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Biological Activity of Plant Anti-Microbial Proteins- Thionin

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Abstract

Many plants, including common crop plants, generate classes of small proteins that protect them from a variety of infections induced by pathogens or stress materials. These proteins are primarily located in the most vulnerable and susceptible tissues of plants and are linked to a variety of biological activities that are significant enough to warrant further investigation, in addition to antimicrobial properties. This article concentrates on the most renowned family of antimicrobial peptides, Thionin, and discloses some of the significant information associated with it, despite the fact that the mode of microbial activity remains obscure.

Keywords: Plant Immunity, Antimicrobial Proteins, Thionins, Plant defence

Introduction

Plants are frequently exposed to many environmental cues like biotic and abiotic stressors. They lack immunity system like mammals and to combat infections. Plants are perpetually affected by a diverse array of microorganisms and engage in constant interaction with them and in return alters emission of many compounds (Sarkar and Sadhukhan, 2023). Under any stressful situation plants have produced different defensive compounds. Plants have the capacity to generate a wide range of chemical compounds, which may be classified into primary and secondary metabolites based on their chemical composition, origin of production, and functional groups (Sarkar *et al.,* 2024). Several plants have formulated some small group of peptides which are found in the infection prone tissues of plants (Campos et al. 2018; Bin Hafeez *et al.,* 2021; Schaller *et al.,* 1996). These groups of small peptides are known as antimicrobial proteins (AMPs) and many of our known crop plants produce these AMPs in a high level in several parts of their body. Technically antimicrobial proteins are a type of defense molecule produced by living organisms to combat microbial infections. These proteins work by targeting and disrupting the growth and survival of microorganisms such as bacteria, viruses, and fungi. Thionins, among them are the most acknowledged group and it consists of various strong AMPs which destroys pathogen infection with great efficacy (Bohlmann and Apel 1991; Stec 2006). At the close of 19th century, initial reports emerged detailing the inhibition of fermentation in bread and beer by some unknown compounds found in grains and it was hypothesized by scientists that wheat flour might contain a substance (Ohtani *et al.,* 1977), detrimental to bread yeast. In 1942, the scientists were successfully able to isolate and crystalize a toxic substance from the endosperm of wheat (*Triticum aestivum*) and later it was named as purothionin derived from two Greek words 'puro' meaning wheat and "thio" meaning sulphur. After that, in recent times numerous other small basic peptides (Campos *et al.,* 2018; Das *et al.,* 2019; Lee *et al.,* 2015) have been extracted from various common crop grains such as wheat, barley, oats, rice and rye as well as some vegetables like spinach and cabbage. The primary focus to explore these small proteins was to learn about their diverse antimicrobial and other biological activities which make them an important component of plant immunity.

Structural Conformation of Thionins:

Thionins can be categorized into more or less four distinct types based on plant species and tissues where they originate, the overall net charge as well as the number of amino acids and disulfide bonds present in the mature protein (Bohlmann and Apel 1991; Stec 2006). Type I thionins am predominantly found in

endosperms of various Poaceae plants and are considered to be highly basic and positively charged. They are commonly known as hordothionins and purothionins (α, β and γ) and were isolated from wheat and other grain endosperms (Mendez *et al.,* 1990). They are composed of more or less 45 amino acids, eight of which are cysteins participating in four disulfide bonds. Type 2 thionins are leaf thionins, isolated from Pyluria pubera leaves consist of 46-47 amino acids and four disulfide bonds and are slightly less basic than type 1. Type 3 thionins or viscotoxins and phoratoxins, retrieved from various mistletoe species, consist of 45-46 amino acids with three or four disulfide bonds and are basic in nature. Type 4 thionin is also known as crambin and purified first among the all. They were isolated from Abyssinian cabbage contains 46 amino acids with three disulfide bonds but are neutral in charge. All of these four types of thionins exhibit significant homology at the amino acid level. The cysteine residues and the tyrosine residue at the 13th position are highly conserved with the positively charged lysine and arginine residues being conserved to a lesser extent. Interestingly, the crambins lack positive net charge at neutral pH and conserved Tyrosine unlike type 1, type 2 and type 3. However, three-dimensional structure analysis has shown that despite these differences, crambins share a similar confirmation with others with significant sequence homology. They contain three cysteine residues involved in the disulfide bonds, an arginine residue, and the conserved tyrosine which is replaced by phenylalanine in crambin and also the distribution of hydrophobic and hydrophilic residues is well conserved. Interestingly, the primary structure analysis of thionins revealed that approximately 12-17% of the amino acids in thionins are cysteins which play crucial role in disulfide bond formation. Studies using CD, 1H NMR and X-ray crystallography declared that purothionin, viscotoxin A3 and crambins have similar secondary structures further confirming the structural similarities in the family, where numerous salt bridges, hydrogen bonds and four disulfide bonds stabilize the internal structure (Orru *et al.,* 1997) of subdomain further making the proteins highly thermos table and chemically stable.

