UREASES ARE MULTIFUNCTIONAL ENZYMES: WHEN ENZYMATIC ACTIVITY DOES NOT EXPLAIN THE BIOLOGICAL EFFECTS.

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Ureases (EC 3.5.1.5) are enzymes that hydrolyze urea to ammonia and CO_2, present in plants, fungi and bacteria. Fungal and plant ureases are hexamers of 90 kDa subunits, while bacterial ureases are multimers of two or three subunits complexes. The high sequence similarity of all ureases indicates similar tertiary structures and catalytic mechanisms. Despite the abundance of ureases in plants, little is known about their physiological roles. We have shown that ureases are multifunctional proteins with biological properties unrelated to their enzymatic activity. Canatoxin (CNTX) is a neurotoxic protein isolated from jackbean *(Canavalia ensiformis*) seeds, lethal to mice and rats if injected, but inactive if given orally. CNTX, a dimer of 95 kDa chains, is an isoform of the major urease in the seed with about 40% of its ureolytic activity. CNTX and jackbean urease display several biological properties independent of their ureolytic effect, such as platelet aggregating activity and interaction with glycoconjugates. These properties are also shared by some bacterial ureases, and may be relevant in diseases such as gastric ulcers due to *Helicobacter pylori* infection. Plant ureases are lethal when ingested by some insects. The kissing bug *R. prolixus,* and crop pests such as the cowpea weevil *C. maculatus,* the green stinkbug *N. viridula* and the cotton stainer bug *D. peruvianus* die when fed on these proteins at 0.1 % w/w levels. These insects have cathepsins as digestive enzymes, which hydrolyze CNTX/urease to release an internal entomotoxic peptide of 10 kDa. No effect was seen in insects relaying on trypsin-like digestive enzymes, which apparently destroy the proteins. A recombinant peptide equivalent to the entomotoxic peptide released from CNTX in the insect midgut was produced in *E. coli*. Besides showing toxicity to *D. peruvianus*, the recombinant peptide was also lethal to the cockroach *B. germanica* and the fall armyworm *S. frugiperda*, which are not affected by intact ureases as they have trypsins as digestive enzymes. The recombinant peptide showed no toxicity after intraperitoneal injection in mice or given intragastrically to neonate rats. We conclude that ureases probably participate in plant defense against insect predation. The potential use of these proteins and derived peptides as bioinsecticides or a putative transgene to engineering insect resistance into plant is presently under consideration.

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