
Review article

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Pathogenesis-related proteins of plants as allergens

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Objective: Many pathogenesis-related (PR) proteins from plants are allergenic. We review the evidence that PR proteins represent an increasingly important group of plant-derived allergens.

Data Sources: A detailed literature search was conducted through PubMed and GenBank databases.

Study Selection: All reports in PubMed and GenBank related to PR protein allergens for which at least partial amino acid sequence is known were included.

Results: Production of PR proteins by plants is induced in plants by stress. Members of PR-protein groups 2, 3, 4, 5, 8, 10, and 14 have demonstrated allergenicity. PR2-, 3-, 4-, and 8-homologous allergens are represented by the latex allergens. Cross-reactivity of PR3 latex allergen, Hev b 6.02, with some fruit allergens may be a reflection of the representation of homologous PR proteins among varied plants. The expression of one of the representative PR5-homologous cedar pollen allergens, Jun a 3, is highly variable across years and geographic areas, possibly because of variable induction of this PR protein by environmental factors. PR10-homologous birch pollen allergen, Bet v 1, is structurally similar to and cross-reacts with PR10 proteins from fruits (eg, Mal d 1) which cause oral allergy syndrome. PR14 allergens (eg, Zea m 14) consist of lipid transfer proteins found in grains and fruits and are inducers of anaphylaxis.

Conclusions: PR-homologous allergens are pervasive in nature. Similarity in the amino acid sequences among members of PR proteins may be responsible for cross-reactivity among allergens from diverse plants. Induced expression of PR-homologous allergens by environmental factors may explain varying degrees of allergenicity. Man-made environmental pollutants may also alter the expression of some PR protein allergens.

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INTRODUCTION

Higher plants protect themselves against various stresses through patterned, physiologic alterations, or “defense responses.” Plant proteins produced as plant defense responses are called “defense-related proteins.”¹ One group of defense-related proteins produced in response to pathogens is

termed pathogenesis-related (PR) proteins or pathogen-response proteins.² PR proteins are characterized by certain common chemical properties, such as low molecular weight, stability at low pH, and resistance to proteases.³ The first PR protein was described in 1970 by Van Loon.⁴ This PR protein was produced in necrotic areas of tobacco (*Nicotiana tabacum* “Samsun”) leaves infected with tobacco mosaic virus. It is thought that this protein binds to tobacco mosaic virus and inhibits the spread to uninfected regions of the plant. In addition to viral infec-

tions, PR proteins are induced by other stresses, including fungal and bacterial infections, drought, flooding, freezing temperature, ozone, ultraviolet B light (UV-B) and mechanical injury.⁵ Thus, PR proteins may have other functions in plant homeostasis and adaptation. The PR proteins are now categorized into 14 groups. This grouping is based on similarities in their amino acid sequences, enzymatic activities, or other functional or physiologic properties (Table 1).² Of particular interest has been the demonstration that at least 20 of the biochemically characterized PR proteins are allergenic.

Plant strains expressing higher levels of certain PR proteins may be more resistant to environmental stress and disease. These properties may provide a selective survival advantage over plants that express PR proteins in lower concentrations.⁶ In some cases, these hardier strains may be selected for agricultural use.⁷ Given the allergenic potential of PR proteins, this selection may increase the allergenicity of cultivated plants. Recently, the Environmental Protection Agency has approved the application of harpin to agricultural plants. An engineered product of the fungus *Erwinia amylovora*, harpin enhances the plants’ tolerance against infection, insect damage, and drought (“Unique protein gives farmers chemical pesticide alternative,” AP, April 29, 2000). The induction by harpin of PR1, 2, and 5 in *Arabidopsis thaliana* (Brassica, mustard family)⁸ may explain the increased disease tolerance and drought resistance in treated plants. Although not

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Table 1. Classification of PR Proteins and Examples of their Function

Family	Representative protein	Accession no.	Properties
PR1	tobacco PR1a	X05959	antifungal
PR2	tobacco PR2	M60460	β -1,3-glucanase and IV
PR3	tobacco P,Q	M29868	type I, II, and IV chitinase
PR4	tobacco R	CAA41437	antifungal, <i>win</i> protein
PR5	tobacco S	JH0230	antifungal, thaumatin, osmotin, zeamatin
PR6	tomato inhibitor I	AAA34198	proteinase-inhibitor
PR7	tomato P69	AW035293	endoproteinase
PR8	cucumber chitinase	AAA33120	type III chitinase
PR9	lignin-forming peroxidase	J02979	peroxidase
PR10	parsley PR-1	X15085	ribonuclease
PR11	tobacco class V chitinase	CAA54373	type V chitinase
PR12	radish Ps-AFP3	T10243	defensin
PR13	<i>Arabidopsis</i> THI2.1	AAC41678	thionin
PR14	barley LTP4	Q43767	LTP

AFP: anti-fungal protein, LTP: lipid-transfer protein

yet examined, it is possible that produce obtained from harpin-treated plants may have enhanced allergenicity. Thus, the use of inducers of PR proteins, although reducing reliance on pesticides, might also increase the risk for unanticipated allergic reactions.

Among the 14 groups of PR proteins, groups 2, 3, 4, 5, 8, 10, and 14 contain allergens. However, the PR proteins within each of the groups have structural homologies in other

families, orders, and even classes of plants (Fig 1). Thus, individuals who are sensitized to PR proteins from the tissue of one plant may demonstrate cross-reactivity to homologous proteins in the same or different tissues from other plants. For example, avocado major allergen, Pers a 1, binds IgE from the sera of latex-allergic patients.⁹ Whereas other latex-derived allergens in the PR3-, 4-, and 8-homology groups have only been described in latex, each has a high

degree of sequence identity to other proteins in their respective groups. Thus, it is likely that other members of these groups will be identified as allergens.

