

REVIEW ARTICLE

Plant Pathogenesis-Related Proteins PR-10 and PR-14 as Components of Innate Immunity System and Ubiquitous Allergens

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Abstract: Pathogenesis-related (PR) proteins are components of innate immunity system in plants. They play an important role in plant defense against pathogens. Lipid transfer proteins (LTPs) and Bet v 1 homologs comprise of two separate families of PR-proteins. Both LTPs (PR-14) and Bet v 1 homologs (PR-10) are multifunctional small proteins involving in plant response to abiotic and biotic stress conditions. The representatives of these PR-protein families do not show any sequence similarity but have other common biochemical features such as low molecular masses, the presence of hydrophobic cavities, ligand binding properties, and antimicrobial activities. Besides, many members of PR-10 and PR-14 families are ubiquitous plant panallergens which are able to cause sensitization of human immune system and cross-reactive allergic reactions to plant food and pollen. This review is aimed at comparative analysis of structure-functional and allergenic properties of the PR-10 and PR-14 families, as well as prospects for their medicinal application.

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1. INTRODUCTION

Pathogenic microorganisms are ubiquitous in the environment. Resistance to pathogens is a prerequisite for survival of species. Although plants lack an adaptive immune system, during evolution they have acquired a number of effective defense mechanisms protecting them from environmental stress. Plants have such defense strategies of innate immunity system as hypersensitive response (HR), cell wall thickening, increasing concentrations of phytohormones, induction of synthesis of antimicrobial peptides (AMPs) and low molecular weight secondary metabolites, as well as pathogenesis-related proteins (PR-proteins) and finally development of local or systemic acquired resistance (LAR or SAR) [1,2]. During the attack of phytopathogens a set of different AMPs and PR-proteins as well as low molecular weight secondary metabolites are synthesized in plants. Most of the PR-proteins possess an-

timicrobial activity, although mechanisms of their action often differ from that of AMPs. Combined interactions between PR-proteins greatly increase antimicrobial effect, extend the repertoire of the suppressed pathogens, and compensate for the absence of adaptive immunity in plants.

Stress-induced PR-proteins are divided into 17 families (PR1-17) in accordance with sequences similarity and biological activities. Many PR-proteins are constitutively expressed in different organs and tissues of plants, however induction of their synthesis takes place in response to abiotic or biotic stress [3]. Antimicrobial PR-proteins affect the cell wall and plasma membrane of pathogens. Some of them have intracellular targets or inhibit enzymes of microorganisms. Among representatives of PR-families are β -1,3-glucanases, chitinases, proteinase inhibitors, thaumatin-like proteins, peroxidases, defensins, thionins, oxalate oxidases, as well as Bet v 1 homologs (PR-10), and lipid transfer proteins (LTPs, PR-14) [3,4]. The members of PR-10 and PR-14 protein families do not show any sequence similarity but have other common bio-

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chemical features such as low molecular masses, the presence of hydrophobic cavities, ligand-binding properties, and antimicrobial activity. Both Bet v 1 homologs and LTPs are multifunctional proteins, and their diverse biological roles in plants are possibly related to the ability to bind different ligands. Many proteins of both families are ubiquitous plant panallergens which cause cross-reactive allergic reactions to plant food and pollen.

In this review we summarized and compared the current data on the PR-10 and PR-14 proteins structural characteristics, ligand-binding capacity, biological activity, possible functions, and the role in plant defense. Special focus is on allergenic properties and prospects for medicinal application of these two PR-protein families.

2. STRUCTURAL FEATURES OF PR-10 AND PR-14 PROTEINS

LTPs and Bet v 1 homologs are small, abundant, multifunctional proteins which have been isolated from a number of species across the plant kingdom. Despite the low amino acid sequence similarity, both families share a common structural architecture with a large internal hydrophobic cavity, which can accommodate different ligands *in vitro* (Table 1).

LTPs are the family of small basic proteins with high structural resistance to denaturants, heat and proteases [5]. LTPs are divided into two structurally-related subfamilies according to their molecular masses: LTP1s (9 kDa) and LTP2s (7 kDa). They display low amino acid sequence homology, except an eight cysteines motif, but have similar spatial structures [6-8]. The fold of LTP1s is composed of four α -helices linked by flexible loops and a stretch of C-terminal residues [9-13] (Fig. 1). LTP2s have the same structural pattern, but contain three α -helices and a region containing two single-turn helices. A large number of intramolecular H-bonds contribute to the stabilization of the LTPs spatial structures. The three or four helices and a long C-terminus form an internal hydrophobic cavity crossing the whole molecule. The cavity in the LTP1 subfamily is shaped as a tunnel holding on the long axis of the protein, while the one in the LTP2 subfamily has the form of a triangular hollow box, which is more flexible than the cavity in the LTP1 subfamily. The triangular cavity of LTP2s exhibits high plasticity in comparison with the tunnel-like cavities of LTP1s [14,15].

The PR-10 family consists of two distinct groups of proteins: intracellular pathogenesis-related proteins

(IPRs) with ribonuclease activity and (S)-norcoclaurine synthases (NCSs). IPRs are subdivided into some subfamilies including homologs of the major pollen allergen Bet v 1 of the white birch, homologs of the major latex proteins (MLPs), cytokinin-specific binding proteins (CSBPs) and recently discovered receptors for abscisic acid (ABA) [4]. The Bet v 1 homologs are the most interesting representatives of the PR-10 subfamily because of their clinical significance as plant allergens. The Bet v 1 homologs are slightly acidic small (154–163 amino acid residues) proteins with molecular masses of ~17 kDa. The Bet v 1-like proteins have sequence identity to Bet v 1 below 25% and similarity below 40% [16]. The Bet v 1 family fold consists of seven-stranded anti-parallel β -sheets wrapped around a long C-terminal α -helix. The β 1 and β 2-sheets are separated by two small consecutive helices. The structure of Bet v 1 homologs contains the conserved motif GxGGxGxxK (amino acid residues 47–55) that forms the P-loop, also known as a glycine-rich loop. The P-loop is a structural element responsible for interaction with nucleotides in some nucleotide-binding proteins [16]. The most important feature of the Bet v 1 spatial structure is a large Y-shaped solvent-accessible cavity penetrating the core of the molecule [4,16]. The Y-shape of the cavity is formed by three tunnels (P, H1 and H2) connecting the cavity with the protein surface [17]. Due to substantial conformational and sequence variability of the C-terminal helices in the Bet v 1-like proteins, their cavity volumes and shapes have remarkable differences.

