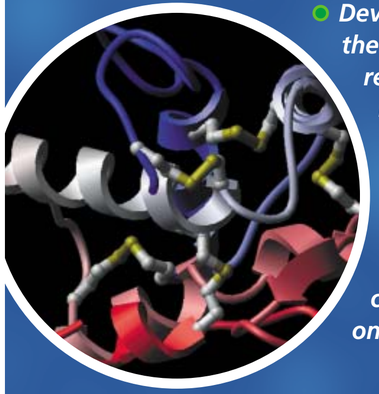
Protall

3 of the main objectives of Protall have been to...

- *Improve our understanding of factors that may be important in predisposing certain plant food proteins to becoming allergens*



- *Develop ideas and strategies to increase the efficiency of processing methods to remove allergenic determinants, thus improving food quality*

- *Compile a searchable database with biochemical and clinical information on plant food allergens*

This newsletter summarises the outputs of the project network and its achievements in working towards these objectives.

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Plant Proteins Families

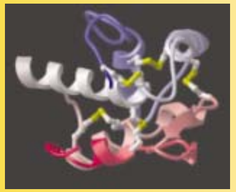
The recently completed genome sequence of the model plant species Arabidopsis has been estimated to encode over 25,000 proteins, which on the basis of their function, can be classified into three groups:

- Structural and metabolic, the vast majority of plant proteins.
- Protective proteins, which defend a plant against invasion by pathogens or feeding by pests.
- Storage proteins, which proved a nutrient store to support germination in seeds.

It is now clear that almost all plant food allergens are either protective or storage proteins.

It is also evident that those proteins that trigger the development of an allergic response through the gastro-intestinal tract belong primarily to two protein superfamilies:

The Cereal Prolamin Superfamily – comprising three major groups of plant food allergens, the 2S albumins, lipid transfer proteins and cereal α-amylase/trypsin inhibitors. This includes major allergens from Brazil nut, fruits such as peaches, and cereals, such as rice and wheat. They have related structures and are stabile to thermal processing and proteolysis.



Wheat α-amylase inhibitor

***Maize non-specific lipid transfer protein* (Zea m 14)**

Aligned structures of three family members of the prolamin superfamily, an α-amylase inhibitor from wheat and a non-specific lipid transfer protein from maize.

The Cupin Superfamily – comprising the major globulin storage proteins from a number of plant species. The globulins have been found to be allergens in plant foods such as peanuts, soya bean and walnut.

3-Dimensional structure of canavalin, a 7S globulin belonging to the cupin superfamily, from seeds of Jack bean.

A second type of plant food allergen is associated with prior allergies to substances such as pollen and latex; a large number fruit and vegetable allergens of this type have been characterised. Of the pollen-related allergens, profilin (a protein, which binds to the actin filaments of the pollen cytoskeleton), and Bet v 1-related allergens, (which are of unknown function in the plant), are amongst the most important. In latex-fruit allergies a group of enzymes, known as class I chitinases, are responsible for the cross-reactivity, because they have a domain in common with the major latex allergen, hevein.

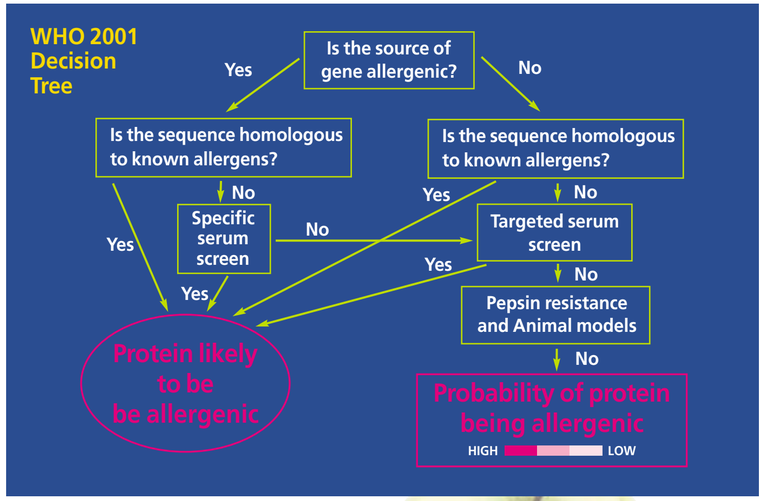
*The major latex allergen proheven (*Hev b 6.01*)*