Because the expression of PR proteins is inducible by environmental stress, plants growing under different conditions may express different levels of the allergenic PR proteins. For example, the amount of the PR5 allergen, Jun a 3, in mountain cedar (*Juniperus ashei*) pollen varies extensively.¹⁰ It is interesting to speculate that alterations in environmental conditions may increase the expression of allergenic PR proteins, thereby enhancing the allergic potency of the plant. Additionally, if man-made pollutants induce PR-protein expression, this may be one mechanism whereby the environment influences the prevalence and severity of allergic disease. Thus, evaluating the role of PR proteins in allergic reactions may help to explain some allergic cross-reactivity, as well as some of the variability in the frequency of sensitization and severity of reactions in sensitized individuals.

In this review, we will describe the current knowledge regarding the structure, function, and allergenicity of the PR proteins. In addition, potentially fertile areas of research on this emerging class of plant-derived allergens will be outlined.

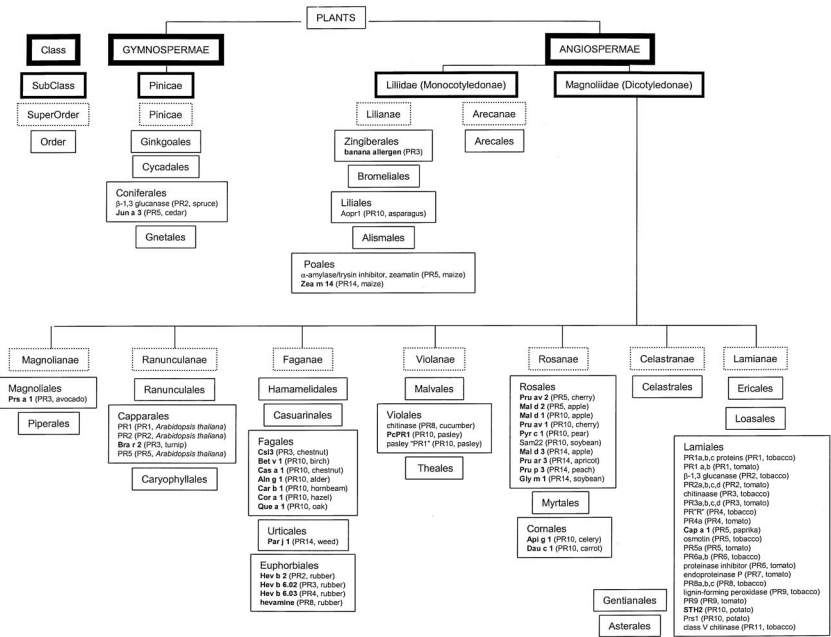


Figure 1. PR proteins with plant taxonomical classification. Allergens are shown in bold.

PR PROTEIN HOMOLOGOUS ALLERGENS

PR2-homologous allergens

Immediate-type allergic reactions provoked by natural rubber products have been increasingly reported around the world. The causative latex products are widespread, including medical devices, dental materials, and household items. Up to 50% of health care workers become sensitized to latex.^{11,12} The symptoms of latex allergy range from mild contact urticaria to asthma and anaphylactic reactions that frequently occur during surgical or endoscopic procedures. Ten allergens (Hev b 1 to 10) have been identified in latex proteins (Official List of Allergens, WHO/IUIS Allergen Nomenclature Subcommittee, 03.01.00). Among these allergens, Hev b 2 (β -1,3-glucanase)¹³ has homology to PR2 proteins. It also has homology to other β -1,3-glucanases from barley (S35156),¹⁴ wheat (S36235),¹⁵ rice (T02210), soybean (T05959), mustard (S42885), and cucumber (T07818). This homology may be responsible for the described cross-reactivity among Hev b 2 and these foods, although allergic reactions to these PR2 proteins have yet to be investigated.

PR3 and 4-homologous allergens

PR3 and PR4 allergens are classified as endochitinases that have antifungal properties. They are most typified by latex allergens. The PR3 and PR4 latex allergens are unique in that they are derived from the same precursor protein, Hev b 6, which has a molecular weight of 20 kD and contains 187 amino acids.¹⁶ Posttranslational modification yields a 5-kD N-terminal fragment, hevein (Hev b 6.02), a PR3 homologous protein, and a 14-kD C-terminal domain fragment, Hev b 6.03, a PR4 homologous protein. Both are reported to be allergenic with multiple IgE epitopes identified.¹⁷ Figure 2 shows the structural relationship between Hev b 6.02 and Hev b 6.03 and their IgE epitopes.

Cross-reactivity of PR3 latex allergens have been identified in the latex-fruit allergy syndrome, which describes the allergic reactions induced

by ingestion of fruit that cross-reacts with latex hevein. Some of the responsible fruit allergens have been identified as proteins of approximately 30 kD in banana, avocado, chestnut, kiwi, peaches, strawberries, and citrus.^{18,19} Allergens from these fruits, for which at least partial amino acid sequences have been determined, are shown in Table 2 and Figure 3. The amino acid sequences of the fruit allergens have 30% to 80% identity to that of Hev b 6.02. Hev b 6.02-like domains have been identified in banana,²⁰ avocado,⁹ chestnut,²¹ and turnip²² by N-terminal and/or internal amino acid sequencing.

Avocado (*Persea americana*) allergens can elicit diverse IgE mediated reactions, including anaphylactic reactions. The serum IgE from 75% of avocado and/or latex allergic patients reacted to Pers a 1,⁹ an endochitinase that inhibits growth and branching of fungi. Pers a 1 has 321 amino acids with 70% identity to Hev b 6.02, and a similar degree of identity with endochitinases from other plants: chestnut, 71.0%; rice, 73.8%; potato, 73.5%; and wheat, 77%. The N-terminal amino acid sequence of the 32-kD avocado class I endochitinase, Pa I 1,²¹ is identical (27 of 27) to that of Pers a 1, suggesting that the two are identical proteins.

Bra r 2 was recently found in chemically (ethephon or salicylic acid) treated turnip (*Brassica rapa*) roots.²² The sequence of its first 32 N-terminal amino acid has 70% identity to Hev b 6.02. The serum IgE from more than 80% of natural rubber latex hypersensitive patients reacted to this turnip allergen in an immunoglobulin (Ig)E-ELISA.

