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Characterization of IgE-binding epitopes of peanut (*Arachis hypogaea*) PNA lectin allergen cross-reacting with other structurally related legume lectins

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ABSTRACT

Sera from peanut allergic patients contain IgE that specifically interact with the peanut lectin PNA and other closely related legume lectins like LcA from lentil, PsA from pea and PHA from kidney bean. The IgE-binding activity of PNA and legume lectins was assessed by immunoblotting, surface plasmon resonance (SPR) and ELISA measurements, using sera from peanut allergic patients as a IgE source. This IgE-binding cross-reactivity most probably depends on the occurrence of structurally related epitopes that have been identified on the molecular surface of PNA and other legume lectins. These epitopes definitely differ from those responsible for the allergenicity of the major allergens Ara h 1, Ara h 2 and Ara h 3, also recognized by the IgE-containing sera of peanut allergic patients. Peanut lectin PNA and other legume lectins have been characterized as potential allergens for patients allergic to edible legume seeds. However, the clinical significance of the lectin–IgE interaction has to be addressed.

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1. Introduction

Allergy to peanut and other legume seeds has now become a major concern of public health not only in children and adolescents, but also in adults with a worrying increase of Quincke oedema or anaphylactic shocks during the last 5 years, especially in industrial countries (Sampson, 2004). The major (glyco)protein allergens responsible for peanut allergy have been identified and characterized. They belong to the different families of vicilins (Ara h 1) (Burks et al., 1991), conglutins (Ara h 2, Ara h 6, and Ara h 7) (Burks et al., 1992), and glycinins (Ara h 3 and Ara h 4) (Rabjohn et al., 1999). Similar allergens also occur in the closely related edible legumes (pea, soybean, kidney bean, and lupine) (de Leon et al., 2003; Goetz et al., 2005; Magni et al., 2005) and other tree nuts (Pecan nut, Brazil nut, and Pistachio) (Wang et al., 2002, 2003; Robotham et al., 2005) that apparently give rise to IgE-binding cross-reactions with the peanut allergens (Barre et al., 2008). However, seed proteins consist of an extremely complex mixture of individual proteins with particular properties (enzyme activity, sugar- or lipid-binding

activity) besides participating in the protein bodies as seed storage proteins (Shewry et al., 1995). Among these proteins, lectins are known for a long time as a widespread group of plant proteins with interesting properties like their ability to trigger some non-specific lymphoblastic transformation in relation to their carbohydrate-binding properties (Stavy et al., 1971). Besides their occurrence in seeds, lectins have been characterized in other plant organs, namely in tubers, bulbs or fruits (Van Damme et al., 1998).

Although lectins are abundantly represented in, e.g. edible fruits (banana and tomato), bulbs (onion, garlic, and shallot), tubers (potato) or in dietary legume seeds (soybean, peanut, kidney bean, and pea), their potential allergenicity has been only scarcely investigated and thereby appears as poorly documented. However, several reports exist on the potential allergenicity of Con A from the Jack bean (*Canavalia ensiformis*) seeds (Gollapudi and Kind, 1975; Siraganian and Siraganian, 1975; Mitchell and Clarke, 1979) and other plant lectins (Barnett et al., 1983; Zavazal and Kraus, 1985; Shibasaki et al., 1992; Burks et al., 1994; Chung and Champagne, 1999; Haas et al., 1999; Gruber et al., 2005). A few cases of type I allergy to elderberry (*Sambucus nigra*) have been recently attributed to a ribosomal activating protein (RIP) of the pollen (Förster-Waldl et al., 2003). In fact, the amino acid sequence stretches available for this so-called RIP suggest that this protein most probably corresponds to a lectin of the ricin B-chain family that also occurs in the elderberry fruits (Van Damme et al., 1997). However, rather conflicting results were reported on the allergenicity of the peanut (*Arachis hypogaea*) lectin PNA. In a previous study on 12 patients with atopic dermatitis and a positive food

Abbreviations: Con A, concanavalin A from Jack bean (*Canavalia ensiformis*); DbL, *Dolichos biflorus* (horse gram) lectin; IgG, immunoglobulin G; IgE, immunoglobulin E; LcA, *Lens culinaris* (lentil) agglutinin; LoLI, *Lathyrus ochrus* (yellow flowered pea) isolectin I; PHA-E, *Phaseolus vulgaris* (kidney bean) erythroagglutinin; PNA, peanut (*Arachis hypogaea*) agglutinin; PsA, *Pisum sativum* (pea) agglutinin; SBA, soybean (*Glycine max*) agglutinin.

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challenge to peanut, PNA reacted in only 50% of the patients and was thus considered as a minor legume allergen (Burks et al., 1994). More recently, a substantial increase in the IgE-binding level of sera was observed in six out of seven patients allergic to peanut (85%) whereas no binding occurred with sera from non-allergic individuals (Gruber et al., 2005). In contradiction with the previous results these data suggest that PNA behaves as a major peanut allergen. According to the high sequential/structural conservation of legume lectins (Van Damme et al., 1998), IgE specific for PNA should also cross-react with other closely related lectins from other legume seeds, e.g. SBL from soybean (*Glycine max*) or PsA from pea (*Pisum sativum*).