Interestingly, the Bet v 1 homologs and LTPs are shown to exist mostly as monomers in solution, although few of them assemble forming dimers or higher order oligomers [18,19].

3. LIGAND BINDING AND TRANSFER

The hydrophobic cavities of both LTPs and Bet v 1 homologs contain potential sites for binding of various ligands [7] (Table 1). The cavity of LTP1s has two entrances, one is small and another is large [11,20]. Arg44 (numbering relative LTP1s from rice *Oryza sativa* and peach *Prunus persica*) localized on the larger entrance of the hydrophobic cavity interacts with the polar heads of lipids. The rice LTP1 contains another basic amino acid residue (Lys35) which is involved in such an interaction [21]. In some proteins Tyr79 was shown to be hydrogen bonded with the polar head group of lipid ligands [22,23], but often it is not the case [24,25]. LTPs show a broad lipid-binding specificity *in vitro*, for a variety of hydrophobic molecules, such as fatty acids, fatty acyl-CoA phospholipids lyso-

Table 1. Comparison of structure-functional properties of plant Bet v 1 homologs and LTPs.

Characteristics	Bet v 1 homologs	LTPs
Mr, kDa	15-18	6-7 (LTP2s) or 9-10 (LTP1s)
Amino acid residues number	154-163	90-95 (LTP1s) or 65-70 (LTP2s)
pI	slightly acidic	basic
Structure stability	labile to heat and proteases	highly resistant to denaturants, heat and proteases
Conservative motif	glycine-rich loop or GXGGXGXXX motif (aa 47-55)	C...C...CC...CXC..C...C
Localization	generally intracellular and cytosolic	generally extracellular
Presence of isoforms in genome	multigene families	
Gene expression	constitutively, induction under stress conditions	
Post-translation modifications	phosphorylation, S-glutathionylation	signal peptide cleavage and formation of disulfide bonds
Oligomerization	generally as monomers, rarely as dimers and oligomers	generally as monomers, rarely as dimers
Spatial structure	three α -helices and seven antiparallel β -strands	three or four α -helices and a flexible C-terminal coil
Hydrophobic cavity:		
Shape	Y-shape	a tunnel (LTP1s)/a triangular hollow box (LTP2s)
Volume	2100-3900 Å ³	180–1000 Å ³
Ligands	lipids, plant hormones, and secondary metabolites	lipids, antibiotics
Signal peptide	-	21-27 aa (LTP1s) or 27-35 aa (LTP2s)
Biological activity:		
RNase activity	+	-
Antimicrobial activity	+	+
Enzyme inhibitor activity	+	+
Possible mechanism of antimicrobial action	degradation of pathogenic or own cellular RNAs and development of HR or LAR	destabilization and permeabilization of membrane of phytopathogen
Possible functions:		
Plant stress response	induced expression in stress conditions	
Defense signaling	interaction of PR-10 with cytosolic receptor and activation of defense signaling pathways	interaction of complex LTP-lipid with membrane-associated receptor and activation of defense signaling pathways
Cryoprotective function	cryoprotective effect on the freeze-labile enzyme (lactate dehydrogenase)	binding and protecting thylakoid membranes from freeze-thaw damage
Metabolism of lipids and other ligands	binding, transfer, storage and release of physiologically relevant ligands	
Plant reproduction	anther development and pollen formation	anther development and pollen formation, adhesion and tip growth of pollen tube, seed formation
Programmed cell death	degradation of own cellular RNAs	release of cytochrome <i>c</i> from mitochondria
Symbiosis	regulation of invasion of symbiotic microorganisms	

