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Protease Inhibitor and Its Role in Pest Management

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SUMMARY

Protease inhibitors are molecules that inhibit the activity of proteolytic enzymes, which are enzymes responsible for breaking down proteins in the insect's digestive system. By disrupting the insect's ability to digest proteins, you can effectively inhibit its growth, development, and survival. They can be used for pest management as biopesticides, transgenic crops, in plant defence mechanisms, via crop rotation and also in insect rearing for biocontrol programms. t's important to note that while protease inhibitors can be effective tools in insect pest management, their use should be part of an integrated pest management (IPM) strategy.

INTRODUCTION

Protease inhibitors (PIs) are small proteins that are quite common in nature. They are natural, defense-related proteins often present in seeds and induced in certain plant tissues by herbivory or wounding (Koiwa *et al.*, 1997). PIs are present in multiple forms in numerous tissues of animals and plants as well as in microorganisms, counted among the defensive mechanisms displayed against phytophagous insects and microorganisms. The defensive capacities of plant PIs rely on inhibition of proteases present in insect guts or secreted by microorganisms, causing a reduction in the availability of amino acids necessary for their growth and development (De Leo *et al.,* 2002). Genes encoding insecticidal proteins have been isolated from various plant species and transferred to crops by genetic engineering, amongst these genes are those that encode inhibitors of proteases (serine and cysteine).

History:

The possible role of protease inhibitors (PIs) in plant protection was investigated as early as 1947. Mickel and Standish observed that the larvae of certain insects were unable to develop normally on soybean products. (Mickel and Standish, 1947). Subsequently the trypsin inhibitors present in soybean were shown to be toxic to the larvae of flour beetle *Tribolium confusum.* (Lipker *et al.*, 1954)

Proteolytic Enzymes

- Most PIs interact with their target proteases by contact with the active (catalytic) site of the protease, resulting in the formation of a stable protease-inhibitor complex that is incapable of enzymatic activity. Proteolytic enzymes are necessary for protein turnover.
- Degradation of damaged, misfolded, and potentially harmful proteins provides free amino acids required for the synthesis of new proteins.
- Furthermore, the selective breakdown of regulatory proteins by the ubiquitin/proteasome pathway controls key aspects of plant growth, development, and defense.
- Proteases are clearly involved in all aspects of the plant life cycle ranging from the mobilization of storage proteins during seed germination to the initiation of cell death and senescence programs.

Protease, Proteinase or Peptidase?

- Several almost-overlapping terms are current for the group of enzymes that hydrolyze peptide bonds. These are peptidases, peptide hydrolase, proteases, proteinases, and proteolytic enzymes. The Nomenclature Committee of the International Union of Biochemistry and Molecular Biology recommended the term peptidase as the general term for all enzymes that hydrolyze peptide bonds.
- These are then subdivided into exopeptidases, which cleave one or a few amino acids from the N- or C-terminus, and endopeptidases, which cleave the internal peptide bonds of polypeptides. The term “protease” will encompass both exopeptidases and endopeptidases while “proteinase” will describe only endopeptidases. In the PLANT-PIs, a database for protease inhibitors and their genes in higher plants, “protease” is adopted as a formal word (De Leo et al., 2002).

Classification of Proteinase

Exopeptidases: Aminopeptidases, Dipeptidases, Dipeptidyl peptidases, Peptidyl peptidases, Serine carboxypeptidases, Metallocarboxypeptidases, Cysteine carboxypeptidases. Omega peptidases

Endopeptidases:

Serine endopeptidases, Cysteine endopeptidases, Aspartic endopeptidases, Metalloendopeptidases and Endopeptidases of unkown catalytic mechanism