Here, we report on immunoblotting, surface plasmon resonance measurements and ELISA using sera from patients allergic to peanut, which confirm the IgE-binding ability of legume lectins and their cross-reactivity with PNA. Exposed IgE-binding epitopic regions were identified on the molecular surface of dimeric (PsA from pea, LcA from lentil) and tetrameric (PNA) legume lectins.

2. Materials and methods

2.1. Chemicals

Sugars were purchased from Sigma and rabbit anti-human IgE was obtained from Dako. Sensor chips (CM 5), HBS (10 mM Hepes, 150 mM NaCl, containing 0.05% surfactant P20 and 3.0 mM EDTA, pH 7.4) and all the chemicals required to activate the carboxymethyl-dextran and immobilize the glycoproteins (100 mM *N*-hydroxysuccinimide, 400 mM *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride and 1 M ethanolamine hydrochloride adjusted to pH 8.5 with NaOH) were from BIAcore AB (Uppsala, Sweden).

2.2. Isolation and purification of lectins

Except for PNA, DbL and PHA-E, which were purchased from Sigma, other legume lectins were isolated from dry seeds and purified by affinity chromatography. SBA was isolated by affinity chromatography on *N*-caproylgalactosamine-Sepharose and subsequent elution with 100 mM galactose, according to Allen and Neuberger (1975). LcA, PsA and LoLI were specifically retained on Sephadex G100 and eluted by adding 100 mM glucose to the Tris-buffered saline (pH 7.6) elution buffer, as previously described (Rougé and Sousa-Cavada, 1984). The purity of the lectin preparations was checked by SDS-PAGE in 12.5% acrylamide gels followed by Coomassie blue staining (Laemmli, 1970).

2.3. Sera from allergic patients

Sera from 16 patients allergic to peanut (Table 1), which had been previously challenged by skin prick tests and oral provocation tests with the offending food, were used as an IgE source for immunoblotting, SRP analyses and ELISA measurements.

2.4. Immunoblotting

One millilitre of each lectin sample used at three concentrations of 50 ng ml⁻¹, 100 ng ml⁻¹ and 200 ng ml⁻¹ in 10 mM PBS (pH 7.4) containing 20 mM Gal and Man, was spotted onto a Hybond ECL nitrocellulose membrane (Amersham) and the membrane was air-dried for 1 h at room temperature. After a pre-incubation in PBS containing 20 mM Gal and Man, 3% (w/v) BSA and 0.1% (v/v) Tween for 1 h, the membrane was soaked in the patient IgE-containing sera diluted 1:10 in the same buffer and incubated for 2 h in a moist chamber. After three washings of 10 min each with the same buffer, the membrane was soaked in rabbit HRP-labelled anti-human IgE

Table 1

Listing of patients allergic to peanut.

Subject	Code	Sex/age (years)	Allergic history ^a	Symptom ^b
J.C.	P1	M/6	PA, TNA, EA	
L.A.	P2	M/4	PA, TNA, MUA, EA, MA, FA, P	PR, E
G.C.	P3	F/15	PA	A
F.E.	P4	F/8	PA, P	E
D.M.	P5	F/6	PA, EA	PR
M.J.	P6	F/6	PA, LA (soybean), EA, P	E
P.A.	P7	M/10	PA, EA, MA, P	A
P.P.	P8	M/11	PA, P	A, PR
O.E.	P9	F/4	PA, TNA, LA (lentil), EA, P	A, PR
G.J.	P10	F/9	PA, TNA, EA, P	E
M.G.	P11	F/19	PA	
G.T.	P12	M/8	PA, EA, P	A, RC
D.R.	P13	M/10	PA, GA, EA, MA, P	A
C.B.	P14	M/4	PA, P	
	P15			
	P16			

^a PA, peanut allergy; TNA, tree nut allergy; LA, legume allergy; GA, gluten allergy; MUA, mustard allergy; EA, egg allergy; MA, milk allergy; FA, fish allergy; P, pollinosis.

^b A, asthma; E, eczema; PR, pollinic rhinitis; RC, rhinoconjunctivitis.

diluted 1/5000 in the buffer and incubated for 1 h under gentle stirring. Following three washings of 10 min each with buffer, the immunolabelled spots were detected using the ECL Plus detection (Amersham) after 1 min exposure in cassette. All the handlings were carried out at room temperature. In order to prevent a possible sugar-binding mediated interaction of the immobilized lectins with the glycoproteins from human sera or the rabbit anti-IgE antibodies that could lead to false positive reactions, the nitrocellulose membrane bearing the immobilized lectins was pre-incubated with PBS containing both Man and Gal at 20 mM concentration. The following incubation steps with the IgE-containing sera and the anti-IgE rabbit antibodies were similarly performed in the presence of both sugars. In these conditions, the revealed interactions do not depend on the carbohydrate-binding properties of the lectins (binding of lectins to the glycan moiety of IgE or other serum proteins) but only depend on a specific lectin-IgE interaction.