Antimicrobial activity of thionins:

As said earlier, plants do not have immunity system but contains some small peptides to perform similar tasks and thionin is one of the most common and diverse group associated with that function. In 1940 during the investigation of substance responsible for the decrease in the baking quality of wheat flour led to testing antimicrobial activity and purothionin was discovered. Upon obtaining the substance in crystalline form, it was found to exhibit toxicity against various microorganisms at relatively low concentrations (Ohtani *et al.,* 1977; Wada *et al.,* 1982). Though these small cysteine-rich peptides have been proposed to serve various functions, this article will mainly focus light on their activity in defence mechanism against pathogenic invaders. Usually, the synthesis of these proteins is abundantly found in the vulnerable plant tissues which are exposed to microbe infection and its accumulation and the enhanced resistance can also be observed in many transgenically expressed engineered plants. However, their higher concentration in growing endosperm and high cysteine content hold proofs of them being storage proteins. Type 1 and 2 thionins have shown toxicity towards both gram-positive, gram-negative bacteria as well as fungi whereas no significant antimicrobial report is published involving type 3 and type 4 thionins.

Though the proper machinery of this antimicrobial activity of these peptides are still unknown, it can potentially be the outcome of pore formation (Hughes *et al.,* 2000). The highly positively charged thionins are suspected to interact with the negatively charged cell membranes and supposedly create pores. The left side of the helical stem and the underside of the molecule contain mainly hydrophobic components while the inner bend between the helical stem and β-arm is predominantly hydrophilic. When these peptides interact with membranes, they tend to form aggregates further forming pores allowing the hydrophobic regions to interact with the polar group of tails while hydrophilic regions extend towards the aqueous phase. The formation of pores ultimately leads to the membrane disruption and leakage resulting the destruction of the cells.

Other mechanisms of the protein

Along with the antimicrobial activities, the thionins tend to have many other activities as well. One possible mechanism involves electrostatic interaction with phospholipids where the interaction occurs between positively charged proteins and negatively charged polar tail groups of phospholipids. Purothionins have demonstrated an ability to impact the redox potential of other proteins such as thioredoxins (Stec 2006). They are also found to activate chloroplasts by facilitating disulfide bond formation. Interestingly, the presence of divalent cations like Ca2+ led to complete inhibition of toxicity

(Oard and Karki 2006). These findings indicate that the toxicity does not solely result from the binding of the membranes suggesting a two-step action is involved, where a non-specific membrane surface binding occurs due to electrostatic interactions followed by a specific interaction with a particular domain within the membrane. Interestingly, the toxicity of purothionin significantly reduced or eliminated by modifying the tyrosine residue, despite the overall charges of the protein unchanged. Additionally, there has been some interaction between thionin and nucleic acids and protein. Type 1 thionins may have potential impact on seed maturation, dormancy and germination (Stec 2006). Thionins can inhibit or activate enzymes, serve as secondary thiol messenger and potentially trigger phosphoinositide-mediated signal transduction and calcium channels in vitro.

Conclusions

Rising resilience towards the existing antimicrobial drugs have put mankind in dire need of new substitutions for the old ones. Also, the synthetic drugs possess some harmful side-effects which make them an unsuitable candidate for drugs. In this scenario, plant AMPs are emerging as the substitutions as they significantly destroy the infection by bacteria, virus as well fungi, they are highly accessible as they are found in various common crops and grains and lastly being our food resources, they often have least to none harmful side effects to human bodies. Lastly if they can be incorporated into bioengineered plants which are important for commercial purpose it will make a huge change in national economy. So, an intense effort is being put into discovering medicinal uses for thionins as well as other AMPs along with improving crop resistance by engineering more potent versions of thionins or translating them to species that do not express them.

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