The Serine Proteinases

- This class comprises two distinct families. The chymotrypsin family, which includes the mammalian enzymes such as chymotrypsin, trypsin, or elastase or kallikrein, and
- The substilisin family, which includes the bacterial enzymes like subtilisin. The serine proteinases exhibit different substrate specificities.
- There are three types of digestive serine proteinases which are distinguished based on their specificity. Trypsin specifically cleaving the C-terminal to residues carrying a basic side chain (Lys, Arg). Chymotrypsin showing a preference for cleaving C-terminal to residues carrying a large hydrophobic side chain (Phe, Tyr, Leu).
- The order Lepidoptera, which includes a number of crop pests, the pH optima of the guts are in the alkaline range of 9-11 where, serine proteinases and metallo-exopeptidases are most active. Additionally, serine proteinase inhibitors have anti-nutritional effect against several lepidopteran insect species. Purified Bowman-Birk trypsin inhibitor at 5% of the diet inhibited growth of these larvae.
- Broadway and Duffey compared the effects of purified SBTI and potato inhibitor II (an inhibitor of both trypsin and chymotrypsin) on the growth and digestive physiology of larvae of *Heliothis zea* and *Spodoptera exigua* and demonstrated that growth of larvae was inhibited at levels of 10% of the proteins in their diet.
- Trypsin inhibitors at 10% of the diet were toxic to larvae of the *Callosobruchus maculatus* and *Manduca sexta* (Shulke & Murdock, 1983).

Bowman-Birk Inhibitor

The Bowman-Birk inhibitor (BBI) and its related family of isoinhibitors comprises a closely related group of serine PIs. The protein was first identified and isolated from soybean seeds by Bowman and further characterized by Birk and associates. Hence the name Bowman-Birk inhibitor (BBI).

The Cysteine Proteinases

- This family includes the plant proteases—such as papain, actinidin or bromelain—several mammalians lysosomal cathepsins, and the cytosolic calpains (calciumactivated) as well as several parasitic proteases (e.g. Trypanosoma, Schistosoma). Papain is the archetype and the best studied member of the family.
- Isolation of the midgut proteinases from the larvae of cowpea weevil, *C. maculatus* and bruchid *Zabrotes subfaceatus* confirmed the presence of cysteine mechanistic class of proteinase inhibitors. Cysteine proteinases isolated from insect larvae are inhibited by both synthetic and naturally occurring cysteine proteinases inhibitors. The optimum activity of cysteine proteinases is usually in the pH range of 5-7, which is the pH range of the insect gut that use cysteine proteinases. Although cysteine proteinase is primarily responsible for protein digestion in *C. maculatus*, it is not clear, how the cowpea and soybean. Bowman-Birk inhibitors exert their antinutritional effects on this organism. The rice cysteine proteinase inhibitors are the most studied of all the cysteine PIs which is proteinaceous in nature and highly heat stable.
- Most cysteine proteinase inhibitors have been found in animals, but several have been isolated from plant species as well including pineapple, potato, corn, rice, cowpea, mungbean, tomato, wheat, barley, rye and millet (Abe *et al.,* 1987)

The Aspartic Proteinases

- Most of aspartic proteinases belong to the pepsin family.
- This family includes digestive enzymes like pepsin and chymosin, lysosomal cathepsins D, processing enzymes like renin, and certain fungal proteases (penicillopepsin, rhizopuspepsin, endothiapepsin). A second family comprises viral proteinases, such as the protease from the AIDS virus (HIV), also called retropepsin.
- This general acid-base catalysis, which may be called a "push-pull" mechanism, leads to the formation of a non-covalent neutral tetrahedral intermediate (Mares *et al*., 1989).

The Metallo-Proteinases

- The metallo-proteinases may be one of the older classes of proteinases and are found in bacteria and fungi as well as in higher organisms.
- They differ widely in their sequences and their structures, but the great majority contain a zinc atom which is catalytically active.
- In some cases, zinc may be replaced by another metal such as cobalt or nickel without loss of the activity.