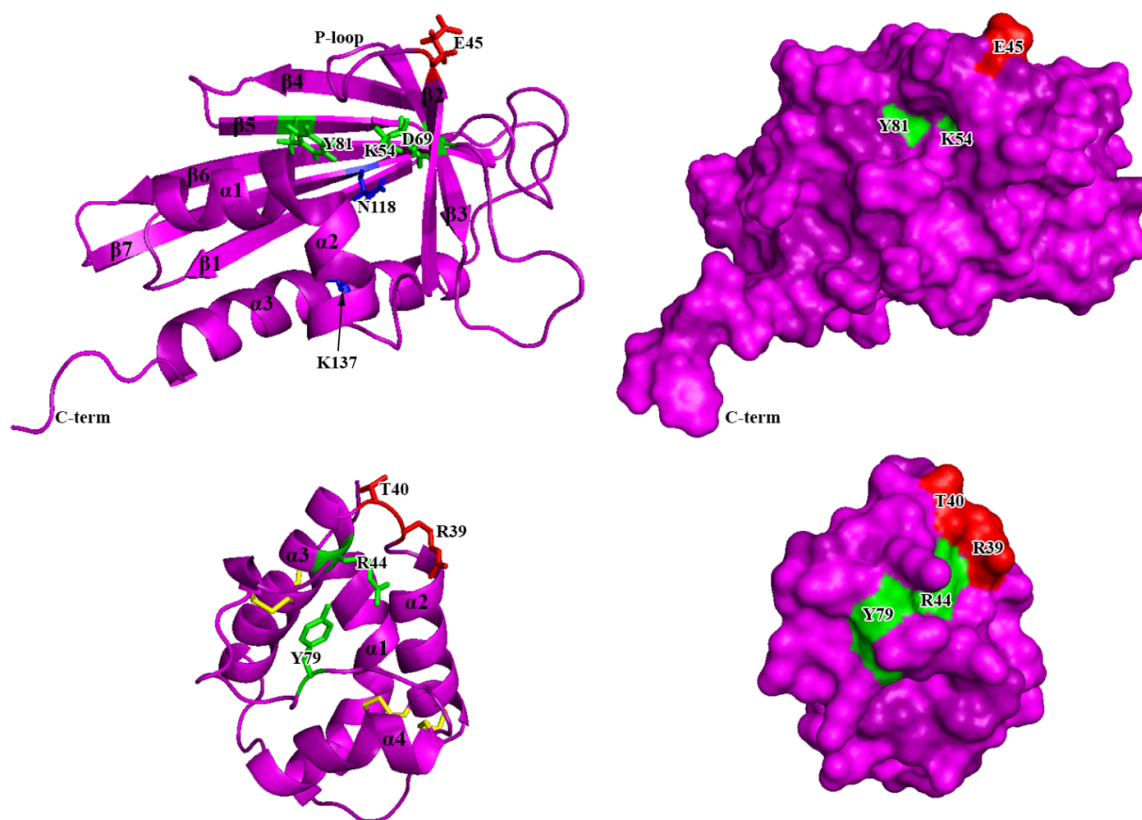


Fig. (1). Crystal structures of the birch Bet v 1 [PDB: 1B6F] (A,B) and the peach LTP Pru p 3 [PDB: 2ALG] (C,D) in ribbon (A,C) and surface (B,D) representations. The Bet v 1 amino acid residues which form kinetin-binding site are colored in green, the residues participating in binding with fatty acids are colored in dark blue. The residues of Pru p 3 participating in lipid uptake are colored in green. Crucial for IgE-binding residues of both allergens are colored in red.

derivatives, glycolipids, hydroxylated fatty acids, prostaglandin B2, amphotericin B, and azole derivative BD56 [15,22,23,26-29]. The hydrophobic cavity of LTP2 family can accommodate large molecules with rigid structures, such as sterols, that are not able to bind to LTP1s [30].

Different LTPs exhibit very similar global folds, however their lipid-binding capacity and interactions with lipid molecules vary notably. Some LTPs can bind one or two ligands at once [31,32], while other members of the family do not bind and transport lipids [33]. Although LTPs typically bind lipids in a non-covalent way, an abundant modified form of LTP1, designated as LTP1b, was found in the wheat and barley seeds. LTP1b contained a covalently bound protein adduct (α -ketol, 9-hydroxy-10-oxo-12(Z)-octadecenoic acid) [34,35]. It should be noted that formation of the covalent complex increases the plasticity of the hydrophobic cavity of the protein as well as its ability to transfer lipids.

In vitro studies have shown that LTPs may transfer and/or exchange amphiphilic molecules between mem-

branes [27]. LTPs are able to transfer phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, and glycolipids [36,37]. Moreover, some LTPs have shown to interact with lipid model membranes and also to modify the permeability of fungal cells [33,38].

In contrast to that of the LTP family, the hydrophobic cavity of Bet v 1 homologs has different binding sites for various ligands, and ligand-specific amino acid residues can be defined. The Bet v 1 homologs may bind complex amphiphilic molecules like fatty acids, flavonoids, cytokinins and brassinosteroids [39,40]. It was shown that the cavity of some members of the Bet v 1 family can simultaneously accommodate two ligands much like LTPs [39]. Different isoforms of the Bet v 1 homologs have shown appreciable differences in binding capacities under the same experimental conditions. Such a functional divergence could be due to structural differences of the binding pocket in various isoforms [41].

Modeling studies of Bet v 1 suggested that Lys54, Asp69, and Tyr81 are the most essential residues for

the affinity to kinetin and sterols. Asn118 and Lys137 located more than 10 Å away from the kinetin binding site are suggested to be the key amino acid residues for fatty acids binding [42]. A binding model indicates that the hydrophobic tail groups of lipids are located in the Y-shaped cavity of the Bet v 1 molecule, while the polar head groups point out of the molecule. Recently the first natural ligand quercetin-3-*O*-sophoroside (Q3OS) of Bet v 1 was identified and the Bet v 1–Q3OS complex was isolated from the mature birch pollen [43]. It was suggested that the Bet v 1–Q3OS complex protects the birch pollen DNA from UV damage, and functional Q3OS may serve as an important signal for unharmed pollen DNA [43]. For some members of the Bet v 1 family participation in the transport of amphiphilic molecules such as ceramides, sphingomyelins, and plant steroids, was revealed [16,42].

4. BIOSYNTHESIS AND LOCALIZATION

Many LTPs and Bet v 1 homologs genes have been shown to be expressed under stress conditions or after wounding, suggesting their involvement in plant defense response. Information regarding the tissue-specific localization of these proteins is of major importance for functional studies. Both LTPs and Bet v 1 homologs are encoded by a divergent multigene family, dispersed in different chromosomes in many plant species. LTPs, in contrast to the Bet v 1 homologs, are synthesized as precursors with the N-terminal signal peptide of 21–35 amino acids and the pre-proteins undergo the secretory pathway [44,45] (Table 1).