2.5. Surface plasmon resonance analysis

The specific interaction of IgE-containing sera from peanut allergic patients with immobilized lectins was performed by surface plasmon resonance (SPR) using a biosensor BIAcore X (BIAcore AB, Uppsala, Sweden). The sera diluted 1:50 in HBS (pH 7.4) containing 20 mM Man and Gal, were injected for 5 min onto the lectin-bound surface of the sensor chip at a flow rate of 5 mL min⁻¹. The change of the SPR response (expressed as resonance units or RU) was monitored at 25 °C for 9.30 min. The same glycoprotein sensor chip surfaces were used repeatedly after removing the remaining immobilized lectin washing with 10 mM HCl for 2 min. For immobilization on the sensor chip, lectins were used at a concentration of 500 µg mL⁻¹ in 5 mM sodium acetate buffer, pH 4.0. According to the change of SPR response as a result of the immobilization of the lectins on the carboxymethylated dextran layer covering the sensor chip, an estimated surface concentration of 5–10 ng mm⁻² of dextran was calculated depending on the immobilized lectins.

The interaction of anti-IgE antibodies with the IgE previously bound to the immobilized lectins was monitored by injecting rabbit anti-human IgE serum diluted 1:10 in HBS (pH 7.4) containing 20 mM Gal and Man, at the beginning of the dissociation phase of the IgE/lectin complex during 5 min at a flow rate of 5 mL min⁻¹, and the change of the SPR response (RU) was monitored at 25 °C during 9.30 min. All SPR measurements were performed in duplicate.

2.6. ELISA

The interaction of IgE with lectins was measured by ELISA on standard 96-well microplates (Linbro). The wells were filled with 50 μ l of PNA solution used at a concentration of 200 ng ml⁻¹ in 10 mM phosphate buffered saline containing 2.7 mM KCl and 10 mM galactose (pH 7.4) (PBSG). After overnight incubation at 4 °C, the wells were washed three times with 100 μ l of PBSG containing 0.1% (w/v) of BSA and 1% (v/v) Tween 20 (pH 7.4) (PBSGT), flooded with the same buffer and incubated for 1 h at room temperature. The wells were loaded with 50 μ l of IgE-containing sera diluted 1:10 in the same buffer and after 2 h of incubation at room temperature; the wells were rinsed three times with PBSGT. Mouse monoclonal anti-human IgE (50 μ l) labelled with alkaline phosphatase (Abcam), diluted 1:2000 in PBSGT, was added. After 1 h of incubation at room temperature, the wells were washed three times with PBSGT and incubated with 100 μ l of 1 mg ml⁻¹ pf *p*-nitrophenyl phosphate in 0.1 M Na₂CO₃–NaHCO₃ buffer (pH 9.8) for 1 h in the dark. The absorbance at 405 nm (*A*₄₀₅) was recorded with an MRX microplate reader (DYNEX Technologies) after the development of the colored reaction was stopped by adding 50 μ l of 4.0 M NaOH. All ELISA measurements were performed in triplicate.

2.7. Peptide synthesis and IgE-binding epitope mapping

Overlapping 15-mer peptides, frameshifted by three residues, covering the entire amino acid sequences of PNA (Accession No. AAM46958), LcA (Accession No. 7428789) and PsA (Accession Nos. 230612 + 230613), were prepared using the SPOT technique (Frank, 1992). The protocol previously described in detail (Laune et al., 2002) was followed, with the exception of the utilization of the Multipip automatic SPOT synthesizer (Intavis). Briefly, peptides were assembled using the Fmoc chemistry on a cellulose membrane harboring an amino polyethylene glycol moiety. The C-terminal residue of each peptide was coupled to the activated membrane. After Fmoc deprotection, the following amino acids were sequentially added. At the end of the synthesis, side chain protecting groups were removed by a trifluoroacetic acid treatment while the linkage of the peptides to the membrane was maintained.

The membrane was soaked overnight in 20 mL of Tris-buffered saline (TBS) containing 2 mL blocking buffer (Roche) and 1 g sucrose (pH 7.0), and then washed three times with TBS containing 0.1% (v/v) Tween (TBSTw). A 1:10 (v/v) diluted pool of patient sera was added in the presence of an anti-protease cocktail (Roche) and the membrane was incubated in a moist chamber for 2 h. After three washes with TBSTw (pH 7.0) the membrane was stirred in a 1:4000 dilution of mouse monoclonal anti-human IgE labelled with alkaline phosphatase (Sigma) for 1 h. After three washes with TBSTw (pH 7.0), the interacting peptide spots were colored for 30 min by adding the 5-bromo-4-chloro-3-indolylphosphate (BCIP) substrate for alkaline phosphate (Promega). The membrane was washed three times with deionized water and dried for scanning. The membrane can be used repeatedly after a regeneration step consisting in one wash in dimethylformamide for 1 min, three washes in deionized water, three washes in 8 M urea containing 1% (w/v) SDS and 1% (w/v) β -mercaptoethanol and three subsequent washes in a mixture of ethanol–acetic acid–water (50:10:40, v/v/v).

2.8. Bioinformatics

Multiple amino acid sequence alignments were based on Clustal X (Thompson et al., 1997) using the Risler's structural matrix for homologous amino acid residues (Risler et al., 1988).

