

IgE BINDING STRUCTURES OF THE MAJOR HOUSE DUST MITE ALLERGEN *DER P I*

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Abstract—The group I allergens *Der p I* and *Der f I* are potent allergens of mites from the genus *Dermatophagoides*. IgE radioimmune dot blots and immunoabsorption with recombinant peptides have been used to define areas of antigenicity. Four linear binding regions comprising residues 15–33, 60–80, 81–94 and 101–111 were found in the *N* terminal domain and one, 155–187, in the *C*-terminal domain, but direct evidence for their discontinuous nature is shown. Firstly, the binding activity of residues 60–80 required either *C*- or *N*-terminal flanking sequences to express reactivity and secondly a discontinuous determinant was directly demonstrated by the two non-overlapping peptides 53–99 and 101–154 which significantly cross absorbed specificities to one another. This also indicates considerable homogeneity in the antibodies recognising these peptides. The IgE binding peptides could be located to equivalent residues on the X-ray crystallographic structure of the homologous proteins actinidin and papain. The residues 81–94 and 101–111 which gave strong reactivity were located on a flexible loop connecting the domains and represent areas in which synthetic peptides could be expected to retain activity.

INTRODUCTION

The inhalant allergen *Der p I* is a cysteine protease excreted in the faeces of the house dust mite *Dermatophagoides pteronyssinus* (Chua *et al.*, 1988; Stewart *et al.*, 1989; Tovey *et al.*, 1981). IgE antibodies to this major allergen together with *Der p II* dominate the response of many mite-allergic patients Chapman *et al.* (1980); Lind *et al.* (1983); Baldo *et al.* (1989); Stewart *et al.* (1986). The response to *Der p I* is also remarkable in that about 50% of allergic patients have greater than 50% of the total anti-mite IgE is directed toward this single allergen (Chapman *et al.*, 1980; van der Zee *et al.*, 1988). It is, therefore, an ideal molecule for studying immunoregulation in allergic disease and in desensitisation therapy.

While it is clear that immunisation can induce antibody with specificity for almost all surface structures, regulatory influences can affect the degree to which a determinant is recognised. For example, mouse anti-lysozyme responses have a preponderance of antibodies with a cross reactive idotype directed to a structure which can be formed by disulphide linked *N*- and *C*-terminal peptides (Harvey *et al.*, 1979). There are also some indications, to be discussed, that at least some allergens have dominant epitopes and private and cross-reactive idiotypes have been reported for mite allergens (Saint-Remy, 1988). Previously we have found by quantitative absorption that *Der p I* produced from cDNA in *E. coli* retains about 50% of the IgE binding activity of

the native molecule, which offers a tool for examining the determinants recognised by a substantial amount of the IgE directed to this molecule. A detailed study using the reactivity of a highly allergic serum to large peptides produced from cDNA fragments has now been undertaken to determine the distribution and nature of the IgE binding residues. Some inference as to the structure of the IgE binding regions can be made by locating the position of equivalent peptides on the X-ray crystallographic structures of the homologous proteins papain and actinidin (Baker, 1980; Baker and Drenth, 1987; Drenth *et al.*, 1971). Strong binding was associated with short regions of a surface loop connecting the two domains of the molecule and discontinuous epitopes were directly demonstrated in a globular domain.

MATERIALS AND METHODS

Fusion peptides

Constructs expressing *Der p I* peptides—1–14, 1–56, 15–94, 34–80, 48–72, 53–99, 57–81, 57–130, 60–111, 95–208, 98–140, 101–154, 131–187, 166–194, 188–222 and 209–222 as fusion proteins from the pGEX vectors have been described (Greene *et al.*, 1990; Greene *et al.*, in press). A further two fragments of nucleotides 76–424 and 425–863 (numbers from Chua *et al.*, 1988, but with 6 nucleotides added to include the 5' *EcoRI* site now found to be an intrinsic part of the coding sequence) were produced by M. Trudinger by introducing a second *Bam* HI site into the cDNA clone half-way along the *Der p I* coding sequence. These fragments were cloned into the *Bam* HI site of pGEX-2T to allow separate expression of the two putative domains comprising residues 1–116 and 117–222 by homology with papain, see below.