LTPs have generally the extracellular localization in plants and are considered as apoplastic proteins [4]. It was shown that some family members are localized at cell walls [46] [47], plasma membranes [46,47] or at the intracellular matrix [48,49]. The extracellular location is consistent with the secretion of LTPs into aleurone and embryogenic cell cultures [50,51]. In contrast, their intracellular location, such as inside the glyoxisomes or protein storage vacuoles, has been proposed in [52]. The members of the LTP family are ubiquitously expressed throughout the plant and have been found in seeds, siliques, leaves, stems, fruits, flowers, and, most rarely, roots. Usually several LTP genes are found per genome, indicating that several isoforms are present in plant species [53]. Different LTP isoforms are expressed in various tissues at certain stages of ontogeny [6]. The highest expression levels have been found in peripheral cell layers surrounding aerial organs, associated with cell wall and cuticle of epidermal tissues [54].

In contrast to the LTP family, the Bet v 1 homologs are generally intracellular and cytosolic [4]. Some Bet v 1 homologs are found in chloroplasts of mesophyll and endodermis, and in the stroma [55]. To date, members of the PR-10 family have been reported in a variety of higher plant species of both monocots [56] and dicots [57,58]. Several Bet v 1 homologs genes are expressed in different tissues and organs during plant growth and development [59,60], such as the pollen grain [57], flower organs [57,61], fruit [62], seeds [63], vegetative organs of roots [64], stems [65] and leaves [65].

5. BIOLOGICAL ACTIVITY AND POSSIBLE FUNCTIONS

The role of PR-10 and PR-14 members in plants is still unclear. Both protein families are widespread in the kingdom Plantae and obviously are very important for plant growth and development. Due to the fact that expression of both protein families genes is induced under abiotic and biotic stress conditions, they are suggested to perform a protective role in plants. Despite the significant difference in structural organization and localization, the proteins of these families possess similar biological activities and often take part in the same processes in plants, including stress response, defense signaling, reproduction, symbiosis, apoptosis, and others (Table 1).

5.1. RNase Activity

Some Bet v 1 homologs possess RNase activity [61]. As mentioned above, the conserved nucleotide-binding motif of the P-loop presents in their structure. However the Bet v 1 homologs do not show any homology with other ribonucleases. Besides, not all members of the Bet v 1 family display the RNase activity [4]. It was suggested that RNAs of pathogenic microorganisms may be potential targets for these RNases, playing a direct role in plant defense. Alternatively, it was supposed that the intracellular PR-10 proteins may degrade cellular RNAs and play a role in development of HR and LAR that block systemic spread of the pathogen across the plant [61]. It was shown that phosphorylation is of much importance for regulation of the activity of PR-10 proteins during defense signaling. Phosphorylation is believed to enhance the specificity of RNase activity of these proteins and potentially prevent their unspecific action within the plant cell [66]. For example, it was determined that phosphorylation of CaPR10 from hot pepper increases its ribonucleolytic activity and gives an option to cleave invading viral RNAs [67]. For the PR-10c protein S-

glutathiolation was shown to play a role in protection of the reactive thiol group, maintenance of RNase activity of the protein, enhancement of the protein resistance to irreversible oxidation and possible inactivation under heavy metal stress conditions [68]. To date, there is no evidence of enzymatic activity of PR-14 proteins.

5.2. Antimicrobial Activity

Some members of PR-10 and PR-14 families display an antimicrobial activity, inhibit growth of phytopathogenic bacteria, fungi and viruses and play an important role in plant defense against infection. Transgenic plants carrying PR-10 or PR-14 genes are resistant to phytopathogens. Mechanisms of antimicrobial action of these PR-proteins are still not fully understood.

LTPs exhibit antimicrobial activity against a wide range of phytopathogenic fungi and bacteria and also are able to inhibit the growth of human pathogenic fungi of the *Candida* genus [69]. LTPs from onion, radish [70] and arabidopsis [71] possess pronounced antimicrobial activity at micromolar concentrations. Disulfide bonds which stabilize LTP structure are essential for antimicrobial activity of PR-14 [72]. A possible target for antimicrobial action of LTPs is cytoplasmic membrane. It is shown that LTPs can cause permeabilization of the model membrane and the cytoplasmic membrane of pathogenic fungi [38]. It was assumed that LTPs like other cationic AMPs are bound to the biological membrane of phytopathogen causing its destabilization and permeabilization [12]. Plant LTPs also possess antiviral activity and inhibit the activity of HIV-1 reverse transcriptase [73].

It was demonstrated that antimicrobial activity of PR-10 proteins was associated with their ability to degrade pathogenic RNAs. Such Bet v 1 homologs as AhPR-10 from peanut [74], Gly m 41 from soybean [75], VpPR-10.1 from *Vitis pseudoreticulata* [59] and TcPR-10 from *Theobroma cacao* [76], possess antifungal activity. It was shown that internalization of the proteins in the sensitive fungi through active uptake without disrupting the membrane is essential for displaying their antifungal activity [74]. It was suggested that PR-10 proteins have non-classical secretory pathway, they are secreted into the intercellular space and degrade there the pathogenic RNAs inhibiting fungal growth. Alternatively, the Bet v 1 homologs can degrade RNAs of haustorium, which is formed and stick into cytoplasm of susceptible plants, inducing programmed death of host cells and limiting pathogen invasion [59,75]. In addition to antifungal activity, some

PR-10 proteins exhibit antibacterial (ocatin from the Andean crop oca [77]) and antiviral (CaPR-10 protein from the hot pepper *Capsicum annuum* [67]) activities.

5.3. Enzyme Inhibitor Activity

Some members of the LTP family were shown to inhibit the activity of endogenous and exogenous enzymes, namely proteases and α -amylases [69,78]. It was considered, that LTPs may be involved in the development and germination of seeds and in plant defense from insects and herbivores. It was found that the PR-10 protein CpPRI from *Crotalaria pallida* roots demonstrated nematostatic and nematocidal effects on nematodes, possibly providing the natural tolerance of plant to these parasites. It was suggested that papain inhibitory activity may be the basis of antinematode action of this protein [79].