The C-terminal domain of latex prohevein (Hev b 6), Hev b 6.03 has homology to PR4 proteins from potato, tobacco, and soybean.²³ Eight distinct IgE epitopes of Hev b 6.03 have been reported, and there is no cross-reactivity to Hev b 6.02. Homology to several of these IgE epitopes was identified in PR4 proteins from potato, tobacco, and soybean. Although no direct association of PR4 proteins with the latex-fruit allergy syndrome has been identified, it has been reported that 40% of latex allergic patients have specific IgE to potato.²⁴

PR5-homologous allergens

The PR5 proteins contain a number of allergenic proteins (Table 3, Fig 4). Although the specific biochemical properties and activities of these proteins are unknown, PR5 proteins have seemingly diverse functions, such as drought and freeze resistance and antifungal activities. They have been found in diverse plants, including the sweet-tasting protein thaumatin from the African shrub, *Thaumatococcus daniellii*,²⁵ osmotin from tobacco,²⁶ α -amylase/trypsin inhibitor from maize,²⁷ anti-fungal protein from maize,²⁸ and zeamatin from maize.²⁹ The proteins within this group identified as allergens include: Jun a 3 from mountain cedar (*Juniperus ashei*) pollen, Pru av 2 from cherry (*Prunus avium*) berry, Mal d 2 from apple (*Malus domestica*) fruit, and Cap a 1 from paprika/bell pepper (*Capsicum annuum*).

Jun a 3 is the only reported PR protein allergen from the Gymnospermae class of plants. Gymnosperms bear seeds that are not enclosed within an

Hev b 6.02 (PR3)		
EQCGRQAGGK	<u>LCPNNLCCSQ</u>	WGWCSTDEY CSPDHCQSN CKDSGEGVGG 50
Hev b 6.03 (PR-4)		
GSASNVLATY	<u>HLVNSQDHGW</u>	DLNAASAYCS TWDANKPYSW RSKYGWTAFC 100
GPVGAHGQPS	CGKCLSVTNT	GTGAKATVRI <u>VDQCSNGGLD</u> LDVNVFRQLD 150
TDGKGYERGH	<u>LTVNYQFVDC</u>	GDSFNPLFSV MKSSVIN 187

Figure 2. Amino acid sequence of prohevein. The amino acid sequence of prohevein, Hev b 6, is shown with the major subunits. Hev b 6.02 (PR3) and Hev b 6.03 (PR4) are shown in bold. IgE epitopes are shown with underlines. Double underlined segments have been reported to be epitopes in two or more reports.

Table 2. PR3-Homologous Allergens

Allergen	Common name	Species	Source	MW (kD) (method)	AA	Family	Order
Hev b 6.02	rubber	<i>Hevea brasiliensis</i>	latex	20 (SDS-PAGE)	187	Euphorbiaceae	Euphorbiales
Pers a 1	avocado	<i>Persea americana</i>	fruit	32.0 (SDS-PAGE)	326	Lauraceae	Magnoliales
Bra r 2	turnip	<i>Brassica rapa</i>	root	25 (SDS-PAGE)	NA	Brassicaceae	Capparales
				18.673 (MS)		(Cruciferae)	
Csl3	chestnut	<i>Castanea sativa</i>	nut	32 (SDS-PAGE)	NA	Fagaceae	Fagales
banana allergen	banana	<i>Musa acuminata</i>	fruit	32 (SDS-PAGE)	NA	Musaceae	Zingiberales

MS: Mass spectrometry. References: Hev b 6.02,¹⁶ Pers a 1,⁹ Bra r 2,²² Bra r 2, Csl3,²¹ banana allergen.²⁰ Csl3 and banana allergen do not have official allergen names. NA: not available.

ovary (vs Angiospermae, which have seeds enclosed in an ovary).^{10,30} IgE ELISAs demonstrated that 42.9% of mountain cedar-hypersensitive patients reacted to Jun a 3, and peripheral blood mononuclear cells from 57.1% of mountain cedar-hypersensitive patients had a proliferative response to Jun a 3.¹⁰ One-third of Japanese cedar (*Cryptomeria japonica*, Taxodiaceae)-hypersensitive patients, who had not been exposed to mountain cedar pollen, also reacted to Jun a 3. This cross-reactivity might be attributable to the presence of unidentified Jun a 3-like proteins in Japanese cedars. Thus, many other gymnosperms may contain similar and potentially cross-reactive PR5 allergens. Additionally, a case of oral allergy syndrome because of tomato and kiwi has been associated with Japanese cedar pollinosis.³¹ Incubation with Japanese cedar pollen extract inhibited IgE binding to the 14-kD protein in tomato extract, suggesting that divergent plant species may contain cross-reacting allergens. Whereas proteins in the pectate lyase family^{32,33} (Jun a 1 from mountain cedar, Cry j 1 from Japanese cedar, and Cha o 1 from Japanese cypress) are responsible for some cross-reactivity among members of Cupressaceae and Taxodiaceae (cedars, cypresses, and junipers) families, PR5 proteins might also cause cross-reactivity among these plant families.

Several fruits contain PR5-homologous allergens. Their functions are unknown, but a sweet taste may be a property of some of these. Thaumatin, which is 100,000 times sweeter than sucrose on a molar basis,³⁴ has been suggested for use as a low-calorie food