Abbreviations: IPTG, isopropyl-B-D-thiogalactopyranoside;
GST, glutathione *S*-transferase.

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The peptides fused to glutathione *S*-transferase (GST), encoded by the Sj26 gene of the pGEX were expressed and affinity purified from glutathione agarose (Smith and Johnson, 1988). SDS-PAGE of the peptides (Laemmli, 1970) was performed using 8–18% (w/v) polyacrylamide gradient gels stained with 0.06% (w/v) Coomassie blue R250 (Sigma). Western blotting using a rabbit anti *Der p* I known to react with all fragments (Greene *et al.*, 1990) at a characteristic *M_r* was used to check clonal purity.

Dot blot radioimmunoassay

Nitrocellulose (Transblot, BioRad, Richmond, CA) was cut to size, soaked in 10 mM phosphate buffer, pH 7.4, containing 150 mM NaCl (PBS), and placed on 3MM paper (Whatman International Ltd, Maidstone, U.K.) before being clamped in a Bio-Dot (BioRad) apparatus. Samples (of 400 µl size) containing 50 µg of fusion peptide were applied by suction. Each well was washed twice with 400 µl of PBS. The nitrocellulose was

then washed with 10 mM Tris-HCl buffer, pH 8.0, containing 150 mM NaCl and 0.05% w/v Tween-20 (TNT) for 30 min and incubated overnight with serum at 4°C. Before use the serum was preabsorbed with vector encoded GST or fusion peptide (see below). After incubation with serum, the filters were washed and bound human IgE detected using ¹²⁵I radiolabelled mouse monoclonal anti-human IgE, Chua *et al.* (1990) at 2 × 10⁶ cpm/ml. When required densitometry was performed by scanning autoradiographs with an Epson GT4000 scanner (Seiko Epson Corp, Nagaro, Japan) set at 80 dots per inch resolution, 256 gray scales and brightness of 2 using the Macintosh Epscan program (Biosoft, Cambridge, U.K.). Scans were analysed using the Scan Analysis (Biosoft) program employing average background subtraction.

Serum

Human serum was obtained from a patient that gave a high RAST score (4+) to mite and had a very high