5.4. Plant Stress Response

As mentioned above, some members of PR-10 and PR-14 families are constitutively expressed while some of them are induced only under stress conditions assuming a protective role of these proteins in plants. Both Bet v 1 homologs and LTPs have been shown to be transcriptionally responsive across pathogen attack and a large range of abiotic stress conditions including drought, salinity, low and high temperatures, heavy metals, wounding, and UV exposure [80-82]. An important role of these PR-protein families in plant defense is also confirmed by enhanced tolerance of transgenic plants overexpressing Bet v 1 homologs [80,83,84] or LTPs [81,82,85] to abiotic and biotic stress. Further proof of the participation of these proteins in plant adaptation to stress is regulation of their synthesis by major stress signaling molecules such as phytohormones abscisic acid, ethylene, jasmonic acid, and salicylic acid [6,66,80-82].

5.5. Metabolism of Lipids and Other Ligands

Due to the fact that both PR-10 and PR-14 proteins are able to bind and transfer different hydrophobic ligands, their involvement in metabolism of such compounds as lipids, hormones and secondary metabolites is suggested [6,16]. The expected functions of the Bet v 1 homologs and LTPs in plants are often linked with biological roles of their ligands. Direct evidences of such a correlation has not been received yet.

It was shown that PR-14 proteins took part in deposition of cuticular wax. LTPs are present at high concentrations in epidermal tissues and are able to bind hydroxy fatty acid (C16 and C18) (LTP1s) and sterols

(LTP2s) required for cutin and suberin biosynthesis, respectively [27,30]. Induction of LTPs synthesis is accompanied by thickening of the cuticle layer [86], and disruption of LTPs genes leads to changes in the lipid composition and a more diffuse cuticular layer structure [87]. It was suggested that LTPs transfer the lipid precursors to the place of suberin and cutin biosynthesis, however the mechanism of transport is still unclear.

It was demonstrated that Bet v 1 binds with low affinity a range of physiologically relevant ligands, including fatty acids, flavonoids, and cytokinins. It was suggested that Bet v 1 may transfer these ligands, take part in ensuring proper hydration and germination of pollen, act as storage bodies for cytokinins in seeds allowing rapid release upon germination of these growth hormones that control differentiation and proliferation of plant cells [40]. It was shown that PR-10 proteins from strawberry, Fra a, may play a role in the control of flavonoid or other secondary metabolic pathways in plants. These proteins bind natural flavonoids which are not only involved in the development of color in fruits and flowers, but also take part in many biological functions in plants, including defense against pathogens, insect attraction, pollination, UV protection, and others. It was suggested that Fra a may perform intracellular transport of flavonoids or play the role in “chemical chaperones”, binding the flavonoid intermediates and making them available for enzymatic transformation [88].

5.6. Defense Signaling

It was shown that both PR-10 and PR-14 proteins are involved in the defense signaling mechanisms in plants during pathogen attack. These proteins interact with immune receptors causing the activation of defense signaling pathways. It was found that during plant infection LTPs are secreted to the apoplast and bind the hydrophobic ligands, for example, the plant hormone jasmonic acid or lipid molecules formed by enzymes of phytopathogenic microorganisms [89,90]. The complex LTP-lipid interacts with membrane-associated immune receptor and activates plant defense response. It was discovered that in the case of the Bet v 1 homologs immune receptors (leucine-rich repeat protein1, LRR1) have cytosolic localization. To date, there are no data about the need for the presence of hydrophobic ligand for interaction of PR-10 proteins with immune receptors and cell death-mediated defense signaling [83].

5.7. Cryoprotective Function

It was shown that PR-10 protein, WAP18 from mulberry *Morus bombycis*, exhibits cryoprotective activity, preventing freeze-thaw damage of the enzyme lactate dehydrogenase, and may be involved in frost-tolerance mechanisms of plant defense [60]. It was found that the member of the WAX9 family of LTPs from cold acclimated cabbage binds and protects the thylakoid membranes from freeze-thaw damage by reducing the fluidity of the membrane lipids [91].

5.8. Plant Reproduction

It was shown that proteins of both PR-10 and PR-14 families, having different localization, may be involved in various stages of anther development and pollen formation. Sporopollenin is a polymer of fatty acids and phenolic compounds and is a major component of the tough outer walls of plant spores and pollen grains. Sporopollenin is synthesized in tapetal cells. It was described that secreted tapetum-specific LTP, OsC6 from rice, plays a crucial role in the development of orbicules and pollen exine during anther development in plants. It was suggested that this LTP transfers lipid molecules, possibly sporopollenin precursors, from metabolically active tapetal cells to other anther cells for orbicule and pollen wall development [92]. The Bet v 1 homolog from the lily also was found during anther development in the tapetal cells. It was suggested that this cytoplasmic protein also takes part in the sporopollenin pathway [56,93].

It was also demonstrated that LTPs from *Lilium longiflorum* [94] and *Arabidopsis thaliana* [95] play a role in adhesion and tip growth of pollen tube and also in seed formation. It was suggested that secreted LTPs act as adhesins between the receptor in the pollen tube wall/plasma membrane and the stylar extracellular matrix. Alternatively, they can take part in adhesion indirectly, for example, as carriers of lipophilic signal molecules [94].

5.9. Symbiosis

The control of bacterial infection by antimicrobial cysteine-rich peptides is a common phenomenon in symbiosis [96]. It was shown that both LTPs and PR-10 proteins are involved not only in plant defense against phytopathogens but also play an important regulatory role in the interaction between plants and symbiotic microorganisms. It was found that LTP from *Medicago truncatula* MtN5 is highly expressed during the early phases of rhizobial symbiosis and is involved in the regulation of root tissue invasion. It was sug-

gested that MtN5 binds lipid molecules and interacts with the rhizobia plasma membrane or participates in the signaling between rhizobia and host cells [97]. The correlation between slightly increased level of the PR-10 protein from the ryegrass *Lolium perenne* and the presence of a normally growing fungal endosymbiont was observed. The PR-10 protein from ryegrass possibly plays a role in controlling the growth of the endophyte in a similar way to LTPs, but there is not direct evidence for this function yet [98].