The surface occupied by the previously predicted sequential IgE-binding epitopes along the amino acid sequence of PNA (PDB ID Code 2PEL) (Banerjee et al., 1996), LcA (PDB ID Code 1LEN) (Loris

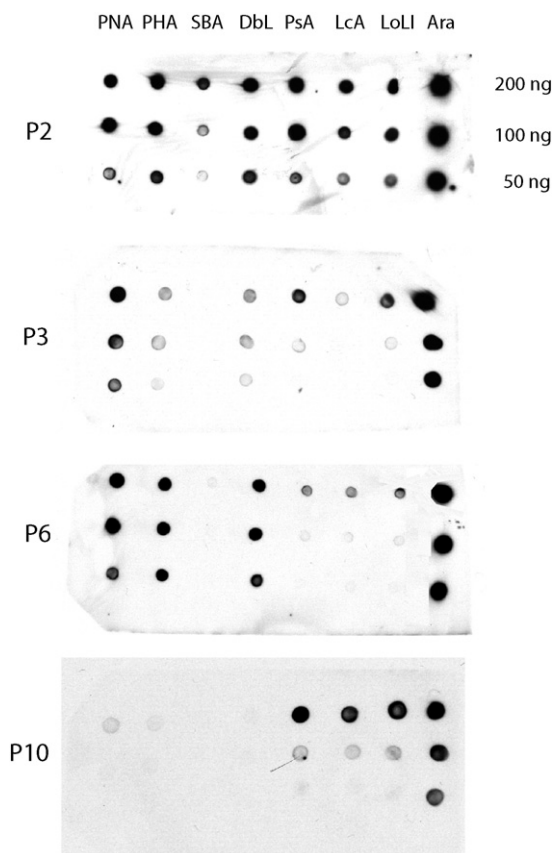


Fig. 1. Dot-blotting performed on nitrocellulose sheets of sera from patients P2, P3, P6 and P10 with increasing concentrations (50 ng, 100 ng, 200 ng) of different legume lectins (PNA from peanut, PHA from kidney bean, SBA from soybean, DbL from horse gram, PsA from pea, LcA from lentil, LoLI from vetch) and a peanut extract (Ara) used as a positive control.

et al., 1994), and PsA (PDB ID Code 2LTN) (Prasthofer et al., 1989) was calculated and displayed on the molecular surface of the proteins with PyMOL (W.L. DeLano, <http://pymol.sourceforge.net>). The overall conformation of the sequential IgE-binding epitopes on the molecular surface was similarly displayed with PyMOL.

Molecular surface and electrostatic potentials were calculated and displayed with GRASP using the parse3 parameters (Nicholls et al., 1991). The solvent probe radius used for molecular surfaces was 1.4 Å and a standard 2.0 Å–Stern layer was used to exclude ions

Table 2

Interaction of IgE from patients allergic to peanut with PNA and other closely related lectins immobilized on nitrocellulose membrane.

Patient	PNA	PHA-E	SBA	DbL	PsA	LcA	LoLI
P1	(+)			+++	+	+	+
P2	+++	+++	+	+++	+++	++	+++
P3	+++	+++		+++	+		+
P4	+++		+	+	+	+	+
P5	(+)	++		+++	+	+	+
P6	+++	+++	+	+++	++		++
P7	++	+++	++	++	++	++	+
P8	(+)	+		+++	+	++	+
P9	+++	++	+		+++	++	++
P10	(+)			+++	++	++	++
P11	+++	+	+	+++	++	+	++
P12	++	+++	+	++	+++	++	++
P13	+++			+++	++	+++	++
P14	(+)	+++		++	++	+	++
P15	++	+++			+++		+++
P16	++				++		++

(+), ++ and +++ correspond to weak, medium and strong interaction, respectively.