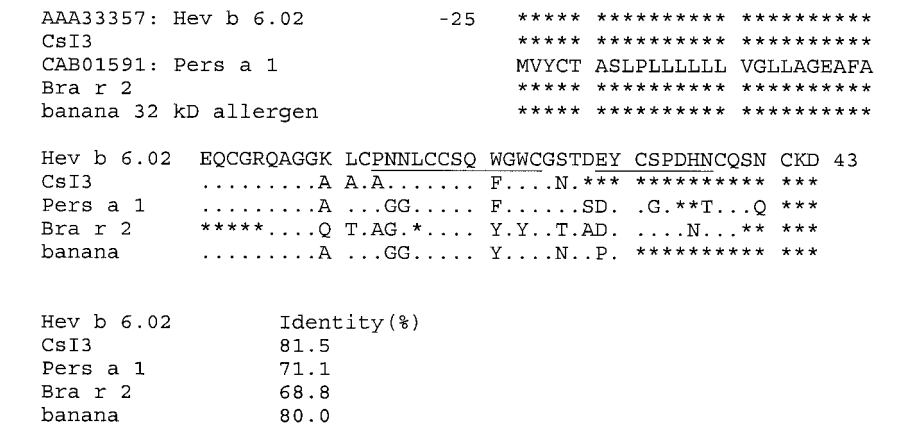


Figure 3. Amino acid sequences of PR-3 allergens. The amino acid sequences and identity to Hev b 6.02 of the PR-3 allergens are shown with the identified IgE epitopes (underlined). Hev b 6.02: rubber latex allergen; CsI3: chitinase from the nuts of chestnut; Pers a 1: avocado fruit allergen; and Bra r 2: turnip root allergen. Dots indicate amino acid identity, asterisks indicate absent/unidentified amino acids.

sweetener. Jun a 3¹⁰ and the next example of PR5 allergens, Pru av 2,³⁵ have approximately 50% amino acid sequence identity with thaumatin. This suggests that the allergenic potential of thaumatin should be tested before widespread use as a food additive.

Pru av 2 is the major allergen in cherries and is suggested as a contributor to oral allergy syndrome.³⁵ Sequence analysis revealed a 49.7% sequence identity to Jun a 3, and 38.8% to thaumatin, from wheat (*Triticum aestivum*). Although Pru av 2 did not possess the putative sweet motif of thaumatin (Y⁹⁵XK⁹⁷XXXXXXXXXK¹⁰⁶), its sweetness has not been tested.²⁹

The apple allergen, Mal d 2, may contain a sweet motif homologous to that of thaumatin.²⁹ Similar to Jun a 3, the amount of this allergen was shown to vary between apple strains and storage conditions. Mal d 2 content increased during ripening, but the Mal d 2 content

has not yet been correlated with allergenicity.³⁶ However, because 75% of apple-allergic patients' sera reacted with this allergen, increasing Mal d 2 levels may potentially increase the risk for allergic sensitizations and reactions.

The last example in this group is Cap a 1 from paprika/bell pepper. Although this allergen was initially identified by its reactivity with sera from patients with the mugwort-birch-celery-spice syndrome, it has neither homology nor cross-reactivity to the birch major allergen, Bet v 1.³⁷ Dried paprika extract and fresh bell pepper both contain 23-kD allergens that have an identical N-terminal amino acid sequence.³⁸

PR8-homologous allergens

The 30-kD latex protein, hevamine, seems to be a relatively minor latex allergen. One patient was reported to have serum IgE directed against hevamine.¹⁷ Hevamine has 61.2% iden-

Table 3. PR5-Homologous Allergens

Allergen	Common name	Species	Source	MW (kD) (method)	AA	Family	Order
Jun a 3	cedar	<i>Juniperus ashei</i>	pollen	30 (SDS-PAGE) 20.941 (MS)	199	Cupressaceae	Coniferales
Pru av 2	cherry	<i>Prunus avium</i>	berry	30 (SDS-PAGE)	222	Rosaceae	Rosales
Mal d 2	apple	<i>Malus domestica</i>	fruit	31 (SDS-PAGE)	245	Rosaceae	Rosales
Cap a 1	paprika/bell pepper	<i>Capsicum annuum</i>	fruit	23 (SDS-PAGE)	NA	Solanaceae	Lamiales

MS: Mass spectrometry. References: Jun a 3,¹⁰ Pru av 2,³⁵ Mal d 2,³⁶ Cap a 1.^{37,38}

P81295:Jun a 3	-26	MARVSE LAFLLAATLA ISLHMQEAGV		
p50694:Pru av 2		***MMK TLVVVLSLSL TI.SFGG.HA		
cac10270:Mal d 2		*MMK.Q V.S..GL... *.PFSG.HA		
Cap a 1		*****		
Jun a 3	VKFDIKNQCG	YTVWAAGLP*G	GGKRLDQ*****GQT WTVNLAAGTA SARFWGRTGC	50
Pru av 2	ATISF..N.P	.M...PGT.TSD	QKPQ.STTGFEELAS.A SFOLDTPVPW NG...A....	
Mal d 2	A.ITTT.N.P	N...PGT.TGD	QKPQ.SLTGFELASKA SRSDV.PSPW .G.....R.	
Cap a 1	AT.EVR.NXP	*****	*****	
Jun a 3	TFDASGKGSC	QTGDC*GGQLS	CTVSGA*VPAT LAEYQ***SDQD YYDVSLVDGF	100
Pru av 2	ST....FV.	A.A..AS..VM	.NGN..IP... ..FNIPAGGG.. F.....	
Mal d 2	ST..A..FT.	E.A..GS..VA	.NGA..VP... .V.I..IAANGG..	
Cap a 1	*****	*****	*****	
Jun a 3	NIPLAINPTN	AQ**CTAPACKA	DINAVCPSEL KVDG*****GCNSAC NVFKTDQYCC	150
Pru av 2	.L.MSVT.QG	GTGD.KTAS.P.	NV..... QKK.SDGSVVA.L... VK.G.P....	
Mal d 2	.L.MSVA.QG	GTGE.KPSS.P.	NV.K...AP. Q.KAADGSVIS.K... LA.GDSK...	
Cap a 1	*****	*****	*****	
Jun a 3	R**NAYVDNCPA	TNYSKIFKNQ	CPQAYSAYKD D*TATFACASG *TDYSIVFCP*	199
Pru av 2	TPPQNTPET..P	...E..H.A	..D....Y.. .KRG..T.NG. P*N.A.T....	
Mal d 2	TPP.NTPET..P	.E..E..EK.	KNS..T.SG. P*..V.T....	
Cap a 1	*****	*****	*****	
Jun a 3	Identity(%)			
Pru av 2	49.7			
Mal d 2	49.2			
Cap a 1	22.2			

Figure 4. Amino acid sequences of PR5 allergens. The amino acid sequences of PR5 allergens are shown with the identified IgE epitopes (underlined). Jun a 3: mountain cedar pollen allergen; Pru av 2: cherry berry allergen; Cap a 1: paprika/bell pepper allergen; Mal d 2: apple fruit allergen. Symbols are as in Figure 2.

tity to another PR8 protein, cucumber chitinase.³⁹ Whether PR proteins of this group prove to be important allergens remains to be seen.