5.10. Programmed Cell Death

It was shown that the maize LTP is able to induce cytochrome *c* release from mammalian mitochondria in the presence of different lipids, similarly to proapoptotic protein Bid of the Bcl-2 family. It was suggested that this LTP transfers lipid molecular and enhances the permeability of the outer mitochondrial membrane providing the consequent release of apoptogenic factors like cytochrome *c* [99]. It was found that the PR-10 protein from rice, PBZ1, is possibly one of the key mediators of programmed cell death. It was shown that PBZ1 induces cell death in plants *via* modulation of its RNase activity inside the cell [100].

6. BET V 1 HOMOLOGS AND LTPS AS ALLERGENS

IgE-mediated food allergies are subdivided into two classes according to the route of sensitization. Class I allergy is due to sensitization *via* the gastrointestinal tract and characterized by systemic reactions, whereas class II allergy results from sensitization to inhalant allergens and is limited to oral allergy symptoms [101]. Complete food allergens have the ability to both sensitize and elicit symptoms and, as a rule, are highly stable proteins inducing class I allergy. In contrast, class II allergy is induced by incomplete food allergens (class II), which are protease- and heat-labile and cannot cause sensitization, but can elicit symptoms because of cross-reactivity with homologous sensitizer [102]. These two classes of allergy have different epidemiology, thus, the class I type of food allergy mainly affects children while the class II is developed in later life [103]. The Bet v 1-related proteins are an example of typical incomplete food allergens inducing mild symptoms (mainly, oral allergy syndrome or OAS) due to their heat lability. In contrast, plant LTPs are resistant to proteases and heating, and tend to induce systemic reactions (Table 2).

One of the most common causes of IgE-mediated allergy in Northern and Central Europe and North

America is birch pollen. In birch pollen allergy, up to 95% of pollen-sensitized patients in Northern Europe display IgE-binding to the allergen Bet v 1, wherein 60% respond exclusively to this allergen [104]. The Bet v 1 homologs are found in pollen of evolutionary related Fagales trees (e.g. alder *Aln g 1*, hornbeam *Car b 1*, chestnut *Cas s 1*, hazel *Cor a 1*, beech *Fag s 1*, oak *Que a 1*) and Apiaceae vegetables (e.g. celery *Api g 1*, carrot *Dau c 1*) as well as botanically unrelated Rosaceae fruits (e.g. apple *Mal d 1*, cherry *Pru av 1*, apricot *Pru ar 1*, pear *Pyr c 1*) and legumes (soybean *Gly m 4*, peanut *Ara h 8*) [105]. The fruits most commonly causing symptoms in Bet v 1-allergic individuals include members of the order Rosaceae, such as apple, pear, cherry, and apricot. As mentioned above, Bet v 1-related allergens induce mostly mild symptoms, like OAS, however some allergens, like Gly m 4, tend to induce severe reactions in allergic patients [106]. Despite low total sequence identity among the Bet v 1 homologs, the protein fold is very conserved within the whole allergen family [107]. To date, the nucleotide-binding motif P-loop, is considered as a major IgE-binding epitope on at least certain Bet v 1 homologs from the Rosaceae fruits which is responsible for their cross-reactivity [108]. E45, the central residue in the P-loop-containing region [108], together with S112 [109] have been shown to be crucial for antibody recognition.

While in Northern and Central Europe and North America the allergy is mostly linked with sensitization to birch pollen, allergic reactions in the Mediterranean area are usually associated with sensitization to peach and its major allergen – Pru p 3, belonging to plant LTPs family [110]. Like the Bet v 1 homologs, LTPs can be found in many fruits (e.g. pear *Pyr c 3*, apple *Mal d 3*, cherry *Pru av 3*, plum *Pru d 3*, etc.), vegetables (e.g. celery *Api g 2*, carrot *Dau c 3*, onion *All c 3*, etc.), nuts (e.g. hazel *Cor a 8*, walnut *Jug r 3*, peanut *Ara h 9*, etc.), as well as in pollen (e.g. ragweed *Amb a 6*, mugwort *Art v 3*, plane tree *Pla a 3*, etc.), grains (e.g. wheat *Tri a 14*, corn *Zea m 14*, etc.) and latex (*Hev b 12*). In contrast to pollen-related food allergy, sensitization to plant food LTPs frequently results in systemic and severe reactions, like urticaria, angioedema, and anaphylaxis [111]. It is believed that the major Pru p 3 IgE-binding epitope responsible for cross-reactivity between many allergenic LTPs is formed by 11-25, 31-45, and 71-80 amino acid residues [112]. R39, T40, and R44 residues of its epitope were shown to be crucial for binding with IgE [113]. Interestingly, the lipid-binding regions of both Bet v 1 and Pru p 3 allergens are located close to their IgE-binding epitopes (Fig. 1).

Table 2. Allergenic properties of plant Bet v 1 homologs and LTPs.

