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Review

Evaluation of available IgE-binding epitope data and its utility in bioinformatics

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This paper reviews the role played by IgE-binding epitopes in eliciting clinical symptoms, the types of IgE-binding epitopes in allergenic proteins, the methods used to identify IgE-binding epitopes, and the availability of IgE-binding epitopes in allergenic sources. Finally, bioinformatics methods to assess protein allergenicity using knowledge of IgE-binding epitopes are discussed.

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1 Introduction

The proteins introduced into all genetically engineered plants that have been put into commerce in the US have been screened by comparing their amino acid sequence with those of known allergens as one of many assessments performed to evaluate product safety [1, 2]. The purpose of this screening is to determine if the introduced protein shares any sequence similarities with known allergen and gliadins that would indicate the protein could elicit a clinical reaction in an allergic population. The extent of sequence similarities between the introduced protein and database sequences of allergens, gliadins, and other proteins can be efficiently assessed using the FASTA sequence alignment tool [3]. Although the FASTA program directly compares amino acid sequences (*i.e.* primary protein structure), the alignment data may be used to infer higher order structure (*i.e.* secondary and tertiary protein structures). Proteins that share a high degree of similarity throughout their entire length are often homologous. Homologous proteins share secondary structure and common 3-D folds [4]. Homologous proteins are more likely to share allergenic crossreactive conformational and linear epitopes than unrelated proteins; however, the degree of similarity (amino acid/structural) between homologs varies widely. Aalberse [5] has noted that proteins sharing less than 50% identity across the full length of the protein sequence are unlikely to be crossreactive, and immunological crossreactivity is likely when the proteins share at least 70% identity.

There is some concern that the FASTA search might miss short regions within a protein that are identical or highly similar in sequence to an existing allergen and have the potential to bind IgE. Since a complete description of IgE-binding epitopes for all known allergens is not available, a theoretical database of all potential epitopes for these same allergens was proposed as a way to address this issue [1]. In essence, a query protein was screened by scanning all overlapping peptides of all the allergens of the database and comparing them in pair-wise fashion to all same-size potential peptides of the test protein using computer software or scanning manually. This can be viewed as a highly conservative and all-inclusive approach as most of the theoretical peptides compared with the query sequence do not represent *bona fide* epitopes. When this approach was first suggested [1], a window size of eight amino acids was recommended. Since this original recommendation, many IgE-binding epitopes have been identified for a number of foods and aeroallergens. Many IgE-binding epitopes have been identified as sequential epitopes, although for many this does not represent the full epitope. While some IgE-binding peptides have been reported to be as short as five amino acids [6, 7], the majority of characterized IgE-linear epitopes are eight amino acids or longer [8–10]. Although many of these reports have demonstrated IgE-binding, few have tested the affinity (avidity) of the binding, or the allergic significance (biological response) of the *in vitro* binding, and it is clear from some reports that high affinity binding requires eight or more amino acids [6, 11].

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Abbreviation: CCDs, crossreactive carbohydrate determinants

In the ensuing years, the appropriate window size for this type of assessment has been debated in the literature [12–14] and by scientific panels (FAO Corporate Document Repository; available at http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/007/y0820e/y0820e00. htm) with a window size of six amino acids being suggested as a more appropriate size for screening. A number of different studies have analyzed the effect of window size on search output when looking for matches between proteins and peptides from protein allergens [12–15]. In each of these studies, it was noted that a sliding window of six identical amino acids yielded exceedingly high match frequencies with query sequences. Based on a six amino acid window search size alone, the aforementioned studies showed that 60–80% of all protein sequences, regardless of their source, might be viewed as potential crossreactive allergens. Recognizing that a consensus in the scientific community had not been reached, the Codex Alimentarius Commission (Food and Agriculture Organization of the United Nations; FAO Corporate Document Repository. http://www.fao.org/documents), an intergovernmental task force whose mission is to harmonize standards for testing of foods derived from biotechnology, recommended that "the size of the contiguous amino acid search should be based on a scientifically justified rationale in order to minimize the potential for false negative or false positive results."