PR10-homologous allergens

Birch (*Betula verrucosa*) pollinosis is one of the main causes of allergic rhinitis and asthma in middle and northern Europe and northern Japan (Table 4). The best characterized allergen in this group is Bet v 1, the major allergen in birch pollen. Many of the other PR10 allergens cross-react with Bet v 1 and cause birch pollen oral allergy syndrome. Approximately 70% of birch pollen-hypersensitive patients have oral allergy syndrome after ingestion of apples, pears, cherries, apricots, hazelnuts, celery tubers, carrots, or other vegetables.⁴⁰ PR10 allergens from Rosaceae (apple and pear),⁴⁰ Prunoideae (cherry and peach),⁴¹ and Apiaceae (celery and carrot)^{42,43} have sequence homology to Bet v 1. Sec-

Table 4. PR10-Homologous Allergens

Allergen	Common name	Species	Source	MW (kD) (method)	AA	Family	Order
Bet v 1	birch	<i>Betula verrucosa</i>	pollen/ leaf	17.0 (SDS-PAGE, Gel chromatography)	159	Betulaceae	Fagales
Aln g 1	alder	<i>Alnus glutinosa</i>	pollen	17.4 (SDS-PAGE)	159	Betulaceae	Fagales
Car b 1	hornbeam	<i>Carpinus betules</i>	pollen	17 (AA)	159	Betulaceae	Fagales
Cor a 1	hazel	<i>Corylus avellana</i>	pollen/ nut	18 (SDS-PAGE)	159	Betulaceae	Fagales
Cas s 1	chestnut	<i>Castanea sativa</i>	pollen	22 (SDS-PAGE)	NA	Fagaceae	Fagales
Que a 1	oak	<i>Quercus alba</i>	pollen	17 (SDS-PAGE)	NA	Fagaceae	Fagales
Mal d 1	apple	<i>Malus domestica</i>	fruit	17.5 (AA)	159	Rosaceae	Rosales
Pru av 1	cherry	<i>Prunus avium</i>	berry	18.0 (SDS-PAGE)	160	Rosaceae	Rosales
Pru ar 1	apricot	<i>Prunus armeniaca</i>	fruit	17.5 (AA)	159	Rosaceae	Rosales
Pyr c 1	pear	<i>Pyrus communis</i>	fruit	17.4 (AA)	158	Rosaceae	Rosales
Api g 1	celery	<i>Apium graveolens</i>	leaf	16.3 (SDS-PAGE)	154	Apiaceae	Cornales
Dau c 1	carrot	<i>Daucus carota</i>	root	16, 18 (SDS-PAGE)	154	Apiaceae	Cornales
PcPR1	parsley	<i>Petroselinum crispum</i>	leaf	17 (SDS-PAGE)	NA	Apiaceae	Cornales
STH2	potato	<i>Solanum tuberosum</i>	root	17 (SDS-PAGE)	NA	Solanaceae	Lamiales

AA: amino acid analysis. References: Bet v 1,^{55,56} Aln g 1,⁵⁷ Car b 1,⁵⁸ Cor a 1,⁵⁹ Cas s 1,⁶¹ Que a 1,⁶² Mal d 1,^{40,42} Pru av 1,⁴¹ Pyr c 1,⁴⁴ Api g 1,^{42,43} Dau c 1,^{47,62} PcPR1,^{63,64} STH2.^{64,65} PcPR1 and STH2 do not have official allergen names.

ondary structure prediction of birch (Bet v 1), cherry (Pru a 1), apple (Mal d 1), pear (Pyr c 1), celery (Api g 1), and carrot (Dau c 1) allergens indicate the presence of similar structural elements, particularly, the phosphate-loop region in a domain common to all these allergens.⁴⁴ Other proteins from Solanaceae (tomato and potato) have similar structure to Bet v 1 that may cause cross-reactivity. Bet v 1 is also

present in low concentration in leaves. Bet v 1 has amino acid sequence identity with the allergens of related species, alder, hazel, hornbeam, and chestnut classified in the Fagales order. The chestnut pollen allergen Cas s 1 cross-reacts with Bet v 1.

Oak (*Quercus ilex*) may also have similar pollen allergens. Anaphylactic reaction occurring after ingestion of holm oak nuts has been reported.⁴⁵

Binding of IgE to the 17-kD major protein of holm oak nuts was inhibited by 62% after absorption of the patient's serum with Bet v 1. The amino acid sequence of the oak nut allergen has not been determined, but homology to Bet v 1 is suspected.

The most common pollen-fruit syndrome is apple allergy in birch pollinosis patients. Approximately 70% of the birch pollen hypersensitive patients react to Mal d 1. IgE epitope analysis by site-directed mutagenesis has shown that Ser¹¹² (Fig 5) was essential for both IgE binding and cross-reactivity between Mal d 1 and Bet v 1.⁴⁶ Figure 5 shows that Ser¹¹² is conserved in all the reported sequences of PR10 allergens.

Pru av 1 from cherries (*Prunus avium*) has 40% to 60% identity to other related tree pollen allergens and other PR10 proteins from parsley (Prl 3), potato (Prs1), and soybean (Sm22), none of which have been demonstrated to be allergens. Site-directed mutagenesis of Pru av 1 revealed that Ser¹¹² is critical for binding IgE from hypersensitive patients.⁴⁴ (Fig 5)

Dau c 1 from carrot (*Daucus carota*) roots, was identified by immunoblotting with sera from carrot-hypersensitive patients.⁴⁷ Three isoforms, all with 154 amino acids, are reported. They have 98% identity to CR16 (carrot root protein), 81% to Api g 1 (celery allergen), 60% to PcPR1 (PR protein from parsley), 38% to Bet v 1, and 39% to Mal d 1 amino acid sequences. Cross-inhibition assays show that Bet v 1 and Api g 1 cross-react with Dau c 1.