Characteristics	Bet v 1 homologs	LTPs
Major allergen of a family	Bet v 1 of white birch (pollen allergen)	Pru p 3 of peach (food allergen)
Class of allergens	II (incomplete)	I (complete)
Allergenic source:		
Pollen	trees	grasses and trees
Plant food	fruits, vegetables, and nuts	fruits, vegetables, nuts, and grains
Allergic reactions	local, OAS	systemic and local
Relevant age	adults	childrens and adults
Sensitizers	-	+
Cross-reactivity	+	+
Allergens from IUIS database:		
Pollen allergens	7	9
Food allergens	18	38
Latex allergen	-	1
Proposed IgE-binding epitopes	P-loop region	11-25, 31-45, and 71-80 regions corresponding to Pru p 3 sequence
Used for component-resolved diagnostics	13 allergens	10 allergens
ASIT prospects (trials on humans)	rBet v 1-FV	rPru p 3 sur, rPru p 3 cys, rPru p 3 G, and rPru p 3 RA

As mentioned above, incomplete food allergens cannot cause sensitization, but are able to elicit symptoms due to cross-reactivity. Thus, with the exception of Dau c 1 from carrot and Cor a 1 from hazelnut, the Bet v 1 homologs are considered to be unable to initiate sensitization in atopic subjects [114]. However, because of IgE cross-reactivity, they cause immediate allergic symptoms in more than 70% of Bet v 1-sensitized patients [115]. In contrast, LTPs are considered as allergens capable of sensitizing *via* the gastrointestinal and respiratory tracts as well as potentially across the skin [110]. For example, it was shown that sensitization to peach Pru p 3, walnut Jug r 3, plane tree Pla a 3, and mugwort Art v 3 is significantly associated with sensitizers of LTP-mediated allergies in Italian patients [116].

In this regard a question arises: which allergen properties are related to sensitization capacity? It was shown for Bet v 1 [117] and peach Pru p 3 [118] that they contained one or more immunodominant T-cell epitopes recognized by more than 50% of allergic pa-

tients. However, such nonsensitizing Bet v 1 homologs like Api g 1 from celery [119] and Mal d 1 from apple [114] do not carry the frequently recognized T-cell epitopes. Correlation between the sensitizing activities of highly homologous members of the Bet v 1 family and the presence of dominant T-cell epitopes was demonstrated by comparison of T-cell epitope mapping [114]. Thus, Bet v 1, the major sensitizing protein of PR-10 family, contains T-cell epitope region (amino acid residues 142-156), which is recognized by 63% of patients with birch pollen allergy [117]. Cor a 1 from hazelnut also contains T-cell epitope region (residues 142-153), which is recognized by 47% of patients with birch pollen allergy and concomitant hazelnut allergy [120], but alongside with cross-reactivity with Bet v 1 it was reported to induce IgE production by itself. Conversely, the allergic response to Api g 1 occurs due to previous sensitization to Bet v 1, and no T-cell epitope in the celery allergen was recognized in more than 33% of the studied subjects [114]. Similar effects were observed for LTPs as well. Study of the cellular and humoral

response to LTPs Pru p 3 from peach and Cor a 8 from hazelnut revealed that Pru p 3 was the origin for sensitization to Cor a 8 in Italian patients with allergies to both foods [121]. For Pru p 3, immunodominant T-cell epitopes located within 12-27, 34-48, and 57-80 regions were described, whereas Cor a 8 contained none [114]. Explanation of the observable diversity in T-cell reactivity of such homologous molecules is still obscure. It may be linked to differences in antigen processing, namely uptake, endolysosomal degradation, and peptide loading to common HLA class II molecules [114].

In summary, the Bet v 1 homologs represent a family of heat-labile proteins which are often denaturated in cooked foods and associated mainly with local allergy symptoms such as OAS. They are relevant in Northern and Central Europe and North America and associated with allergic reactions to fruit and vegetables, although primary sensitizers are considered to be caused by pollen. In contrast, LTPs are proteins stable to heat and digestion, causing allergy reactions to cooked foods. They are often associated with systemic and more severe reactions in addition to OAS, can be sensitizers to both pollen and foods, and are often associated with allergic reactions in Southern Europe.

7. APPLICATION OF PANALLERGENS: BET V 1 HOMOLOGS AND LTPS AS MODELS

One of the upcoming trends of allergen application is development of next generation of test systems, based on individual allergens. Such an allergy testing is known today as “component-resolved diagnostics”. Discovery of novel allergens underlies development of the assays for comprehensive analysis of sensitization and cross-reactivity profiles of allergens from food, pollen and other sources. For example, allergen biochip ImmunoCAP ISAC enables a single-step measurement of IgE antibodies to the fixed panel of 112 components from 51 allergen sources [122]. Today such Bet v 1 homologs like Cor a 1.0401 (hazelnut), Ara h 8 (peanut), Gly m 4 (soybean), Act d 8 (kiwi), Api g 1 (celery), Mal d 1 (apple), Pru p 1 (peach), Dau c 1 (carrot), Fra a 1 (strawberry), Aln g 1 (adler), Bet v 1 (birch), Art v 1 (mugwort), and Cor a 1.0101 (hazel pollen) are used for diagnostics of pollen-food allergy syndrome. Likewise, Cor a 8 (hazelnut), Jug r 3 (walnut), Ara h 9 (peanut), Tri a 14 (wheat), Pru p 3 (peach), Fra a 3 (strawberry), Ole e 7 (olive), Pla a 3 (plane tree), Art v 3 (mugwort), and Par j 2 (wall pellitory) are employed for diagnostics of LTP-related allergic reactions. Precise knowledge of the origins of allergic reactions is

necessary to prescribe a further allergen-specific immunotherapy (ASIT) correctly (Table 2).

Currently, plant food and pollen extracts as well as chemically modified allergoids are widely used in ASIT. Classical ASIT, based upon subcutaneous injections of aqueous food extracts is effective but has proven to be accompanied in some cases by anaphylactic side-effects. Treatment with recombinant or individual natural allergens delivers benefits with respect to pharmaceutical quality, standardization and dosing. Currently available data for the recombinant birch pollen and grass pollen allergens suggest that the ones can effectively replace natural allergen extracts [123]. Nevertheless, in spite of obvious advantages, ASIT with the use of individual allergens and their mixtures has not been introduced in medical practice and currently is just for clinical trial [124]. An in-depth knowledge of allergen cross-reactivity is imperative when selecting allergens for immunotherapy. As the limiting number of allergens in a vaccine preparation may be adequate to achieve the optimal therapeutic effect for each one, the cross-reactivity of clinically relevant allergens should be taken into account when making allergen selections for a vaccine compounding. When cross-reactivity between different pollens is substantial, selection of a single allergen within the cross-reactive pollen genus or subfamily may suffice. The observed effect may be caused by molecular similarity of allergenic proteins from different cross-reactive genera and species. It was shown that the birch pollen immunotherapy prevents anaphylaxis to the cross-reactive apple allergen Mal d 1 in mice [125]. Thus, a cross-reactivity opens the way to minimization of the number of allergenic proteins in a vaccine preparation. This ASIT model can be based on administration of most cross-reactive ubiquitous panallergens.