Astwood *et al.* [2] have recommended a sequence comparison to a database of known IgE-binding epitopes as a way to reduce the possibility of missing a known epitope smaller than eight contiguous identical amino acids and to minimize the potential for false positive results. In this chapter, we review the role played by IgE-binding epitopes in eliciting clinical symptoms, the types of IgE-binding epitopes in allergenic proteins, the methods used to identify IgE-binding epitopes, and the availability of IgE-binding epitopes from allergenic sources. Finally, bioinformatics approaches to assess protein allergenicity using knowledge of IgE-binding epitopes are discussed.

2 Types of IgE-binding epitopes

The requirement that IgE be crosslinked on effector cells in order to release the mediators of allergic symptoms dictates that there should be at least two high affinity IgE-binding epitopes on a single allergen [16]. Two general categories of IgE-binding epitopes, linear and conformational, are accepted to occur in food allergens. While there is a tendency to place epitopes into one or the other category it should be noted that epitopes can have both conformational and linear characteristics. Conformational epitopes occur when either secondary or tertiary structure of the allergen is required before IgE binds. In contrast, linear epitopes require only the primary amino acid sequence of the allergen for IgE to bind. While conformational IgE-binding epitopes are prevalent and important to the aetiology of aeroallergen-mediated allergic reactions, in some cases linear epitopes are important to food allergens, mainly because the immune system will encounter them only after they have been partially denatured and digested by the human GI tract. Therefore, the linear IgE-binding epitopes of food allergens have garnered more attention than conformational epitopes, atleast partly due to the difficulty in studying conformational epitopes. However, Vila *et al.* [17] have shown in comparison to tolerant patients, milk-allergic children with persistent symptoms had a significantly higher ratio of specific IgE antibodies to linear epitopes than to native alpha- and beta-casein proteins. These results illustrate the importance of linear IgE-binding epitopes in persistent food allergy and the potential predictive value of conformational epitopes.

Conformational epitopes on food allergens play an important role in pollen-associated food allergic reactions by acting as crossreactive structures for IgE-binding. Bet v 1, a birch pollen allergen, is the major cause of pollen-associated food allergy [18]. The tertiary structure of Bet v 1 contains three surface patches that have conserved-amino acid sequences and structures with proteins from a variety of foods including cherry, apple, hazelnut, peach, carrot, celery, and soya [19]. One of these conserved-IgE crossreactive regions is the P-loop located between β-strands 2 and 3 of Bet v 1 and its homologs [20, 21]. Elicitation of clinical allergy symptoms appears to be dependent on IgE-binding to these structures, which is in direct contrast to that observed for other food allergens such as those indicated in Table 1.

The vast majority of IgE-binding epitopes are linear or conformational arrays of amino acids. However, there is evidence that the Asn-N linked glycan moiety of glycoproteins can bind IgE [22] and are known as crossreactive carbohydrate determinants (CCDs) [23–25]. The major IgE-binding glycan moiety is composed of a Man(3)GlcNAc(2) backbone with beta 1→2 xylose and/or alpha 1→3 fucose branches. These types of glycans are commonly found in plants and invertebrates, and IgEs recognizing CCDs are found in some patients sensitive to pollens and plant-derived foods. The clinical relevance of IgE directed against CCDs is open to debate as there is some question as to whether they can precipitate an allergic response [26].

3 Methods for determining IgE-binding epitopes

Methods exist for determining both types of IgE-binding epitopes in sufficient detail to allow biochemical character-