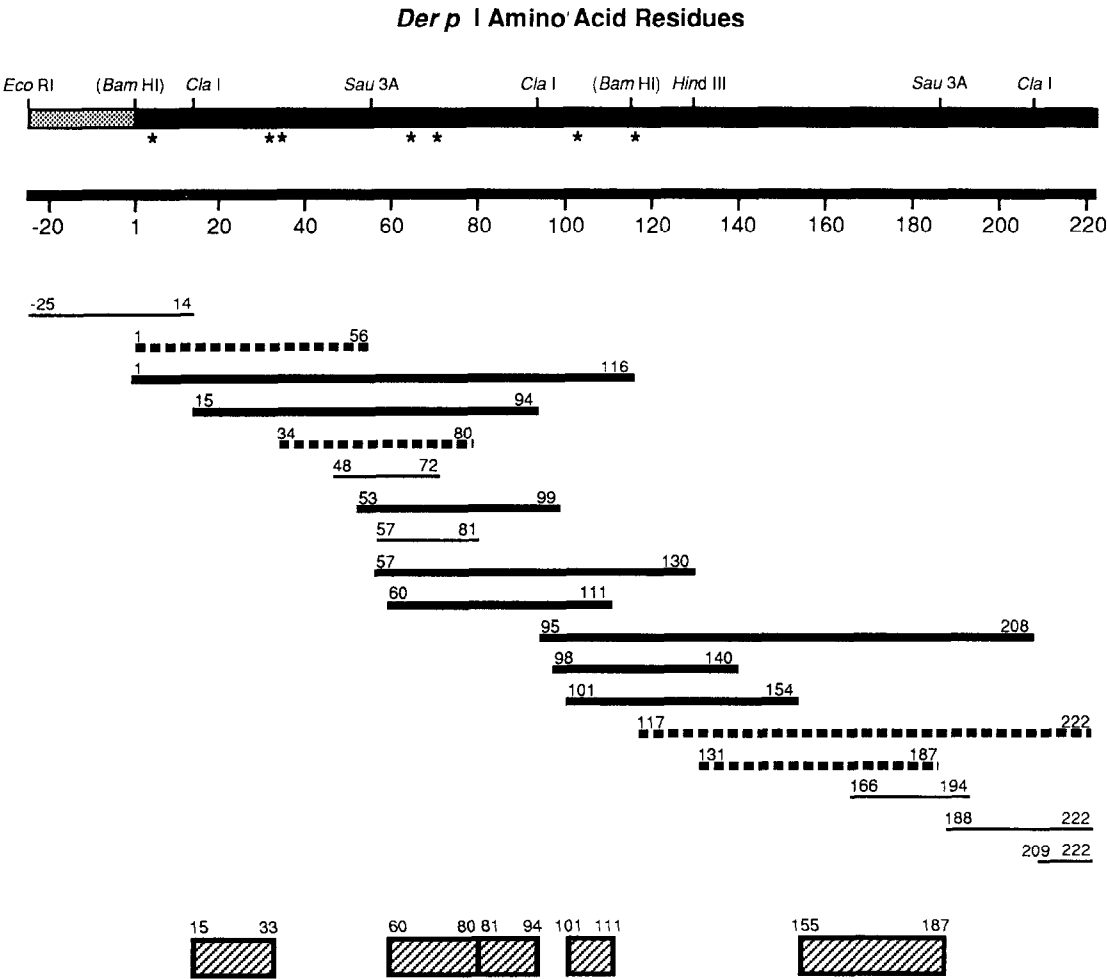


Fig. 1. Map showing *Der p* I sequence position of the 18 pGEX expression clones which cover the entire mature *Der p* I coding region represented by the solid bar. Peptides with densitometric IgE binding readings of more than 10,000 gray scale units are shown in black and readings of less than 500 are shown as lines. Broken bars are those with intermediate readings. Twelve peptides which bound IgE were used in a cross-absorption analysis on allergic serum (see Table 1). Boxes correspond to regions of *Der p* I deduced to account for IgE reactivity as explained in the Table 1 legend. Cysteines at positions 4, 31, 34, 65, 71, 104 and 117 are marked by asterisks. Minus residues are from the proenzyme sequence and are not found in native *Der p* I.

reactivity to *Der p* I by dot blot IgE immunoassay. The serum was used diluted 1:2 in *E. coli* lysate (see below) containing 5% w/v skim milk powder and 0.1% w/v sodium azide.

Serum was absorbed with affinity purified GST fusion peptides expressed by *E. coli* HB101 transformed with pGEX constructs. GST or GST fusions were added to serum at 1 mg/ml final concn, incubated with rotation overnight at 4°C and then clarified by centrifugation (10,000 g, 15 min).

RESULTS

GST-*Der p* I fusion peptides

The 18 clones shown in Fig. 1 expressed overlapping peptides fused to the C-terminus of GST which collectively comprise the entire *Der p* I sequence. The level of recombinant peptide synthesised varied from an estimated 10–25% of total *E. coli* protein as judged by SDS-PAGE and had discrete major bands at their predicted molecular weight. Yields of fusion peptide affinity purified using glutathione agarose varied from 0.2–20 mg/L of culture depending on the peptide. The affinity purified preparations were examined by SDS-PAGE for stability. Some peptides (60–111, 15–94, 1–56 and 166–194) did show significant breakdown, but had 20–30% of the fusion with the expected M_r and except for 166–194 had IgE binding activity.