Mechanism of Binding Enzyme and Enzyme Inhibitors

- The adverse effects of protease inhibitors in foods are more complex than simply reducing the proteolytic activities of the digestive proteases.
- Trypsin inhibitors in animal diets have been known for some time to evoke increased pancreatic secretions, implying that active trypsin plays a role in normal regulation of pancreatic function by a monitor peptide that is secreted into the gut, cholecystokinin (CCK). When CCK is released from the intestinal wall into the blood stream, it control processes such as pancreatic secretion, gall-bladder contraction, gut mobility and appetite.
- Thus, the presence of high levels of protease inhibitors on a continual basis can lead to chronic hyper secretion by the pancreas, loss of proteolytic activity in the gut, loss of appetite, starvation and eventually death.
- The secretion of protease in insect guts depends upon the midgut protein.
- PIs inhibit the protease activity of these enzymes and reduce the quantity of proteins that can be digested and also cause hyper-production of the digestive enzymes which enhances the loss of sulfur amino acids as a result of which, the insects become weak with stunted growth and ultimately die.

General Properties of Plant PIs

- Generally speaking, plant PIs vary from 4 to 85 kDa, with the majority in the range of 8 to 20 kDa. Plant PIs usually have a high content of cysteine residues that form disulfide bridges and confer resistance to heat, extremes in pH, and proteolysis.
- Studies on the biosynthesis of several plant PIs demonstrated these PIs are synthesized as either prepro-proteins or pre-proteins that are processed *in vivo* either during or after synthesis to produce the native PIs.
- Many PIs are produced in response to various stress conditions, e.g. pathogens, insects, wounding, and environmental stresses such as salt.
- A common opinion is that most known plant PIs do not inhibit endogenous plant proteases but have specificities for animal or microbial enzymes.
- These observations may result from the fact that most studies used commercially available proteases, e.g. trypsin, chymotrypsin, elastase, and subtilisin from animal or microbial sources such as the test enzyme in the activity assays.
- However, none of these test enzymes are likely to be the true physiological target enzymes for most of the characterized plant PIs.

Insect resistant transgenic plants expressing PIs

- A large number of protease inhibitor genes with distinct modes of action have been isolated from a wide range of crop species.
- Considering the high complexity of protease inhibitor interactions in host pest systems and the diversity of proteolytic enzymes used by pests and pathogens to hydrolyze dietary proteins or to cleave peptide bonds in more specific processe, the choice of an appropriate proteinase inhibitor (PI) or set of PIs represents a primary determinant in the success or failure of any pest control strategy relying on protease inhibition (Graham & Ryan, 1997)

Commercial Applications of Plant PIs

- As stated above, plant PIs are involved in plant defense, regulation of endogenous proteinases, and protein storage.
- Whether plant PIs can be used in commerce has drawn great attention, and by 1991, plant PIs had already appeared in therapeutic use and laboratory applications.

- Several plant PIs such as soybean trypsin inhibitor, which are readily available from commercial sources or conveniently prepared in relatively large quantities at low cost, have been successfully used for the affinity purification of their inhibited proteases from a wide variety of sources.
- **Insect Rearing:** In some cases, protease inhibitors can be used in the rearing of insects for biological control programs. By adding protease inhibitors to the diet of predatory or parasitoid insects, you can inhibit the growth and development of the pests they are meant to control, making the biological control agents more effective.

Advantages

- The use of PIs in developing insect resistance in transgenic plants is of dual benefit, as they inhibit insect mid-gut proteinases, thereby protecting other defense proteins from proteolytic degradation. PIs blocks proteinases in insect guts and starve them of essential amino acids.
- They also affect a number of vital processes, including proteolytic activation of enzymes.
- PIs are present in the leaves and storage tissues, and are shown to be induced upon wounding, thereby significantly reducing the insect attack.

CONCLUSION

The continuous use of pesticides for crop protection had resulted in damaging impact on biological ecosystems. The use of target specific compounds with low persistence of intrinsic plant resistance mechanisms are safer alternative strategies for effective insect pests management. Complete understanding of the structural bases of inhibitor interactions will also enable site directed mutagenesis of existing inhibitors or design of synthetic peptides to yield inhibitors specific to a small number of pests thereby, minimizing the possible environmental side-effects of the transgenic technology. In addition, many plant PIs have been shown to act as defensive compounds against insects by direct assay or by expression in transgenic crop plants.

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