Table 1. Linear IgE-bindung epitopes of major allergens

Source	Allergen	Epitope	Position	Reference
Plant				
Peanut	Ara h 1	AKSSPYQKKT	25–34	[10]
		QEPDDLKQKA	48–57	
		LEYDPRLVYD	65–74	
		GERTRGRQPG	89–98	
		PGDYDDDRRQ	97–106	
		PRREEDWRQP	107–116	
		EDWRRPSHQQ	123–132	
		QPRKIRPEGR	134–143	
		TPGQFEDFFP	143–152	
		SYLQEFSRNT	294–303	
		FNAEFNEIRR	311–320	
		EQEERGQRRW	325–334	
		DITNPINLRE	344–353	
		NNFGKLFEVK	393–402	
		RRYTARLKEG	498–507	
		ELHLLGFGIN	525–534	
		HRIFLAGDKD	539–548	
		IDQIEKQAKD	551–560	
		KDLAFPGSGE	559–568	
		KESHFVSARP	578–587	
	Ara h 2	HASARQQWEL	15–24	[28]
		QWELQGDRRC	21–30	
		DRRCQSQLER	27–36	
		LRPCEQHLMQ	39–48	
		KIQRDEDSYE	49–58	
		YERDPYSPSQ	57–66	
		SQDPYSPSPY	65–74	
		DRLQGRQQEQ	115–124	
		KRELRNLPQQ	127–136	
		QRCDLDVESG	143–152	
	Ara h 3	IETWNPNNQEFECAG	33–47	[11]
		GNIFSGFTPEFLEQA	240–254	
		VTVRGGLRILSPDRK	279–293	
		DEDEYEYDEEDRG	303–317	
Soy	G2 Glycinin	KLVLSLCFLLFSGCP	3–17	[35]
		NGPQEIYIQQGNGIF	83–97	
		QQGNGIFGMIFPGCP	90–104	
		RFYLAGNQEQEFLKY	177–191	
		LKYQQQQQGGSQSQK	189–203	
		VKGGLRVTAPAMRKP	255–269	
		LDFPALWLLKLSAQY	337–351	
		LKLSAQYGSLRKNAM	345–359	
		MFVPHYTLNANSIIY	358–372	
		NANSIIYALNGRALV	367–381	
		QHTFNLKSQQARQVK	469–475	
		GGSILSGFTLEFLEHAFSV	217–235	[36]
		GAIVTVKGGLSVI	253–265	
		SGFAPEFLKEAFGVN	219–233	[37]
	P34/Gly m Bd 30K	FLVLLLFSLL	3–12	[38]
		PQEFSKKTYQ	110–119	
		RCKANKIQDK	229–238	
		INHFVLLVGY	299–308	
		GYIWIQRNTG	311–340	
Wheat	ω-5 Gliadin	QQIPQQQ		[39]
		QQLPQQQ		
		QQFPQQQ		
		QQSPEQQ		
		QQSPQQQ		
		QQYPQQQ		
		PYPP		

Table 1. Continued

Source	Allergen	Epitope	Position	Reference
Pollen	Jun a 1	IFSQNMNIKLKMP	71–83	[40]
		AFNQFGPNAGQR	217–229	
		MPRARYGL	229–237	
		WRSTRDAFING	295–306	
	Par j 1	QGKEKEP	20–26	[41]
		SKGCCSGAKRLDG	27–39	
		KTGPQRV	42–48	
		PKHCGIVD	72–80	
		PAHKARLE	120–127	
	Par j 2	CLHFVK	14–20	
		VKGEEKEPSK	19–28	
		CSGTKKLSEE	30–40	
		EEVKTTEQ	38–45	
		KREACKCIVR	46–55	
		CIVRATKGI	52–60	
		KKCDIKTT	73–80	
		SKIQSTIF	91–98	
Tree nuts	Jug r 1	QGLRGEEMEEMV	33–44	[42]
	Ana o 1	AIMGPPTKFSFSLFL	1–15	[43]
		CKVQRQYDEQQKEQC	41–55	
		EQQKEQCVKECEKYY	49–53	
		KECEKYYKEKKGRER	57–71	
		EKKGREREHEEEEEE	65–79	
		DEAEEEDENPYVFED	145–159	
		RRGEGPKIWPFTEES	337–351	
		NITKGGMSVPFYNSR	393–407	
		TKIAIVVSGEGCVEI	409–423	
		SSHPSYKKLRARIRK	433–447	
		EEFFFQGPEWRKEKE	521–535	
Animal				
Egg	Ovalbumin	LAMVYLGAKDST	38–49	[44]
		DVYSFSLA	95–102	
		EDTQAMPFRV	191–200	
		VLLPDE	243–248	
		GLEQLESIIN	251–260	
	Ovomucoid	TDGVTYTNDCL	32–42	[45]
		DCLLCAYSIEF	40–50	
		KEHDGECKETV	56–66	
		SSYAN	71–75	
		DGKVMVLCNRA	80–90	
		TYDNE	101–105	
		KRHDGGCRKE	121–130	
		KTYGNKCNFCNAVVES	159–174	
		TLSHFGKC	179–186	
Shrimp	Tropomyosin	VHNLQKRMQQLEN	44–55	[46]
		ALNRRIQLLEEDLER	88–102	
		RSLSDEERMDALEN	133–146	
		ERMDALENQLKEARF	139–154	
		ESKIVELEEELRVV	188–198	
		LQKEVDRLEDEL	249–261	
		KYKSITDE	267–274	
		ELDQTFSEL	274–282	
Milk	αs2-Casein	SKENLCSTFCKEVV	31–44	[47]
		VVRNANEEEYSIGS	43–56	
		NEIN QFYQKFPQYLQYLY	83–100	
		PQYLQYLYQGPIVL	93–106	
		VLNPWDQVKR	105–114	
		VPITPTLNREQL	117–128	
		STEVFTKKTKLTEEEK	143–158	
		KNRLNFLKKISQRYQ	157–172	
		KKISQRYQKFALPQYLKTVYQHQK	165–188	
		KPWIQPKTKV	191–200	