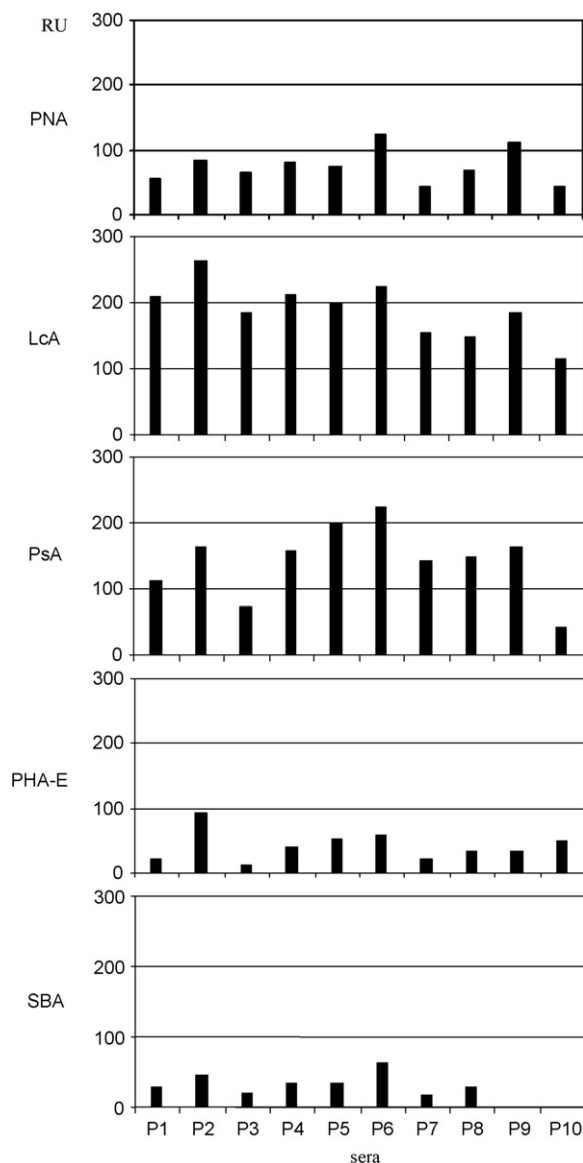


Fig. 2. BIAcore measurements (expressed as RU) of the interaction of sera from different patients (P1–P10) with the peanut (PNA), lentil (LcA), pea (PsA), kidney bean (PHA-E) and soybean (SBA) lectins. Each value is the mean of two independent measurements.

from the molecular surface (Gilson and Honig, 1987). The inner and outer dielectric constants applied to the protein and the solvents were, respectively, fixed at 4.0 and 80.0 and the calculations were performed keeping a salt concentration of 0.145 M.

3. Results

The patient sera that strongly reacted with a crude extract from peanut seeds also interacted strongly with PNA and other lectins in immunoblotting experiments (Fig. 1). The best interaction occurred with PNA and lectins from the edible legumes pea (PsA) and lentil (LcA), and the non-edible yellow flowered pea (LoL1). A weaker interaction also occurred with lectins from kidney bean (PHA-E) and horse gram (DbL). SBA from soybean cross-interacted weakly with the IgE-containing sera. No interaction (SBA) or a faint interaction (other lectins) occurred with those patient sera, which weakly interacted with a crude peanut extract (Table 2). In addition, no interaction occurred with two IgE-containing sera from patients allergic to apple and kiwi fruits. Except a weak and apparently non-

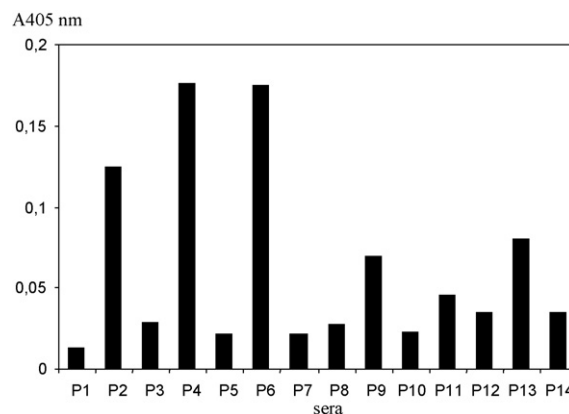


Fig. 3. ELISA measurements at 405 nm of the interaction of sera from different patients (P1–P14) with PNA. Each value is the mean of two independent measurements.

specific interaction with DbL, PsA and LcA, no interaction occurred between other lectins and rabbit HRP-labelled anti-human IgE diluted 1/5000 used as a control.

Very similar results were obtained in ELISA experiments (Fig. 2) showing a strong interaction of IgE with PNA, PsA, LcA and LoL1. Like in immunoblotting experiments, rabbit HRP-labelled anti-human IgE weakly interacted with a few lectins, e.g. DbL, PHA-E and SBA.

All the patient sera more or less interacted with PNA and other immobilized lectins in SPR experiments (Fig. 3). As shown from experiments in which an additional injection of rabbit anti-human IgE serum enhanced the RU response, this interaction results from the specific interaction of IgE from the checked sera with the lectins (result not shown). However, the increase in RU response was rather weak suggesting that an additional interaction with IgG most probably occurred in these SPR measurements. In this respect, sera from patients allergic to other unrelated food, e.g. banana, apple or kiwi, readily reacted with some immobilized lectins even though the interaction is much more lower.

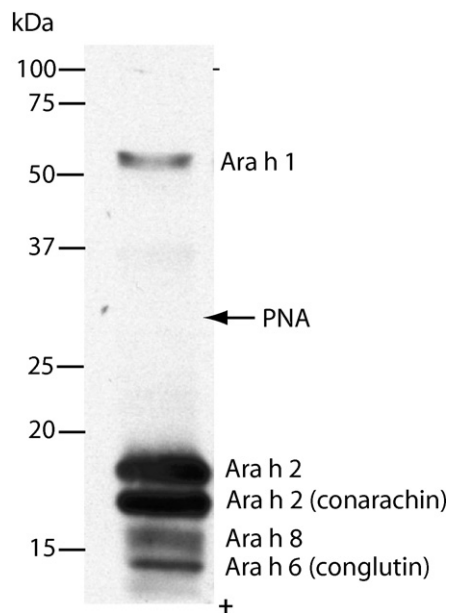


Fig. 4. Immunoblotting of a peanut extract treated by a pool of sera from peanut allergic patients previously treated with a huge amount of PNA. The anti-PNA IgE exhausted serum still reacts with the major and minor allergens of the peanut extract but no more with PNA.