Latex -fruit syndrome allergen Class I chitinase

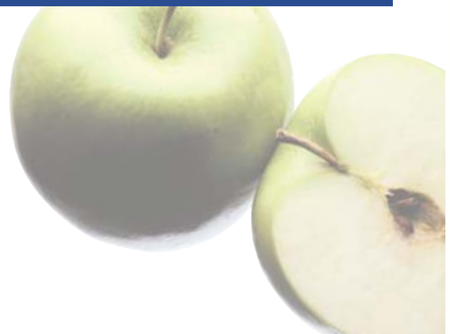
The structural relationship between the major latex allergen, prohevein, and Class I chitinase from fruit.

Assessment of Allergenic Risk

A joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology was held in Rome 22-25 January 2001. The consultation resulted in a report 'Evaluation of Allergenicity of Genetically Modified Foods' which proposed a decision tree for assessing the allergenic risks posed by novel proteins, which is based primarily on direct and indirect comparison of allergen structure.



A comparison of the amino acid sequence of a new food protein with those of known allergens forms a part of this tree. However, sequence comparisons used alone can be misleading. The outputs of Protall regarding allergen family structures, relationships and properties (such as the source of the protein, stability to digestion, and stability to processing) complements this approach ensuring a realistic assessment can be made. Computing -based *(in silico)* approaches to assessing protein allergenicity must also be combined with other experimental methods, such as allergic serum screens.



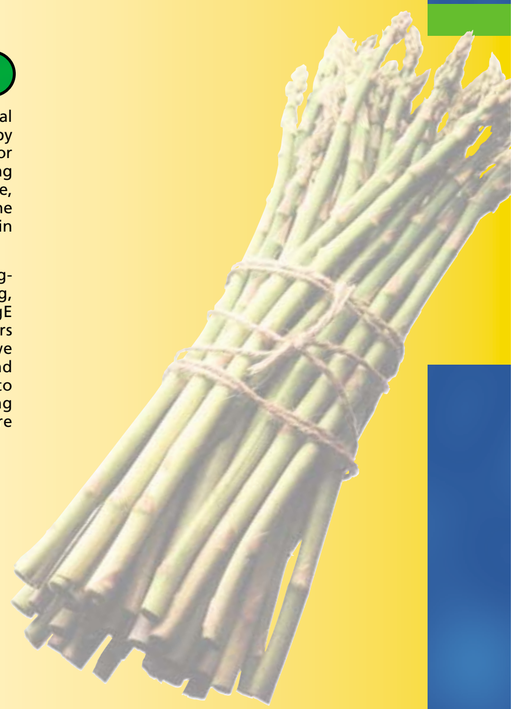
Processing and Allergen Structure

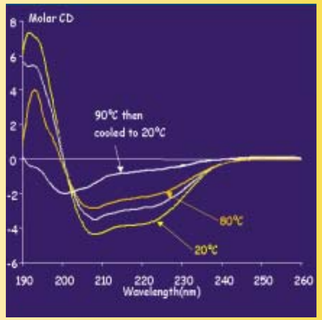
Epitopes are the small structural elements on an allergen recognised by IgE. They can be either linear or conformational, the former comprising the primary sequence of a polypeptide, whilst in conformational epitopes the three-dimensional structure of a protein is important.

Processing may affect the allergenicity of a protein by removing, destroying or masking these IgE epitopes. If we understand the factors that determine epitope integrity we will be able to develop novel and more specific approaches to decreasing food allergenicity using processing, a number of which are highlighted below.