ization and manipulation of specific regions of an allergenic protein. However, conformational IgE-binding epitopes are much more difficult to study when compared with linear IgE-binding epitopes. This is primarily due to the extensive knowledge of the 3-D structure of the allergen required to localize and reconstruct conformational epitopes. Because our knowledge of the 3-D structure of allergens is limited, the identification of conformational IgE-binding epitopes has lagged behind the identification of linear IgE-binding epitopes.

Most linear IgE-binding epitopes have been mapped using methods designed to identify specific regions on the allergen that bind IgE. Initially, epitopes could only be localized to relatively large peptide fragments based on protease digestion of a purified allergen followed by identification of the IgE-binding peptides by biochemical methods. This method was limited by the position of protease cleavage sites within the target amino acid sequence, size of the peptide that could be resolved in a polyacrylamide gel matrix, and the quantity of peptide required for amino acid sequence analysis. The techniques of molecular biology provided a refinement to this relatively crude localization of IgE-binding epitopes. Molecular biology technology allowed the amplification and subsequent expression of smaller protein fragments. It also eliminated the need to rely on proteases to generate peptides and, theoretically, could be used to generate any peptide designed by the investigators. In this manner, a more precise map of the IgE-binding regions on a specific allergen could be determined. More recently, advances in peptide chemistry allowed for the cost-effective synthesis of overlapping peptides that represent the entire allergen amino acid sequence. Bovine αS1-casein was one of the first allergens to which this technology was applied to determine the exact sequence of IgE-binding epitopes [27]. Using the known amino acid sequence of this protein, a set of 188 overlapping sequential decapeptides, each shifted by one amino acid, were manually synthesized on polyethylene pins using Fmoc chemistry. Serum IgE from 15 cow's milk-allergic patients was then used to determine which of the peptides are IgE-binding epitopes.

A further improvement on this technique allowed individual peptides to be synthesized on a derivatized membrane. Cycles of coupling, blocking, and deprotection reactions resulted in the construction of a series of peptides on a medium that could be readily probed with IgE. One of the first examples using this improved technology to study food allergy was the work by Stanley *et al.* [28], in which the authors defined ten linear IgE-binding epitopes of the major peanut allergen, Ara h 2, utilizing sera from 15 peanut-sensitive patients. Since this time, many linear IgE-binding epitopes from a variety of food allergens have been identified using this technology (Table 1). Unfortunately, there are no obvious biochemical characteristics (sequence, charge, size) that can be attributed to these peptides that could be used to predict protein fragments that are IgE-binding epitopes.

While the delineation of linear IgE-binding epitopes using these methods has led to a better understanding of allergen biochemistry, there are limitations to this technology that need to be considered. These limitations include the length of the peptide that can be synthesized and the use of serum IgE pools to identify epitopes. For example, most peptide synthesis technologies utilizing Fmoc chemistry recommend production of peptides no longer than 15 amino acids. This is a direct consequence of the fact that each coupling reaction that adds an additional amino acid is not 100% efficient. Even coupling reactions that occur at 97% efficiency will result in some nonsense peptides as the length of the chain is increased. The number of nonsense peptides begins to become significant after 15 coupling reactions. Therefore, the chemistry of the reaction limits the size of the peptide that can be synthesized and therefore epitopes that require more than 15 amino acids for IgE to bind would not be detected.