Table 3

Amino acid sequence stretches identified as sequential IgE-binding epitopic regions along the amino acid sequences of PNA, LcA and PsA, using the SPOT technique. Surface-exposed residues are in black boxed white letters. Residues exposed on the surface of the lectin monomer but not exposed on the surface of the tetramer are grey boxed. Both LcA and PsA are continuously numbered as single chain lectins but they correspond in fact to two-chain lectins with distinct numbering for their heavy (β) and shorter light (α) subunits. Underlined residues are missing in the three-dimensional structure of the lectins.

IgE-binding epitopes of PNA:	IgE-binding epitopes of LcA:	IgE-binding epitopes of PsA:
31NIQLTNLNKVN SV GRVLY AM EVRI WSS ATGNVA63	1 TETTSFSIT KFS PD Q15	1 TETTSFLIT KFS PD Q15
106LG VSD TKGA114	22GDSY TTK GL TLT KAVK STV GRAL45	22GDSY TTK EL TLT KAVK NTV GRAL45
145V DSV KTV PWN SVSGA159	50PIH52	59 GNV ANFV IS 67
166IY DS STK TL 174	80VAD GF TFFI88	77 SYN VAD GF TFFI88
190Q VVD L KAK L PER V KF 204	101YLG VFN SK EYD K112	104V FN SA EYD K112
208AS GS LG RQ I HL IR SWS F TS TLIT T232	131 PSN 133	122D TF YNA AWD PS N 133
	137HIGIDVNS IKSV NTK SWN L QNG ERANV163	140IDVNS IKSV NTK SWK L QNG EE160
	194 LKD V PEW VRIG205	173 VLT V SL TY PNV T184
	215AAQ EVH SW SFNS QL GHT SKS234	194 LKD V PEW V202
		215AA HEV L SWS F HSEL SG TSS SKQ236

In order to assess the specific character of IgE towards PNA and other legume lectins, a pool of sera from peanut allergic patients previously treated with a huge amount of PNA was checked for its capacity to recognize the major peanut allergens in immunoblotting experiments. As shown in Fig. 4, the anti-PNA IgE exhausted serum still reacted with the major allergens Ara h 1 and Ara h 2 (and other minor allergens like Ara h 6 and Ara h 8) of the peanut extract but gave no reaction with PNA.

Different IgE-binding regions were identified along the amino acid sequences of PNA, LcA from lentil, and PsA from pea, using sera from peanut allergic patients in SPOT experiments (Table 3). Mapping of the IgE-binding stretches previously identified by the SPOT technique on the molecular surface of the lectins allowed us to define more accurately those amino acid residues sufficiently exposed to readily interact with the corresponding IgE (Fig. 5). Finally, exposed residues in each of the IgE-binding stretches were considered as forming the IgE-binding epitopic regions of the

lectins. They correspond to more restricted sequence stretches sufficiently exposed on the surface to interact with polyclonal IgE from allergic patients. Most of these epitopic stretches consist of hydrophilic and essentially electropositively charged residues, e.g. Arg and Lys residues. Accordingly, they mostly coincide with the electronegative potentials arrayed on the molecular surface of the allergens (Fig. 5).

The dimeric LcA and PsA lectins contain more IgE-binding regions, 9 and 10, respectively, than the tetrameric PNA (6), even though they possess a less extended molecular surface (Table 3 and Fig. 5). The IgE-binding regions of both LcA and PsA exhibit a high degree of sequence similarity that should account for some IgE-binding cross-reactivity among these lectins (Table 4). Less pronounced similarities occur with the IgE-binding amino acid sequence stretches identified along the PNA sequence, that should decrease the IgE-binding cross-reactivity between dimeric (LcA, PsA) and tetrameric (PNA, SBA) lectins as well (Table 4).

Table 4

Comparison of the sequential IgE-binding epitopes identified at homologous positions along the amino acid sequence of PNA, LcA and PsA. Identical residues are in black boxed white letters. Structurally homologous residues according to the Risler's matrix (Risler et al., 1988) are grey boxed.

Epitopes:	Amino acid sequences:
PNA epitope 1	31 NIQLTNLNKVN SV GRVLY AM EVRI WSS ATGNVA 63
LcA epitope 2	22 GDSY TTK GL TLT KAVK STV GRAL 45
PsA epitope 2	22 GDSY TTK EL TLT KAVK NTV GRAL 45
PNA epitope 2	106 LG VSD TKGA 114
LcA epitope 5	101 YLG VFN SK EYD K 112
PsA epitope 5	104 VFN SA EYD K 112
PNA epitope 3	145 VDSV KTV PWN SVSGA 159
LcA epitope 7	137 HIGIDVNS IKSV NTK SWN L QNG ERANV 163
PsA epitope 7	140 IDVNS IKSV NTK SWK L QNG EE 160
PNA epitope 5	190 Q VVD L KAK L PER V KF 204
LcA epitope 8	194 LKD V PEW VRIG 205
PsA epitope 9	194 LKD V PEW V 202
PNA epitope 6	208 AS GS LG RQ I HL IR SWS F TS TLIT T 232
LcA epitope 9	215 AAQ EVH SW SFNS QL GHT SKS 234
PsA epitope 10	215 AA HEV L SWS F HSEL SG TSS SKQ 236