PR14-homologous allergens

Plant PR14 proteins have functional properties of lipid transfer proteins.⁴⁸ Lipid transfer proteins are present in relatively high concentrations in vascular tissue and in the outer cell layers of plants and inhibit bacterial and fungal pathogens. Allergens in this group can cause Rosaceae (apples, peach, and apricot) fruit allergy in individuals without birch or grass pollen hypersensitivity (Table 5, Fig 6).⁴⁹ Patients who are allergic to PR14 allergens in fruits tend to have a higher rate of anaphylaxis (36%)

P15494:Bet v 1	M GVFNYETETI SVIPAARLFK AFILDGDNLF PKVAPQAIS SVENIEGNNGP	50
P38948:Aln g 1	A..P.....K.L.....E.V.....	
P38950:Car b 1	A..P.....NK.I.....S.....V.....	
Q08407:Cor a 1	V..P.....SYV.....K.I.....T.....V.....	
AAB29092:Cas s 1	*...TX.SQE...P.....V.....*****	
D53288:Que a 1	*...TF.N.SQE...AP.X.....LF.....*****	
P43211:Mal d 1	...YTF.N.F...E..PS.....V..A...I...I.....K QA.IL.....	
AAC02632:Pru av 1	...T..S.F...E..PP.....V..A...V...I.....K HS.IL..D..V	
AAB97141:Pru ar 1	...T..S.F...PEK.....V..A...I...I.....T.VK GT.IL..D..V	
AAC13315:Pyr c 1	...LYTF.N.F...E..PP.....V..A...I...I.....K HA.IL.....	
P49372:Api g 1	...QTHVL.L...SVS.EKI.Q G.VI.V.TVL...A..G.YK...*K.D..A	
CAB06416:Dau c 1	...AQSHSL.I...SVS.EKI.S GIV..V.TVI...A..G.YK...*VK.D..A	
CAB31086:PcPR1	...QKS.V...SVS.EK...GLC..I.T.L...Q.L.G..K...S.TL..D..V	
S35161:STH2	...TS.TH...TP.APT...LVV.S...I...LM...*K...AE.D*	
Bet v 1	GTIKKISFPE GPFKVKVDR VDEVDHTNFK YNYSVIEGGP IGDTEKISN	100
Aln g 1	T...S.....RV...SF.....A V..A...VC.	
Car b 1	T.S...S.V...E...E.I.....T....D. L..K...V.H	
Cor a 1	N.T.G...S.Y...E...N...T...S.T....D. L..K...VCH	
Cas s 1	*****	
Que a 1	*****	
Mal d 1	T.G...SQYG...H. I.SI.EASYS...S.TL...DA LT..I...NY	
Pru av 1	T.G...SQYG...HK I.SI.KE.YS...S.TL...DA L.....Y	
Pru ar 1	VT.G...SQYA...H...GI.KD.LS...S.TL...DA LS.VI.N.AY	
Pyr c 1	T.G...SQYG...H...SI.EASYS...A.TL...DA LT..I...Y	
Api g 1	...L.I.TL.D...G.ITMTL...I.G.NKEALT FD...D.DI LLGFI.S.E.	
Dau c 1	...VRI.TL...S.ITSMVT...T.A.NKEALT...DST...D.DI LLGFI.S.ET	
PcPR1	...V.LVHLGD AS...TM.QK...AI.KAT.T...S.I.D.DI LLGFI.S.N.	
STH2	...S.*MN.V...S.I...L.HK IHV...DK.LV TK..M...DV L..K...S.Y	
Bet v 1	EIKIVATPDG GSILKISNKY HTKGDHEVKA EQVKASKEMG ETLRLAVESY	150
Aln g 1	A...F.....IN...I.IE.KA VG..K....	
Car b 1	...L...A.G...V...S.F...A.Y...N...EM.GA...A..K.....	
Cor a 1	...L...A.G...S.F...A...IN...EM.GA...A..K.....T.	
Cas s 1	*****	
Que a 1	*****	
Mal d 1	...T.L...CGS*...TI.SISH...NI.I.E...H..VG..KA HG.FKLI...	
Pru av 1	...T.L...S.S...I.S.TSH...V.I.E...H..G..KA SN.FKLI.T.	
Pru ar 1	...D.L...S...V.TTSH...V.I.E...G..KA AG.FKLI.A.	
Pyr c 1	...A.L...SGS*...TI.SISH...I.I.E...H..G..KA HG.FKLI...	
Api g 1	...HVVL.P.A...C.TTAIF...AV.PE...NI.YAN.QN TA.FK.L.A.	
Dau c 1	...HLV.P.A...T.TTAIF...AV.PE...NI.FADAQN TA.FK.L.A.	
PcPR1	...HFTA.PNA...CTV.STIIF...AV...NI.FANDQN L.IFK...A.	
STH2	...DL..E.HGN...CVC.SITE...YVL.D...EHNEGGKQ...ME.FKI..A.	
Bet v 1	LLAHSDAYN Identity (%)	159
Aln g 1	81.8	
Car b 1	TAE...76.1	
Cor a 1	AE...73.6	
Cas s 1	*****70.8	
Que a 1	*****63.6	
Mal d 1	...K..P....56.3	
Pru av 1	...KG.P....59.7	
Pru ar 1	...NP....59.7	
Pyr c 1	...KD.P....57.0	
Api g 1	...I.N*****41.8	
Dau c 1	...I.N*****38.6	
PcPR1	...I.N*****42.9	
STH2	...NPSV.A 47.1	

Figure 5. Amino acid sequences of PR10 allergens. The amino acid sequences of PR10 allergens are shown with the identified IgE epitopes underlined. Bet v 1: birch pollen allergen; Aln g 1: alder pollen allergen; Car b 1: hornbeam pollen allergen; Cor a 1: hazel pollen and nuts allergen; Cas s 1: chestnut pollen allergen; Que a 1: white oak pollen allergen; Mal d 1: apple fruit allergen; Pru av 1: cherry berry allergen; Pru ar 1: apricot berry allergen; Pyr c 1: pear fruit allergen; Api g 1: celery allergen; Dau c 1: carrot allergen; PcPR1: parsley allergen; STH2: potato allergen.