As individual allergens retain all moieties relevant for allergy reactions, treatment with hypoallergenic variants is considered to be the most perspective oncoming approach for ASIT that allows to reduce the risk of IgE-mediated therapy-induced side effects [126]. Modifications of allergen molecules aim to remove the B-cell epitopes and thus prevent cross-linking of IgE on allergic effector cells, including mast cells and basophils. Such hypoallergenic variants assure safe immunotherapy compared to crude allergen extracts or even individual allergens. Several hypoallergenic analogues of Bet v 1-related allergens (Gly m 4 [127], Pru av 1 [128], Mal d 1 [109]) were engineered by directed mutagenesis and produced. rBet v 1-FV became the first hypoallergenic variant of PR-10 family allergens,

and the first trial on humans was started [126]. Phase II of the clinical trials revealed no difference between effects of Bet v 1 analogue rBet v 1-FV and a natural pollen extract. At the same time, rBet v 1-FV can be administered in higher doses than the native protein with no increase in adverse effects. Several hypoallergenic LTPs (e.g. Par j 1/Par j 2 [129] and Pru p 3 [130]) were developed for more safe immunotherapy as well. Hypoallergenic analogues of Pru p 3 (rPru p 3 sur, rPru p 3 cys, rPru p 3 G, and rPru p 3 RA) are going to be tested in phases I/IIa and IIb of clinical trials under the FAST (Food Allergy Specific Immunotherapy) project, which aims to develop a safe alternative by replacing food extracts with hypoallergenic recombinant analogues of major allergens [131] (Table 2). This ambitious project will be carried out by consortium of six participating clinical centers in six countries.

In summary, both Bet v 1 homologs and LTPs represent significant panallergen families in terms of application in modern diagnostic kits and more safe immunotherapy approaches.

CONCLUSION

Both Bet v 1 homologs and LTPs belong to the superfamily of PR-proteins, which are the components of plant innate immunity system and play an important role in plant defense. Induction of their synthesis takes place in abiotic and biotic stress conditions and is regulated by major phytohormones involved in plant defense response. Protective function of PR-10 and PR-14 proteins in plants is also confirmed by the presence of antimicrobial activity, involvement of these proteins in plant adaptation to stress and enhanced tolerance to stress of transgenic plants overexpressing members of these families.

Bet v 1 homologs and LTPs do not show any structural similarity with the exception of existence of hydrophobic cavity, which has potential sites for the binding of various ligands. Bet v 1 homologs as well as LTPs seem to be rather nonspecific binders *in vitro* and their ability to form protein-ligand complexes depends on the hydrophobic cavity size, amino acid residues constituting the cavity and spatial structure of the ligand. Direct evidence of complex formation *in vivo* is not yet received. Nevertheless, it is believed that some functions of these proteins are linked with their ability to bind, transfer, storage and release such hydrophobic ligands as lipids and phytohormones as well as secondary metabolites in the case of some members of PR-10 family.

Bet v 1 homologs as well as LTPs are ubiquitous in various plant organs and tissues and are encoded by multigene families. PR-10 and PR-14 proteins have different cell localization, but are characterized by a similar spectrum of biological activities and are presumably involved in the same processes in plants. Bet v 1 homologs have intracellular localization and their biological activity as well as mechanism of antimicrobial action are linked with an ability to degrade RNAs. At the same time, LTPs are generally secreted to apoplast and their action is often related to an ability to affect the membrane permeability. Both Bet v 1 homologs and LTPs possibly take part in such processes in plants as stress signaling and response, metabolism of lipids, secondary metabolite biosynthesis, reproduction, symbiosis, and others.

Both Bet v 1 homologs and LTPs are abundant pollen and food allergens. They both display allergenic cross-reactivity responsible for manifestation of allergic reactions in majority of patients with food allergies to at least one representative of the corresponding family. The reason for this phenomenon is a high structural homology of the members of each family. At the same time, PR-10 and PR-14 proteins have significant structural differences between each other. LTPs have high heat- and protease-resistant spatial structures, stabilized by 4 disulphide bridges, while Bet v 1 homologs have labile spatial structures, which are denatured during thermal processing of foods and in gastrointestinal tract. There are structural differences that may lead to distinct epidemiology and severity of the allergic reactions caused by members of PR-10 and PR-14 families. Structural properties of LTPs may allow them to act as sensitizers and cause severe allergic reactions, while Bet v 1 homologs are not able to sensitize the immune system, but can only elicit allergic reactions due to cross-reactivity and cause mild symptoms (mainly OAS). To date, such panallergens as LTPs and Bet v 1 homologs become substantial element for modern component-resolved diagnostics and are considered to be promising candidates for safe allergen-specific immunotherapy (ASIT).

ABBREVIATIONS

AMPs	=	Antimicrobial peptides
ASIT	=	Allergen-specific immunotherapy
FA	=	Fatty acids
HR	=	Hypersensitive response
LAR or SAR	=	Local or systemic acquired resistance
LTPs	=	Lipid transfer proteins
OAS	=	Oral allergy syndrome
PR	=	Proteins pathogenesis-related proteins

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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