One approach to identify conformational IgE-binding domains was demonstrated using the major natural rubber-latex allergen hevein. In this approach, domains from the hevein allergen were engineered into a structurally similar but nonallergenic homolog to determine if the newly engineered protein bound IgE from latex-allergic patients. This work demonstrated that the IgE-binding ability of hevein is primarily determined by conformational eptiopes on the N-terminal and C-terminal regions of the protein [29].

Conformational IgE-binding epitopes on the house dust mite-allergen Der p 2 were first demonstrated by disrupting disulfide bonds known to stabilize the tertiary structure of the allergen [30, 31]. Further localization of the IgE-binding region was accomplished using hydrogen exchange NMR imaging followed by alanine scanning mutagenesis of the identified region to determine which amino acid residues are important to antibody binding [32].

Many studies have utilized pooled serum IgE from allergic patients to identify the IgE-binding epitopes of a specific allergen [33]. Utilizing this practice the investigator can conserve the limited availability of patient sera by using pools and still map the major IgE-binding epitopes of an allergic population. However, by pooling patient sera, IgEs in low concentrations or that are specific to a single patient may be diluted to such an extent that they will not be detected. This could lead to an under representation of the IgE-binding peptides of a specific allergen. Conversely, using pooled patient sera may lead to incorrect identification of certain epitopes as being overrepresented in the population.

4 Potential bioinformatic uses for IgE-binding epitopes

The use of an eight amino acid search window in the bioinformatics assessment of novel proteins was introduced as a means to identify proteins that did not share a significantly broader homology with known allergens. This was conceived as a conservative approach to identify potential IgE-binding epitopes within a larger amino acid sequence at a time when very few IgE-binding epitopes were known. In the ensuing years, linear IgE-binding epitopes were experimentally determined in a number of different allergens. Most IgE-binding epitopes were described as at least eight amino acids long, but some were as small as four amino acids in length (Table 1). This new information led to an FAO/WHO scientific panel recommendation that a six amino acid window should be used for this type of analysis (FAO Corporate Document Repository; available at http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/007/y0820e/y0820e00.htm) to ensure that potential cross-reactive proteins were not missed. However, a number of subsequent studies have analyzed the effect of search size window on the frequency of matches between proteins and peptides derived from a dataset containing protein allergens [12–15]. In each of these studies, it was noted that a sliding window of six identical amino acids yielded exceedingly high match frequencies with query sequences. Given that this search overestimated potential protein allergen frequencies that approached two of every three proteins found in a dataset, Stadler and Stadler [14] proposed that a motif-based allergen prediction scheme be used in place of this type of search. Likewise, observing matches in two of every three proteins, Kleter and Peijnenburg [13] attempted to justify the use of a six amino acid window size search by utilizing Hopp and Woods [34] antigenicity predictions on those proteins that are selected in this manner. Each of these approaches recognized the fallacy of using a search window that led to many false positive matches and proposed that an additional screen using a structure-based algorithm be utilized. However, these approaches may be flawed in that they utilize structure-based motifs that have not been proven to be associated with IgE-binding.

An alternative approach would be to perform a sequence comparison using the query protein and known IgE-binding epitopes. In this manner, the theoretical search using a small search window would be eliminated and replaced by a bioinformatics search using known IgE-binding epitopes. This approach has the advantage of using experimentally determined IgE-binding data. In addition, most of the allergens from the major food allergies have been mapped, so the major IgE-binding epitopes would be included in the search. However, there are also caveats to this approach. For example, the methods used to determine IgE-binding epitopes do not guarantee that minor epitopes recognized by IgE from a small subpopulation of allergic individuals would be detected. In addition, the IgE-binding epitopes of all allergenic proteins have not been determined yet. Furthermore, conformational epitopes would not be included in such a database. These limitations could severely restrict the utility of this type of bioinformatics search in identifying proteins with the potential to cross-react with known allergens. Finally, whatever approach is developed, it should be remembered that bioinformatics is but one tool in the array of analytical methods for determining potential allergenicity, by itself it does not imply a novel protein to be an allergen.

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