IgE epitope localisation

A total of 18 fusion peptides (Fig. 1) were tested for IgE reactivity using serum from a highly allergic patient BN, six of which, minus 25–14, 48–72, 57–81, 166–194, 188–222 and 209–222, were unreactive as measured by IgE dot blot radioimmunoassay. A hyper IgE serum known to lack anti *Der p* I antibodies used at 1000 IU/ml of IgE did not react with any of the peptides. To define IgE B cell epitopes of *Der p* I cross-absorption experiments were performed by absorbing aliquots of a mite-allergic serum (BN) with each of the 12 IgE reactive peptides. IgE dot blot radioimmunoassay was used to assess the changes in reactivity. The results are summarised in Fig. 1 and Table 1. Based on a linear sequence analysis detailed in the legend, 5 regions 15–33, 60–80, 81–94, 101–111 and 155–187, were deduced to contain residues responsible for IgE binding. Binding regions were delineated by positive reactivity of a peptide or by the removal or persistence of reactivity to a fusion peptide after serum absorption with other positive overlapping peptides. Details are included in Table 1, but for example, 101–111 was inferred to account for the reactivity of the peptide 101–154 because absorption with 60–111 removed all reactivity to itself and 101–154. The reactivity of BN was mainly to the N-terminal domain of *Der p* I, which contains 4 of the 5 IgE binding regions. This observation is supported further by an absorption study which showed that the peptide 1–116 removed almost all the IgE reactivity to the full length recombinant molecule whereas 117–222 removed very little (Fig. 2).

Table 1. Cross-absorption of serum BN IgE reactivity using overlapping *Der p* I peptides

IgE reactivity with peptides comprising residues:	BN serum absorbed with <i>Der p</i> I peptides comprising residues:											
	1–56	1–116	15–94	34–80	53–99	57–130	60–111	95–208	98–140	101–154	117–222	131–187
1–56	–	–	–	+	+	+	+	+	+	+	+	+
1–116	+	–	–	+	+	+	+	+	+	+	+	+
15–94	+	–	–	+	+	+	+	+	+	+	+	+
34–80	+	–	–	–	–	–	–	–	–	–	–	–
53–99	+	–	–	+	–	–	–	–	–	–	–	–
57–130	+	–	–	+	–	–	–	–	–	–	–	–
60–111	+	–	–	+	–	–	–	–	–	–	–	–
95–208	+	–	–	+	–	–	–	–	–	–	–	–
98–140	+	–	–	+	–	–	–	–	–	–	–	–
101–154	+	–	–	+	–	–	–	–	–	–	–	–
117–222	+	–	–	+	–	–	–	–	–	–	–	–
131–187	+	–	–	+	–	–	–	–	–	–	–	–

Cross-absorption of serum BN *Der p* I-specific IgE reactivity using overlapping *Der p* I peptides. BN serum was absorbed with each of twelve affinity purified fusion peptides (shown in Fig. 1). Changes in IgE reactivity of peptides after absorption was assessed by dot blot radioimmunoassay and the results used to deduce regions responsible for IgE binding. Region 15–33 was inferred because the peptide 1–56 retained antigenicity after absorption with peptide 34–80, and because 15–94 and 60–111 removed reactivity to 34–80; region 60–80 because absorption with 60–111 abrogated reactivity to peptide 34–80; region 81–94 since the peptide 53–99 retained reactivity after absorption with 34–80 but not 15–94; region 101–111 because peptides 98–140 and 101–154 were rendered non-reactive after absorption with 60–111; and region 155–187 because the reactivity of 131–187 was not inhibited by peptide 101–154.

+ + Positive reactions; + reduced reaction; – no reaction.