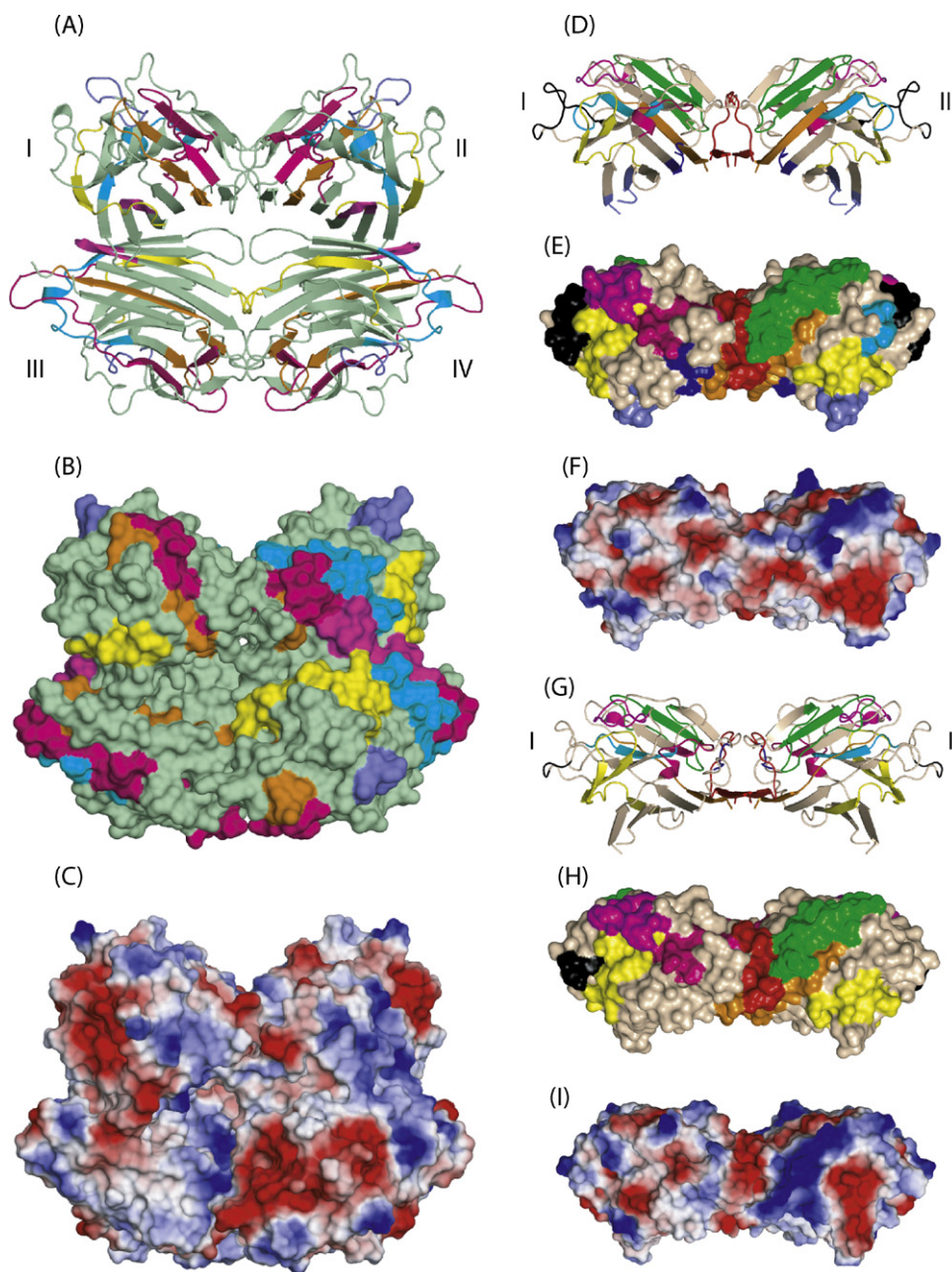


Fig. 5. (A) Ribbon diagram of the PNA tetramer showing the localization (differently colored) of the IgE-binding epitopes identified on the molecular surface. (B) Surface exposition of the IgE-binding epitopes (differently colored) identified on the molecular surface of the PNA tetramer. (C) Mapping of the electrostatic potentials on the molecular surface of the PNA tetramer. Electropositively and electronegatively charged regions are colored blue and red, respectively; neutral regions are colored white. (D) Ribbon diagram of the PsA dimer showing the localization (differently colored) of the IgE-binding epitopes identified on the molecular surface. (E) Surface exposition of the IgE-binding epitopes (differently colored) identified on the molecular surface of the PsA dimer. (F) Mapping of the electrostatic potentials on the molecular surface of the PsA dimer. Electropositively and electronegatively charged regions are colored blue and red, respectively; neutral regions are colored white. (G) Ribbon diagram of the LcA dimer showing the localization (differently colored) of the IgE-binding epitopes identified on the molecular surface. (H) Surface exposition of the IgE-binding epitopes (differently colored) identified on the molecular surface of the LcA dimer. (I) Mapping of the electrostatic potentials on the molecular surface of the LcA dimer. Electropositively and electronegatively charged regions are colored blue and red, respectively; neutral regions are colored white (For interpretation of the references to color information in the figure caption, the reader is referred to the web version of the article).

