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Glycine-Rich Analogues of *Cucurbita maxima* Trypsin Inhibitor (CMTI-III) Substituted by Valine in Position 27 Display Relatively Low Antitrypsin Activity

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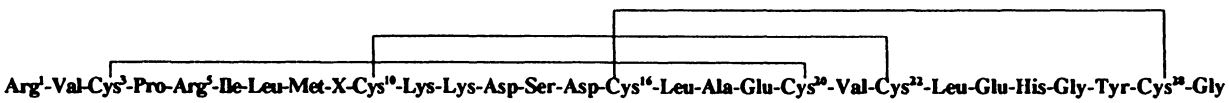
Summary: Five new analogues of the trypsin inhibitor CMTI-III were synthesized by the solid-phase method. All analogues containing a valine residue in position 27 and glycine residues in some or all of the positions 9, 11, 14, 17, 19, 29 as well as in two cases a norleucine residue in position 8 displayed association equilibrium constants by 6–7 orders of magnitude lower than the native CMTI-III inhibitor.

Key terms: Trypsin inhibitor, solid-phase peptide synthesis.

In the seeds of plants from the Cucurbitaceae family, a number of polypeptide serine proteinase inhibitors occur, consisting of 28–32 amino acid residues, six of which are cysteines forming 3 disulfide bridges[1]. The first inhibitors belonging to this group, CMTI-I and CMTI-III (Cucurbita maxima trypsin inhibitor), were isolated in 1980[2] from squash seeds. The amino acid sequences of these were published in 1983[3] and the three-dimensional structure of CMTI-I in 1989[4–6]. Both inhibitors differ only in one position, Lys^9 in CMTI-III being replaced by Glu^9 in CMTI-I.



CMTI-I: X = Glu; CMTI-III: X = Lys

Using the procedure of solid-phase peptide synthesis we obtained synthetic counterparts of the two native trypsin inhibitors CMTI-III and CMTI-I[7–9]. We also demonstrated that the substitution of certain amino acid residues at the reactive center of CMTI-III (Arg^5-Ile^6) resulted in the change in the specificity of the inhibitor. In this way, the introduction of the valine residue instead of arginine in position 5 led to an elastase inhibitor[10] and the introduction of phenylalanine in this position led to a chymotrypsin inhibitor[11]. In addition, we have shown that replacing some of the amino acid residues of CMTI-III in the region far from the reactive center did not affect the specificity but affected the efficiency of formation of disulfide bridges (proper folding of the polypeptide chain)[11]. Some simplifications of the CMTI-III mol-

Enzyme:
Bovine β-trypsin (EC 3.4.21.4).

Abbreviations:
CMTI, *Cucurbita maxima* trypsin inhibitor; Acm, acetamidomethyl; Bz, benzoyl; E, residual enzyme concentration; HPLC, high performance liquid chromatography; I_0, initial inhibitor concentration; K_a, association equilibrium constant; RP, reversed phase; RT, retention time.