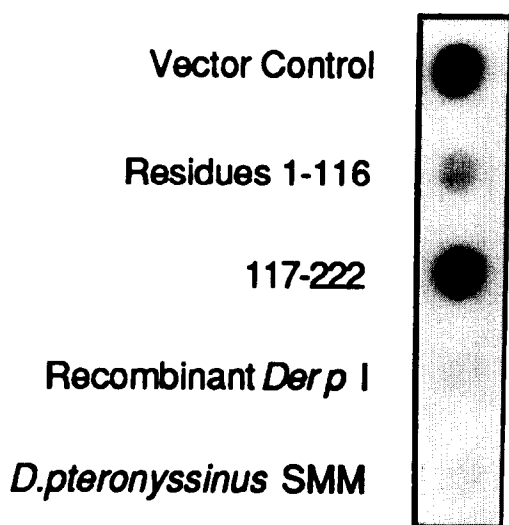


Fig. 2. Absorption of *Der p* I-specific IgE reactivity from allergic serum BN. Serum was absorbed with peptides 1–116 and 117–222, full length *Der p* I, vector control, or *D. pteronyssinus* spent mite medium (SSM) and tested by radio-immunoassay with 50 μ g of recombinant *Der p* I. Most of the IgE reactivity occurs in the left domain (residues 1–116). All reactivity of GST-*Der p* I was removed by both itself and native *Der p* I contained in the spent mite medium.

Discontinuous nature of *Der p* I epitopes

Absorption analysis revealed an interesting example of cross-inhibition between the non-overlapping peptides 53–99 and 101–154 (Fig. 4), a result which suggested that these two peptides contribute residues to a larger, discontinuous determinant recognised by a relatively homogeneous population of IgE antibodies. Peptide size in some regions also appears critical for IgE binding since although the short (25 residue) peptides 48–72 and 57–81 did not react with IgE from patient BN, they did in fact contain the reactive region 60–80. This is demonstrated by the observation that peptide 34–80 retained reactivity when absorbed with the vector control, or 1–56, but lost all reactivity when absorbed with itself (34–80) or 60–111 (Fig. 3). The antigenic determinant must, therefore, be located between residues 60–80 because they are the only residues common to the peptides. Thus the IgE determinant in this region can be

expressed provided either of the flanking sequences are present.

DISCUSSION

Five IgE-binding determinants of *Der p* I have been identified by the reactivity of peptide fusions expressed in *E. coli* using serum with high reactivity to *Der p* I. By using immunoabsorption, a map of the regions responsible for IgE reactivity was constructed. The sites were limited to four regions, 15–33, 60–80, 81–94 and 101–111 located in the *N*-terminal half and one, 155–187, in the *C*-terminal. Since serum absorbed with recombinant *Der p* I displays about half the IgE binding activity to native *Der p* I (Greene *et al.*, in press) they represent a substantial amount of the allergic reactivity. The concentration of recombinant protein used for inhibition was also similar to that reported by Lind (1985) to produce inhibitions of solid phase assays with purified *Der p* I. That at least some of the binding to recombinant *Der p* I required tertiary structure was shown by absorption studies which revealed the region 60–80 to be responsible for the IgE reactivity of the peptide 34–80 yet peptide 57–81 contained this region and was non-reactive. The increased reactivity which increasing peptide length may reflect the effect of size on stabilisation of the conformation complementary to the antibody. Reactivity of a fragment may also depend, in part, on the point of cleavage relative to the structure of an antigenic-allergenic determinant. Other aspects that could influence antigenicity is the requirement for a proenzyme sequence for complete folding and the possible *N*-glycosylation of the Asn–Gln–Ser at 52–54. Although this has not been studied in detail the fact that the signal sequence for glycosylation is conserved in *Der p* I and *Der f* I despite numerous basic changes indicates it has some importance (Dilworth *et al.*, 1991). A similar analysis on the requirement for flanking regions could not be performed for the other binding regions from the material available, but will be of some interest and practical importance.