- **Removal of epitopes** - An effective method achieved by removing parts of a food which are rich in allergen. Thus in peach allergy removing the peel which is rich in peach allergens, reduces the allergenicity of fruits.
- **Destruction of epitopes** - Epitopes may be destroyed using physical processing, the heat causing the allergen to become unfolded or denatured. Some proteins unfold easily, but others, such as the lipid transfer-proteins, are thermostable and must be heated to over 90° to begin to unfold.

  Proteases have also been used to destroy allergenic epitopes in foods, although an undesirable side effect of this type of treatment is that proteolytic cleavage damages the functional properties of food proteins, such as the baking quality of wheat flour.
- **Masking of epitopes** – enzymes can also be used to modify proteins (e.g using transglutaminase to cross-link allergens in wheat) reducing their allergenicity by "masking" IgE epitopes, which become unavailable for IgE-binding. However such approaches may also present problems in altering the functional properties of proteins.





Circular dichroism (CD) spectrum of maize LTP allergen Zea m14 after heating showing the protein unfolds completely at 90°c

www.ifr.bbsrc.ac.uk/protall

Welcome to the Protall Database!

What is the database? This database contains biochemical and clinical information about plant food allergens involved in classical IgE-induced hypersensitivity reactions - about 77 allergens from 48 plant species. There are many foods for which a case history of an allergic reaction has been reported but the allergens responsible have not been described. These are not included in the database.

Who compiled the information? The information has been compiled by the following members of Protall: Rene Crevel (Unilever-SEAC, Colworth, UK), Rafael Monsalve (Universidad Complutense, Madrid, Spain), Rosa Sanchez-Monge (Universidad Politécnica, Madrid, Spain), Karin Hoffman-Sommergruber and Heimo Breiteneder (University of Vienna, Austria), Hub Noteborn and Ad Peijnenberg (RIKILT-DLO, Wageningen, The Netherlands), Jaap Aakerdass (University of Amsterdam, The Netherlands), Shmuel Yannai (Technion, Haifa, Israel), Arthur Tatham LARS (Long Ashton Research Station, Bristol, UK), Elide Pastorello (Hospital Maggiore, Milan, Italy), Photini Papageorgiou (University of Athens, Greece).

This information has subsequently been edited by Clare Mills (co-ordinator), John Jenkins (IFR, Norwich, UK) and Charlotte Madsen (Danish Veterinary and Food Administration, Søborg, Denmark). The IT expertise has been supplied by Andy Walker (IFR, Norwich, UK), assisted in the initial phases by Marcos Alcocer (University of Nottingham, UK).

Who is it for? The information in the database is understandable by a graduate level scientist or health professional, although anyone is free to search it. If you have a problem understanding some of the terms used in the database records there is a comprehensive glossary of words used in relation to allergy research at (put in web address) or you can email a query to us.

You can find the database at **www.ifr.bbsrc.ac.uk**/protall

Designed by IFR Communications

.....An Example Record on the Protall Database.....An Example Record on the Protall Database.....An Example Record on the Protall Database.....