Table 5. PR14-Homologous Allergens

Allergen	Common name	Species	Source	MW (kD) (method)	AA	Family	Order
Zea m 14	maize	<i>Zea mays</i>	grain	13.1 (AA)	119	Poaceae	Poales
Pru ar 3	apricot	<i>Prunus armeniaca</i>	fruit	9.3 (AA)	91	Rosaceae	Rosales
Pru p 3	peach	<i>Prunus persica</i>	fruit	9.178 (AA)	91	Rosaceae	Rosales
				9.138 (MALDI)			
Mal d 3	apple	<i>Malus domestica</i>	fruit	9.058 (MALDI)	91	Rosaceae	Rosales
				9 (SDS-PAGE)			
Par j 1	weed	<i>Parietaria judaica</i>	pollen	10.6, 14.7 (AA)	631, 794	Urticaceae	Urticales
Gly m 1	soybean	<i>Glycine max</i>	hull	7.0, 7.5 (SDS-PAGE)	NA	Fabaceae	Fabales

AA: amino acid analysis; MALDI: matrix-assisted laser desorption/ionization. References: Zea m 14,⁶⁶ Pru p 3,⁶⁷ Mal d 3,⁶⁷ Par j 1,⁶⁸ Gly m 1.⁶⁹

than patients having fruit allergy attributable to PR10 allergens (18%).

The weed pollen allergen Par j 1 is also a weaker PR14 homolog. The pollen of *Parietaria judaica* is the main cause of immediate hypersensitivity in the Mediterranean area, affecting 50% of allergic patients. The dominant IgE epitope was identified in the first 30 amino acids of Par j 1, and Cys¹⁴ and Cys²⁹ were found to be essential for IgE binding.⁵⁰ The molecular structure of Par j 1 has been modeled after that of the soybean protein, hydrophobic seed protein (HPS), which is thought to be identical to the allergen, Gly m 1. Gly m 1 has an identical N-terminal amino acid sequence to HPS. HPS crystal and Par j 1 model structures show they have an overall α - α - α - β secondary structure with four disulfide bonds that presumably maintain a stable core structure.⁵¹ This structure may be important for maintaining conformational IgE epitopes as evidenced by the loss of the major epitope after disruption of the Cys¹⁴ and Cys²⁹ crosslinks. The similarity of Par j 1 to Gly m 1 and their divergence from other PR14 allergens has led some authors to suggest that they should be classified in a distinct PR group.²

INDUCTION OF PR PROTEINS BY ENVIRONMENTAL STIMULI

Plants continuously produce active oxygen species (AOS) as a consequence of normal cellular metabolism. Under normal conditions, plants rapidly metabolize these AOS with the help of constitutive antioxidant enzymes or metabolites. However, when subjected to environmental stress, excess AOS is

generated.⁵² AOS-regulated pathways include systemic-acquired resistance and the hypersensitive response which are characterized by induction of PR proteins. A list of reports describing induction of PR proteins by environmental and biologic agents is shown in Table 6. Among these stimuli, some can be attributed to human activity. Increases in UV-B light and ozone might induce PR1, 2, 5, and 10 proteins in some regions. Salicylic acids used as a rubber softeners and ethephone and ethylene used as pesticides tend to induce PR1, 2, 3, 5, and 10. Harpin, a bacterial-derived inducer of

proteins of the PR1, 2, and 10 groups has been approved for use as a biopesticide in agriculture. Similarly, salicylic acid and ethephone have been studied for their ability to induce disease resistance in other food crops. The allergenic PR protein, Bra r 2^{21,22} (a turnip allergen) has been shown to be induced when the turnip defense mechanisms were activated by these agents. Because IgE antibodies against latex allergen prohevein (PR3) cross-react with Bra r 2, patients with rubber latex allergy might potentially have allergic reactions to turnips treated with these chemicals.

P19656:Zea m 14	M	ARTQQLAVVA	TAVVALVLLA	AATSEAAISC	GGVASAIAPC	ISYARGGGSG	50
P81651:Pru ar 3	*	*****	*****	*****	.T.	.G.V. . . .	AV
P81402:Pru p 3	*	*****	*****	*****	.T.	.P.V. . . .	AV
CAB96874:Mal d 3	*	*****	*****	*****	.T.	.Q.T.S. . .	AV
Q40905:Par j 1	MRTVSARSSV	.LVVIV.A.L	VWTSSASVAP	.P.PGSEET.	.T.VG.LM.	LPFVQ.KEKE	
CAA59370:Par j 2	*	*****	*****	*****	*****	*****	
AAB34755:Gly m 1	*	*****	*****	*****	.LIT	RPSXPDLISX	LN*IL. .SL.
Zea m 14	PSAGCCSGVR	SLNNAARTTA	DRRAACNCLK	NAAAGVSGLN	AGNAASIPSK		100
Pru ar 3	.P.*. .N.I.	NV..L....P	..T.....	GLSGSI..V.	PN...A..G.		
Pru p 3	.P.*. .N.I.	NV..L....P	..Q.....	QLS.S.P.V.	PN...AL.G.		
Mal d 3	.P.*. .N.I.	TI.GL....	..QT.....	.L..SI..V.	PN...GL.G.		
Par j 1	..K....AK	R.DGETK.GP	G.VH..E.IQ	T.MKTY.DID	GKLVSEV.KH		
Par j 2	..K....AK	R.DGETK.GP	Q.VH..E.IQ	T.MKTY.DID	GKLVSEV.KH		
Gly m 1	TVDDXXALIG	G.XDX*****	*XAIV*****	*****	*****		
Zea m 14	CGV***SIPYTIS	TSTDCSRVN*	*****	*****	*****		119
Pru ar 3	...***N...K..	A..N.AT.K*	*****	*****	*****		
Pru p 3	...***...K..	A..N.ATEK*	*****	*****	*****		
Mal d 3	...***NV..K..	...N.AT.K*	*****	*****	*****		
Par j 1	..IVDSKL.*P.D	VNM..KTLGV	LHYKGN****	*****	*****		
Par j 2	..IVDSKL.*P.D	VNM..KT.GV	VPRQPQLPVS	LRHGPVTGPS	DPAHKARLER		
Gly m 1	*****	*****	*****	*****	*****		
Zea m 14	*****	***	Identity (%)				
Pru ar 3	*****	***	63.7				
Pru p 3	*****	***	62.6				
Mal d 3	*****	***	62.6				
Par j 1	*****	***	28.8				
Par j 2	PQIRVPPAP	EKA	35.5				
Gly m 1	*****	***	14.3				