Although the three dimensional structure of *Der p* I is not known the position of epitopes could be estimated

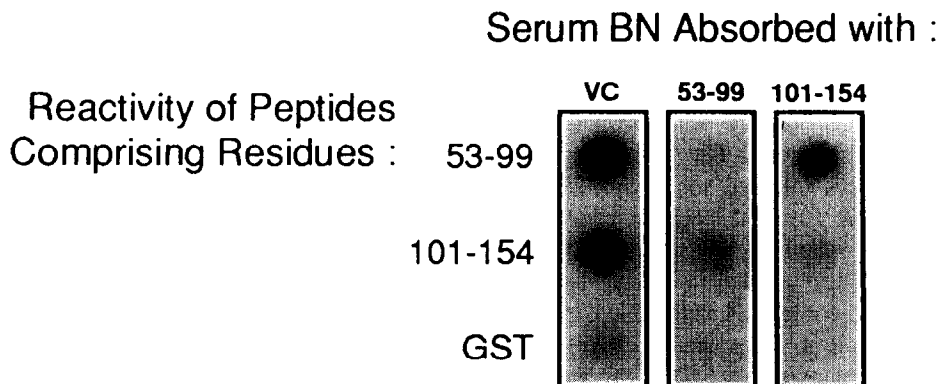


Fig. 3. Cross-absorption of serum IgE reactivity by the non-overlapping peptides 53–99 and 101–154. Aliquots of serum BN were absorbed with affinity purified GST vector control (VC) or fusion peptides 53–99 and 101–154 at 1 mg/ml and tested for binding activity to each peptide.

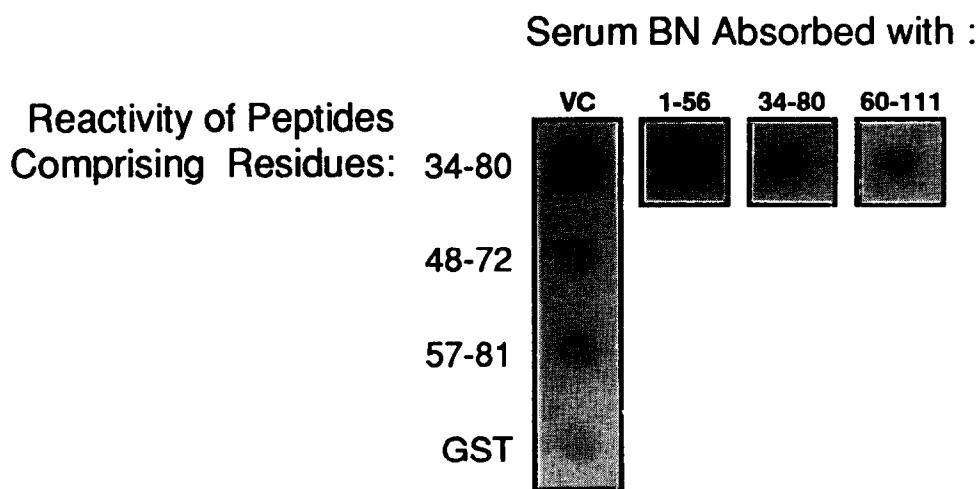


Fig. 4. Effect of flanking sequences on ability to bind *Der p* I-specific IgE. Serum BN absorbed with vector control GST (VC) shows IgE reactivity to 34–80 but reactivity to peptides 48–72 and 57–81 indistinguishable from the GST fusion control. The region 60–80 is IgE reactive, however, since reactivity to 34–80 is removed by absorption with itself and 60–111, but not by 1–56.

by aligning the IgE binding sequences with the corresponding residues of the X-ray crystallographic structures of the homologous enzymes papain and actinidin (Fig. 5) (Drenth *et al.*, 1971; Baker, 1980; Baker and Drenth, 1987). Cysteines for two of the three disulphide bonds present on papain and actinidin are conserved in *Der p* I and in the *L* domain (residues 1–123) 13/14 of the hydrophobic core residues found in actinidin and papain are conserved in *Der p* I and 13/15 in the right domain. The conserved half-cystines are both in the *L* domain. Notable differences are the additional nine *N*-terminal residues of *Der p* I which include a cysteine residue. A further cysteine (residue 117) is located in the *R* domain,

