# Indigestion is a plant's best defense

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Over three decades ago, Green and Ryan (1) reported that insect herbivory induces the accumulation of proteinase inhibitors (PIs) in tomato leaves, a discovery that initiated the concept that plant defenses were dynamic processes. Their seminal finding also showed that a key mechanism of plant defense was targeted at limiting an herbivore's ability to digest and use essential nutrients from its host plant. More recently, microarray-based approaches have shown that scores of genes are up-regulated by herbivory and/or the wound signal jasmonic acid (JA); yet very few gene products definitively have been shown to play a role in antiherbivore defense. In this issue of PNAS, Chen *et al.* (2) elegantly demonstrate that two tomato plant enzymes mediate antinutritive defenses against an insect herbivore (*Manduca sexta*). In the broader context, this article demonstrates a new level of coordination and complementation of plant defenses heretofore not fully appreciated.

Chen *et al.* use a novel proteomics/mass spectrophotometry approach to identify plant proteins that are stable (or undigested) in the herbivore's digestive system (i.e., midgut). The midgut of many caterpillars, including the *Manduca sexta* used in this study, is a hostile environment for ingested proteins; it is highly alkaline (pH 8–11) and filled with a complex milieu of serine and metalloproteases. Surprisingly, the JA-inducible plant proteins are among the most abundant that accumulate in the insect's digestive system and include a suite of inhibitors of digestive proteases and several enzymes. Two of the enzymes are arginase (ARG) and threonine deaminase (TD), which are remarkably active at very high pH and catalytically degrade the essential amino acids arginine and threonine, respectively. The insects are unable to recover arginine or threonine from the products of these enzymes and thus suffer the consequences of nutrient deprivation. Chen *et al.* (2) then employ transgenic plants that overexpress ARG to provide more definitive evidence for the defensive role of ARG. Larvae feeding on the plants that overexpressed arginase grew more slowly than those feeding on the wild-type plants. Midgut levels of arginine were also lower in the larvae feeding on the overexpressing plants.

**Table 1. Inducible antinutritional proteins of the tomato plant**

| Plant protein | Putative mode of action | Stability in insect gut | Induced by herbivory or wounding |
|---|---|---|---|
| Arginase | Enzymatic removal of arginine | Yes | Yes |
| Cathepsin D | Inhibition of aspartyl proteases and overproduction of proteases. Likely targets are insects with acidic digestive systems (e.g., beetles). | Yes | Yes |
| Cysteine protease inhibitors | Inhibition of cysteine proteases and overproduction of proteases. Likely targets are insects with acidic digestive systems (e.g., beetles). | Yes | Yes |
| Lipoxygenase | Formation of lipid peroxides and breakdown products with strong electrophilic action. Potential loss of amino acids. | Unknown | Yes |
| Leucine aminopeptidase | Liberation of arginine from N terminus of peptides; possibly acts in tandem with ARG. | Yes | Yes |
| Peroxidase | Formation of quinones and subsequent reactions with nucleophilic side chains of amino acids. Protein cross-linking. | Possible | Yes |
| Phenylalanine ammonia lyase | Enzymatic removal of phenylalanine. | Unknown | Primarily pathogen-induced |
| Polyphenol oxidase | Formation of quinones and subsequent reactions with nucleophilic side chains of amino acids (e.g., lysine, histidine, cysteine). | Yes | Yes |
| Serine protease inhibitors I | Inhibition of serine protease; hyperaccumulation of inhibitor-insensitive proteases; amino acid deprivation. | Yes | Yes |
| Threonine deaminase | Enzymatic removal of threonine. | Yes, activated in gut | Yes |

Chen *et al.* (2) provide an intriguing linkage to the initial studies of Green and Ryan (1). In addition to ARG and TD, multiple plant serine, aspartyl, and cysteine protease inhibitors accumulate in the gut. Not only are the PIs, ARG, and TD all regulated by JA, but their biological activities against insect herbivores may be complementary or even synergistic (see Table 1). The mechanism of action of PIs in insect herbivores has been proposed to result from hyperaccumulation of PI-resistant proteases in the insect gut, thus placing an increased demand for essential amino acids necessary for the insect's protein synthesis (3, 4). A dietary deficiency or

the lack of bioavailability of two essential amino acids, lysine and arginine, was considered a significant factor in determining the toxicity of PIs to herbivores (5). Thus, ARG would reduce the availability of arginine required as part of the herbivore's compensatory response to PIs. Yet another JA-inducible protein, leucine aminopeptidase A found in the gut, may act in tandem with ARG. The enzyme liberates free arginine from the N terminus of peptides, thus provid-

ing further substrate for catabolism by ARG.

There is evidence that additional JA-regulated enzymes such as polyphenol oxidases (PPOs) (6) and lipoxygenases (LOXs) may further exacerbate the effect of nutrient deprivation. PPOs (and peroxidases) catalyze the oxidation of phenolics to form strongly electrophilic quinones that may irreversibly bind to the nucleophilic side chains of some amino acids (e.g., lysine, histidine, and thiol amino acids) (7). PPO is highly active and stable in the insect gut (2, 8) and may further contribute to the irreversible loss of essential nutrients. The role of LOX in wound signaling and the JA pathway is well established (9), but its direct role in producing defense metabolites is not widely appreciated. Many lipid peroxidation products (e.g., hydroperoxides and aldehydes) possess potent electrophilic activity and are formed by LOXs and/or reactive oxygen species (10, 11). These products can then react with the nucleophilic side chains of amino acids producing further loss of essential amino acids (11, 12). Ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) is generally the most predominant protein in plant foliage and is believed to provide the major source of amino acids to herbivores (13). In the case of this protein, it is down-regulated by JA (14), providing an additional argument that a major mechanism of plant defense strategy is the limitation of the availability of essential amino acids.

While Chen *et al.* (2) convincingly demonstrate the roles of ARG and TD in plant antiherbivore defense, their work provides compelling evidence supporting the notion that a major plant defense mechanism involves numerous proteins acting coordinately to starve herbivores of key nutrients. The nutritional quality of foliage is an important component of herbivore growth and development (13, 15); however, the prevailing view has been that small-molecular-weight toxins (e.g., alkaloids, phenolics, glucosinolates, etc.) (16) or volatile compounds that attract natural enemies (17) are the prime determinants of plant defense. In addition to the loss of amino acids incurred by the aforementioned proteins, there are a host of plant proteins that potentially degrade other essential nutrients such as fatty acids, minerals, ascorbic acid, and other vitamins. The loss of essential nutrients caused by these defensive plant proteins is predicted to be one of the most ecologically and evolutionarily stable forms of plant defense. Scientists interested in developing insect-resistant plants should follow the cues being uncovered in the defensive strategy of the tomato plant.

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