4. Discussion

All the assayed legume lectins consist of the quaternary association of two (PsA, LcA and LoLI) or four protomers (PNA, PHA-E, DbL and SBA) built from a curved seven-stranded β -sheet (front face of the protomer) associated with a flattened six-stranded β -sheet (back face of the protomer) by turns and loops (Bourne et al., 1990). An additional four-stranded β -sheet, also known as S-sheet (Banerjee et al., 1996), connects the two β -sheets on both sides of the protomer. Four convergent loops located at the top

of the protomer build up the carbohydrate-binding site responsible for the specific recognition of simple or complex sugars by the lectins (Van Damme et al., 2007). Legume lectins roughly differ by: (1) the occurrence of a single (single-chain lectins) or two chain (two-chain lectins) in the protomer and, (2) the dimeric (12-stranded β -sandwich structure) or tetrameric (dimer of dimers) association of the protomers. Accordingly, legume lectins exhibit either two (divalent lectins) or four (tetravalent lectins) identical carbohydrate-binding sites (Van Damme et al., 1998).

With a few exceptions, most of the assayed legume lectins interacted more or less strongly with IgE-containing sera from patients allergic to peanut in various experiments including immunoblotting, SPR analyses and ELISA measurements. This interaction was sugar-independent and did not depend on the dimeric or tetrameric organization of the lectins. It revealed the occurrence of IgE-binding epitopes sufficiently exposed at the surface of the legume lectins to be specifically recognized by the IgE paratopes. However, the molecular basis responsible for the allergenicity (antigenicity) of legume lectins has been poorly documented so far with respect to the occurrence and characterization of their IgE-binding epitopes.

Despite the amounts of results that have been accumulated on the three-dimensional structure of legume lectins, little if not all is known on the epitope analysis of these plant proteins. Except for the yellow flowered pea (*Lathyrus ochrus*) isolectin LoLI for which the sequential IgG-binding epitopes have been characterized and mapped on the three-dimensional structure (Lueken et al., 1988, 1989), none of the other legume lectins have been investigated in this way. The characterized IgG-binding epitopes of the *L. ochrus* lectin all occur in nicely exposed loops located between the front and back face of the protomer and some of them coincide with the four loops forming the carbohydrate-binding site of the lectin (Lueken et al., 1988, 1989). This is not surprising since dimeric lectins like LoLI possess an extremely compact rod-like β -sandwich structure from which protrude exposed loops that roughly correspond to IgG-binding epitopes. Additional SPOT experiments performed with PNA, LcA and PsA, allowed us to identify 6, 9 and 10 sequential IgE-binding epitopes, respectively, along the amino acid sequences of the lectins. As shown from the electrostatic potentials mapping on their molecular surface, most of these IgE-binding regions coincide with the surface-exposed electropositively charged residues. These experimentally characterized IgE-binding epitopes fairly well coincide with the amino acid sequence stretches predicted as exposed epitopes according to their hydrophilicity, flexibility, solvent exposure and charged residue content. According to the extremely structurally conserved character of these proteins, one can assume that some IgE-binding epitopes of other legume lectins also coincide with these exposed loops protruding outside the molecular surface of both the dimeric or tetrameric proteins.

Both dimeric and tetrameric legume lectins consist of tightly packed proteins extremely resistant to heat denaturation (cooking) and to proteolysis by the digestive enzymes pepsin, trypsin and chymotrypsin (Morari et al., 2008). As a result, they should retain most of their IgE-binding properties along the digestive tract to trigger some allergenic response upon consumption of cooked legume beans by previously sensitized individuals.

Finally, lectins from currently consumed legume seeds (peanut, soybean, kidney bean, pea, and lentil) have been demonstrated to contain IgE-binding epitopes susceptible to specifically interact with IgE from peanut allergic patients. As shown in immunoblotting experiments performed with exhausted sera of allergic patients, IgE that specifically recognize legume lectins do not interact with IgE-binding epitopes of the major peanut allergens, e.g. Ara h 1, Ara h 2 or Ara h 3. In this respect, PNA does not share common epitopes with the other seed protein allergens like vicilin (Ara h 1), legumin (Ara h 3) or 2S albumin (Ara h 2). However, the question is still pending to discriminate between the ability of lectins to sensitize susceptible individuals or to simply trigger an allergic reaction in people previously sensitized to either vicilin (Ara h 1) or legumin (Ara h 2) or their homologous proteins from other edible legumes. However, the reported ability of various dietary lectins to induce *in vitro* release of IL-4 and IL-13 from human basophiles brings some credit to the first hypothesis that lectins could actually sensitize susceptible subjects. Recently, the horse gram (*Dolichos biflorus*) lectin DbL (or DBA for *Dolichos biflorus* agglutinin) has been iden-

tified as an allergen susceptible to induce *in vivo* the activation of mast cells from atopic subjects presenting an allergic reaction to horse gram (Pramod et al., 2006). More recently, PHA was identified as responsible for a severe case of anaphylaxis to white kidney bean (*Phaseolus vulgaris*) along with phaseolin, a vicilin seed storage protein homologous to the major peanut allergen Ara h 1 (unpublished result).

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