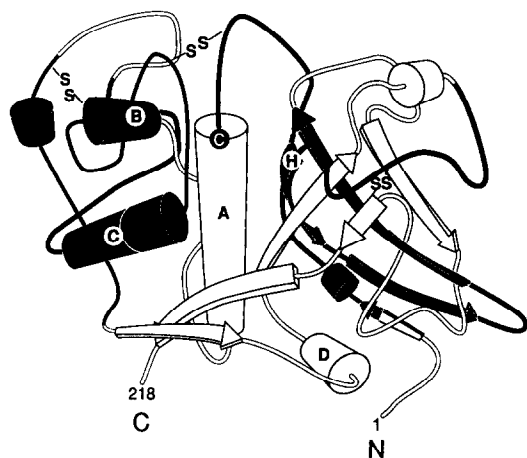


Fig. 5. Schematic representation of the polypeptide folding in papain and actinidin, modified from Baker and Drenth (1987). These enzymes have similar structures consisting of two globular domains of about 110 residues separated by a catalytic cleft. The *L* (left) domain consists of three major helical segments while the *R* domain is comprised of beta-pleated sheets. Alpha-helices are represented by cylinders (A–D), and strands of beta-sheet by arrows. C and H represent the catalytic residues cysteine 34 and histidine 170 respectively. Areas in black indicate those regions of *Der p* I deduced to be responsible for reactivity of IgE from serum BN.

which does not correspond to the half-cystine in the *R* domain of actinidin or papain.

The five regions deduced to be responsible for reactivity with IgE from serum BN are shown in Fig. 5. Region 15–33, based on homology with the cysteine proteases papain and actinidin, consists of a loop close to the *N*-terminus. BN also recognised residues 60–80 which comprises helix B and the loop connecting to helix C, 81–94 which is part of helix C and an adjoining loop structure and 101–111 which forms part of the surface loop connecting the two domains. The remaining region in the right domain, 155–187, comprises a loop connected to a beta-pleated sheet. Together, the regions 60–80, 81–94 and 101–111 appear to be an intensive area of IgE binding since folding of the polypeptide chain brings them into close proximity (Fig. 5). Further evidence that this region comprises a larger, discontinuous epitope was provided by an analysis which revealed cross-absorption between peptides 53–99 and 101–154 despite their lack of sequence overlap. Antibodies to this region, therefore, may have important contact residues to both peptides. This observation also implies that IgE antibodies are relatively homogeneous with a significant proportion possessing epitopes which require the two peptides. The cross absorption was, however, not complete so each of these peptides did have independent binding specificities. The existence of immunodominant IgE determinants is also suggested by studies of other allergens, namely codfish *Gad c* I Elsayed (1972) and hazel *Cor a* I Elsayed (1989) which appear to elicit IgE that binds predominantly to discrete peptides. The extreme dependence of other allergens such as *Amb a* I (Olsen and Klapper, 1986) and *Der p* II (Lombardero *et al.*, 1990; Chua *et al.*, 1991) on an intact structure for binding may also suggest a limited repertoire of antibodies.

The finding that high IgE binding was located on peptides comprising the flexible loop connecting the domains (residues 81–94, 101–111) may be because of

the ability to adopt a number of configurations in the native molecule increases their antigenicity or because these are the regions which because of their flexibility conserve their antigenicity when expressed in *E. coli*. Alternatively, they may be regions which have less constraints on their structure and are evolutionary more divergent than the homologous mammalian enzymes cathepsins H and L as already pointed out by Baker and Drenth for papain and actinidin. They also may for similar reasons be easy to express in recombinant molecules and synthetic peptides and experiments to determine if flanking regions are required are planned. Since peptides 98–140 and 101–154 containing these structures have also given strong reactivity in other sera (Greene, in press) they may provide a basis for efficiently engineering a synthetic allergen.

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