BIOCHEMICAL DATA	
Botanical name	Arachis hypogea
Trivial name	Peanut; ground nuts; monkey nuts
Allergen designation	Major
Allergen name	Ara h 1
Allergen sequence known?	Yes
Allergen accession number(s)	P43237 L38853 P43238 L34402
Accession Number web link(s)	*http://ca.expasy.org/cgi-bin/niceprot.pl?P43237* *http://ca.expasy.org/cgi-bin/niceprot.pl?P43238*
Allergen 3D structure accession number	Not determined
Molecular weight	65,000
Allergen epitopes	Immunodominant epitopes: Ara h 1 25-34 (KSSPYQKK) Ara h 1 65-74 (EYDPRLVY) Ara h 1 89-98 (ERTRGRQP) Ara h 1 498-507 (RRYTARLKEG) Other IgE epitopes: Ara h 1 48-57 (QEPDDLKQKA) Ara h 1 89-98 (ERTRGRQP) Ara h 1 97-105 (GDYDDDRR) Ara h 1 107-116 (RREEGGRW) Ara h 1 123-132 (EREEDWRQ) Ara h 1 134-143 (EDWRRPSHQQ) Ara h 1 143-152 (PRKIRPEG) Ara h 1 294-303 (PGQFEDFF) Ara h 1 311- 320 (YLQEFSRN) Ara h 1 325-334 (FNAEFNEIRR) Ara h 1 344-353 (QEERGQRR) Ara h 1 393-402 (DITNPINLRE) Ara h 1 409-418 (NNFGKLFEVK) Ara h 1 461-470 (GNLELV) Ara h 1 525-534 (ELHLLGFGIN) Ara h 1 539-548 (HRIFLAGDKD) Ara h 1 551-560 (IDQIEKQAKD) Ara h 1 559-568 (KDLAFPGSGE) Ara h 1 578-587 (KESHFVSARP) Ara h 1 597-606 (EKESPEKED)

Allergen stability

Ara h 1 (also known as con-arachin) belongs to the 7S (or vicilin) family of seed storage globulins. It is a trimeric acidic glycoprotein with a subunit molecular weight of 65,000, which associates into homotrimers in high salt. It is, like beta-conglycinin from soya, a 'large' vicilin, with a N-terminal extension of over 100 amino acids. Most of the major IgE epitopes are contained within this extension region.

The experimentally determined Mr is smaller than that calculated from the gene sequence, or observed for recombinant Ara h 1. It is thought that the anomalous Mr of conarachin on SDS-PAGE probably results from glycosylation.

The thermal stability has been studied by Koppelman et al. (1999) but its allergenic properties are reported to be unaffected by thermal denaturation. The protein shows only minor secondary structural changes in 5M urea. It is resistant to pepsin hydrolysis.

Nature of main cross-reacting proteins

As a consequence of the homologies between the 7S globulins of legumes, there are cross-reactions with 7S proteins with a range of legumes. In particular, around 80% of anti-Ara h 1 antibodies bind to the 7S globulin of soya, beta-conglycinin.

Allergen properties & function in plant

Ara h 1 is one of the major storage proteins in peanut.

Allergen purification

A number of different approaches have been taken to purification:

1. Extract defatted peanut meal with 50mM Tris-HCl, pH 8.2, containing 0.2M NaCl (1:10, w:v) and purified by gel filtration on a Superose 6 HR10/30 column attached to a FPLC system. (Mills *et al* 1997 Food Agric Immunol 9:37-50.)
2. Extract defatted roasted (163-177C) peanut meal with 8M urea, 1MNaCl in 20mM sodium phosphate, pH7.0. Following dialysis into 20mM Tris-bis-propane (pH7.2) containing 8Murea the sample was fractionated on a MonoQ10/10 column attached to a FPLC system and eluted with a 0-1.5MNaCl gradient. (cf Burks *et al* 1991 below).

Biochemical references

Burks AW, Williams LW, Helm RM, Connaughton C, Cockrell G, O'Brien T (1991) Identification of a major peanut allergen Ara h 1 in patients with atopic dermatitis and positive peanut challenge. J Allergy Clin Immunol 88:172-9.

Burks AW, Shin D, Cockrell G, Stanley JS, Helm RM, Bannon GA (1997) Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. Eur J Biochem 245:334-339.

For more information on the project look at our web-site

www.ifr.bbsrc.ac.uk/protall

or contact the co-ordination team.

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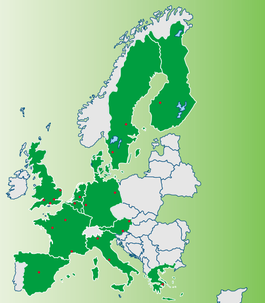

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