa and Laskowski [10] first showed that various trypsin inhibitors could be partially cleaved at their reactive sites by incubation with catalytic amounts of trypsin at acid pH. Other workers have also shown that limited hydrolysis of the reactive peptide bonds Leu–Asx [4] and Leu–Ser [11] could be achieved in chymotryptic inhibitors by incubation with chymotrypsin under similar conditions. In the preliminary experiments reported here we were to show that very limited (< 15%) hydrolysis of potato Chymotrypsin Inhibitor I and its purified subunits occurred only at the Met ^{60}–Asp^{61} bond, and that a 24 residue fragment corresponding to residues 61–84, was released during prolonged incubation with chymotrypsin at pH 3.0.

Acknowledgements

We gratefully acknowledge financial support from the Science Research Council and the Smith, Kline and French Foundation. Our thanks are also due to Professor D. Boulter for the provision of certain facilities.

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