Figure 6. Amino acid sequences of PR14 allergens. Amino acid sequences of PR14 allergens are shown. Zea m 14: maize grain allergen; Pru ar 3: apricot fruit allergen; Pru p 3: peach fruit allergen; Mal d 3: apple fruit allergen; Par j 1: weed pollen allergen; Gly m 1: soybean hull allergen.

Table 6. PR Protein Induction by Environmental Factors

Environmental stimulus	PR-protein	Source	Reference
UV-B	1, 2, 5	<i>Arabidopsis</i> , rice	5, 70
UV-C	10	lupine	71
Ozone	1, 2, 3, 10	tobacco, Norway spruce, parsley	72–75
Drought	1, 3	tomato	76, 77
TMV	1, 5	tobacco	78, 79
TPMV	5	tomato	73
Harpin	1, 2, 5	<i>Arabidopsis</i>	8
Salicylic acid	1, 2, 3 (Bra r 2), 5, 10	tobacco, turnip, lupine, bean, <i>Arabidopsis</i>	5, 22, 71, 78–80
Acetylsalicylic acid	1	tobacco	81
Salicylhydroxamic acid	1	tomato	82
Ethylene	1	tobacco, tomato	77, 79, 83
Copper chloride	2, 5	rice	70
Ethephone	3 (Bra r 2)	turnip	22
Sodium chloride	5	tobacco	26
Glucose	3	tobacco	84
Sucrose	3	tobacco	84

Allergic proteins are shown in bold.

TMV: tobacco mosaic virus, TPMV: tomato planta macho viroid

The content of Jun a 3 (PR5 protein from mountain cedar pollen) among widely collected pollen from different years and different regions was compared with total pollen protein.¹⁰ The proportion of pollen protein attributable to Jun a 3 varied from 1.8% to 38.0%. This variation may have been attributable to environmental conditions during pollen formation, and might be an example of how environmental changes could alter the incidence and/or severity of allergic disease.

Air pollutants from industry and automobiles are considered cofactors contributing to the recent increase in allergic disease and asthma. For example, pollutants have been shown to enhance IgE responses to Japanese cedar pollen.⁵³ In an attempt to examine the effect of air pollutants on Bet v 1 expression, the levels of Bet v 1 in birch pollen were examined as a function of distance from industrial pollutant point sources.⁵⁴ That investigation failed to demonstrate a correlation. Those authors concluded that other factors, including shading, soil properties, and genetics may have had stronger influences on the composition of birch pollen allergens. However, this single study could not exclude industrial or

other sources of pollutants as factors affecting pollen allergen expression.

CONCLUSION

We have reviewed the currently identified PR protein allergens. The PR proteins represent an important, emerging group of allergens in humans. They are important because of their propensity to induce allergic reactions and, because similar PR structures in different plant species may be responsible for a high frequency of cross-reactivity among plant allergens. Use of chemical inducers of PR proteins in agriculture and man-made pollutants in the environment may induce the production of PR protein allergens, potentially resulting in enhanced sensitization and severity of allergic reactions.

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CME Examination

1–5, T Midoro-Horiuti, EG Brooks, and RM Goldblum. 2001;87:261-271. Answers found on page 310.

CME Test Questions

1. Which of the following can induce PR proteins?
 - a. Fungal infection.
 - b. Bacterial infection.
 - c. Drought.
 - d. High temperature.
 - e. Ozone.
 2. Which plants produce PR proteins?
 - a. Tobacco.
 - b. Tomato.
 - c. Maize.
 - d. Birch.
 - e. Cedar.
 3. Why are PR proteins important in allergy?
 - a. They are the cause of cross-reactivity between plant allergens and animal allergens.
 - b. They are the cause of cross-reactivity among diverse species of plants.
 - c. The content of PR proteins in the same species is stable.
 - d. All 14 groups of PR proteins have homologous amino acid sequences and similar enzyme activity.
 - e. Plant strains which produce less PR proteins have been selected for agriculture.
 4. Which of the following statements is true about PR10 proteins?
 - a. About 30% of the birch pollen-allergic patients react to allergens contained in fruits and nuts.
 - b. Birch pollen allergy is more common in the United States than in northern Europe.
 - c. Birch pollen allergen, Bet v 1, mainly causes atopic dermatitis.
 - d. The oral allergy syndrome induced by PR10 homologous allergens is mainly because of banana, avocado, and kiwi allergens.
 - e. The oral allergy syndrome induced by PR10 homologous allergens is because of allergens contained in tree nuts.
 5. Which of the following statements is true about PR-homologous allergens?
 - a. Fruit allergy patients without birch pollen allergy have a lower rate of anaphylaxis.
 - b. PR-homologous allergens of fruits are the cause of cross-reactivity among latex, cedar pollen, and birch pollen.
 - c. Cedar pollen allergen and birch pollen allergen cross-react because of cross-reacting PR proteins.
 - d. The latex-fruit allergy syndrome is not common in middle and northern Europe.
 - e. The turnip allergen, Bra r 2, was reported to be inducible.
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