Zeamatin Inhibits Trypsin and \( \alpha \)-Amylase Activities  

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Zeamatin is a 22-kDa protein isolated from *Zea mays* that has antifungal activity against human and plant pathogens. Unlike other pathogenesis-related group 5 proteins, zeamatin inhibits insect \( \alpha \)-amylase and mammalian trypsin activities. It is of clinical significance that zeamatin did not inhibit human \( \alpha \)-amylase activity and inhibited mammalian trypsin activity only at high molar concentrations.

Zeamatin is a 22-kDa protein isolated from corn (*Zea mays*) seeds and meal (7, 10, 11) that has significant amino acid homology to thaumatin and to thaumatin-like proteins, including pathogenesis-related group 5 (PR-5) proteins (10). Zeamatin has potent antifungal activity in vitro against a number of human and plant pathogens (7, 8, 10, 11). Recently, zeamatin, in synergy with nikkomin Z and fluconazole, was shown to be effective in vivo in both a systemic murine candidosis model (8) and a vaginal murine candidosis model (8; D. Stevens et al., submitted for publication). Zeamatin has the potential to be used not only as a human therapeutic agent but also in transgenic plants to increase in planta resistance to pathogens. To use zeamatin as a therapeutic agent, it is important to understand its properties, including potential inhibition of mammalian enzymes.

Zeamatin binds \( \beta \)-1,3-glucans (9) and has antifungal activity because it can permeabilize fungal cells, leading to cell death (7). Zeamatin is identical to a previously isolated bifunctional \( \alpha \)-amylase and trypsin activity inhibitor (6). However, thaumatin and several other thaumatin-like proteins with significant homology to zeamatin, e.g., PR-R and PR-S, do not inhibit trypsin or \( \alpha \)-amylase activity (3, 5).

To determine if zeamatin is both an \( \alpha \)-amylase and trypsin inhibitor and an antifungal protein, we tested highly purified zeamatin for inhibition of \( \alpha \)-amylase and trypsin activities. We extracted zeamatin from corn (*Zea mays*) meal and purified it by two reversed-phase chromatography steps to apparent homogeneity (12). *Tribolium castaneum* larvae were provided by Sue Haas (Kansas State University, Manhattan); *Bacillus* species, human saliva, porcine pancreas, and barley malt \( \alpha \)-amylases were obtained from Sigma-Aldrich (St. Louis, Mo.). *Tribolium* \( \alpha \)-amylase was prepared by lysing larvae in ice-cold buffer (20 mM NaH_2PO_4, pH 6.0, containing 6 mM NaCl) using a Dounce homogenizer; the supernatant from a 1,000 g, 10 min, 4°C centrifugation was used as a source of \( \alpha \)-amylase activity. The amount of \( \alpha \)-amylase protein in the lysate was determined by densitometry of Coomassie-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels (data not shown). Zeamatin, an \( \alpha \)-amylase inhibitor from a biological source (A-1520; Sigma-Aldrich) or buffer (20 mM NaH_2PO_4, pH 6.0, containing 6 mM NaCl) was incubated with the various \( \alpha \)-amylases at 25°C for 20 min, and \( \alpha \)-amylase activity was assayed by the method of Bernfeld (2). Consistent with the results of Blanco-Labra and Iturbe-Chiñas (4), we found that zeamatin strongly inhibited (95%) \( \alpha \)-amylase activity from *T. castaneum*, slightly inhibited (30%) the activity of *Bacillus* \( \alpha \)-amylase, but did not inhibit the \( \alpha \)-amylase activity from human saliva, porcine pancreas, or barley malt (Table 1). Thaumatin at a 120:1 molar ratio to *Tribolium* \( \alpha \)-amylase and a 1,800:1 molar ratio to porcine pancreas \( \alpha \)-amylase did not inhibit \( \alpha \)-amylase activity (data not shown). Since zeamatin did not inhibit the activity of any of the commercially available \( \alpha \)-amylases except *Bacillus* sp. \( \alpha \)-amylase, and that only slightly and at a high molar ratio, it is not surprising that researchers have not seen \( \alpha \)-amylase inhibition by zeamatin.

Purified zeamatin also was incubated with trypsin, and trypsin activity was assayed using a spectrophotometric assay (1) which measures trypsin digestion of \( N \)-\( \alpha \)-benzoyl-L-arginine ethyl ester (BAEE). In a total volume of 200 \( \mu \)l, porcine pancreas trypsin (0.15 nmol in 1 mM HCl) was mixed with either buffer (67 mM NaH_2PO_4, pH 7.6), a commercially available trypsin inhibitor (Sigma T9003) (0.15 or 1.5 nmol), zeamatin (0.15, 1.5, 4.5, or 15 nmol), thau-matin (15 nmol) (Sigma-Aldrich), bovine serum albumin (15 nmol), or lysozyme (Sigma L-6876) (15 nmol). To these mixtures, 1 ml of BAEE (0.25 mM BAEE in buffer; Aldrich Chemical Co, Milwaukee, Wis.) was added and the A_{233} was measured over time against a blank containing the identical components except 1 mM HCl in place of trypsin. Zeamatin at a 100:1 molar ratio to trypsin inhibited trypsin activity by 62% \( \pm \) 4% (average \( \pm \) standard deviation) and slightly inhibited trypsin activity (29% \( \pm \) 9%) at a 30:1 mole ratio. Zeamatin had no effect on trypsin activity at a 10:1 or 1:1 molar ratio (1% \( \pm \) 9% and 7% \( \pm \) 9% inhibition, respectively). In contrast, a 100:1 molar ratio of thaumatin, bovine serum albumin, or lysozyme to trypsin did not inhibit trypsin activity (4% \( \pm \) 7%, 3% \( \pm \) 1%, and 11% \( \pm \) 8% inhibition, respectively). In comparison, the trypsin inhibitor from Sigma inhibited trypsin activity at both a 1:10 and a 1:1 molar ratio...
(93% ± 7% and 44% ± 5%, respectively). Again, not surprisingly, inhibition of trypsin activity by zeamatin has not been observed by other researchers since high molar ratios of zeamatin to trypsin are required. Importantly, inhibition of insect α-amylase and mammalian trypsin appears to be unique to zeamatin and not shared by thaumatin and other PR-5 proteins.

In conclusion, in addition to its antifungal activity, zeamatin can inhibit the activities of Tribolium α-amylase and porcine pancreas trypsin, in agreement with the results originally reported by Richardson et al. (6) and Blanco-Labra and Iturbe-Chiñas (4). Since zeamatin did not inhibit fungal α-amylase (4) and fungi do not contain trypsin, zeamatin’s antifungal activity is not the result of inhibition of these enzymes. Zeamatin did not inhibit mammalian α-amylase and inhibited trypsin activity only at high molar ratios; this effect is not likely to lead to clinically relevant toxicity, even at high oral or intravaginal doses of zeamatin.

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REFERENCES


