

Solution Structure of Der f 2, the Major Mite Allergen for Atopic Diseases*

(Received for publication, July 16, 1997, and in revised form, October 8, 1997)

Saori Ichikawa‡§, Hideki Hatanaka‡¶, Toshifumi Yuuki||, Namiko Iwamoto||, Soichi Kojima**, Chiharu Nishiyama||, Kenji Ogura‡, Yasushi Okumura||, and Fuyuhiko Inagaki‡

From the ‡Department of Molecular Physiology, Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113, the §Department of Material and Biological Science, Faculty of Science, Japan Women's University, 2-8-1 Mejirodai, Bunkyo-ku, Tokyo 112, the ||Bioscience Research & Development Laboratory, Asahi Breweries, Ltd., 2-13-1, Ohmori-kita, Ohta-ku, Tokyo 143, and the **Laboratory of Gene Technology and Safety, Tsukuba Life Science Center, The Institute of Physical and Chemical Research (RIKEN), Koyodai, Tsukuba, Ibaraki 305, Japan

House dust mites cause heavy atopic diseases such as asthma and dermatitis. Among allergens from *Dermatophagoides farinae*, Der f 2 shows the highest positive rate for atopic patients, but its biological function in mites has been perfectly unknown, as well as the functions of its homologs in human and other animals. We have determined the tertiary structure of Der f 2 by multidimensional nuclear magnetic resonance spectroscopy. Der f 2 was found to be a single-domain protein of immunoglobulin fold, and its structure was the most similar to those of the two regulatory domains of transglutaminase. This fact, binding to the bacterial surface, and other small pieces of information hinted that Der f 2 is related to the innate antibacterial defense system in mites. The immunoglobulin E epitopes are also discussed on the basis of the tertiary structure.

Mites are the closest animals to human life and their relation is inseparable in the modern residential environment. House dust mites cause heavy atopic diseases such as asthma and dermatitis, which are rapidly increasing worldwide, especially among the children in developed countries. *Dermatophagoides farinae* and *D. pteronyssinus* are recognized as the main sources of house dust allergens. Among their allergens, the group-2 allergen proteins, Der f 2 and Der p 2, show the highest positive rate for atopic patients (1) so they are called major allergens. Their sequences are 88% identical (2, 3), and their cross-reactivity was well confirmed (1). They are also homologous to the major allergen from the mite *Lepidoglyphus destructor* (4), which is important in farming environments. These proteins are 125–129 amino acid residues long and have three intramolecular disulfide bonds.

Although the properties of these proteins related to their allergenicity have been well characterized, their biological function in mites is unknown. Homologous proteins were also found in human epididymis, cow milk, and moth trachea (5–7) and named HE1, EPV20 and esr16, respectively, the first two of which are glycoproteins unlike the group-2 mite allergens. However, there have been no clues for their functions except for

their expression patterns. This is in contrast to the case of the other major allergen group including Der f 1 and Der p 1, because they were found to be cysteine proteases, and their activity was suggested to be involved in the induction of allergic responses (8). Therefore, the innate functions of Der f 2 and Der p 2 have interested many allergy researchers.

It is generally accepted that allergic symptoms are initiated by the specific binding of allergens to immunoglobulin E (IgE)¹ antibodies, which cross-link the high affinity IgE receptors on mast cells and basophils (9). So monovalent ligands to allergen-specific IgE are expected to block IgE receptor aggregation. Structure determination of allergens will offer the basis of design of such drugs. The tertiary structures of some pollen allergens have been previously reported including ragweed allergens Amb t 5 and Amb a 5, a major birch allergen Bet v 1, and a minor birch allergen profilin (10–14). However, the tertiary structure of Der f 2 and the following drug-design processes are exceptionally urgent considering the serious mental influences on atopic children.

In this study, we have determined the tertiary structure of Der f 2 by multidimensional nuclear magnetic resonance (NMR) spectroscopy. Unexpectedly we found that Der f 2 is a single-domain protein of immunoglobulin fold. There are few single-domain proteins of this fold, and this protein is soluble and monomeric. We feel it is of interest also in terms of the evolution of protein folds. The structural similarity to the two regulatory domains of transglutaminase and other small pieces of information prompted us to suppose that Der f 2 is a component of the innate antibacterial defense system in mites. Furthermore, we found that Der f 2 binds to the surface of bacteria, which is the first clue to the biological function of this class of proteins. We also discuss previous work related to the immunoglobulin E epitopes on the basis of the tertiary structure.

EXPERIMENTAL PROCEDURES

Sample Preparation—*Escherichia coli* BL21 cells harboring pFLT1 (pGEMEX1 derivative containing cDNA for clone 1 of Der f 2; see Ref. 15) were cultured in M9 minimum medium in the presence of [¹³C]glucose and [¹⁵N]ammonium chloride. Labeled recombinant Der f 2 was expressed and purified as described (16). NMR samples contained 1.5 mM of Der f 2 in 90% H₂O, 10% D₂O, 140 mM N-octyl-β-D-glucoside, 0.01% NaN₃ at pH 5.6.

NMR Spectroscopy—NMR spectra were acquired at 55 °C on a Varian Unityplus 600 NMR spectrometer equipped with a triple resonance pulse field gradient probe. The sequential assignment of the ¹H, ¹³C and

* This work was supported in part by the Tokyo Metropolitan Government (to F. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The atomic coordinates and restraints (codes 1AHK, 1AHM, 1IAHKMR) have been deposited in the Protein Data Bank, Brookhaven National Laboratory, Upton, NY.

¶ To whom correspondence should be addressed. Tel.: 81-3-3823-2101; Fax: 81-3-3823-1247; E-mail: hatanaka@rinshoken.or.jp.

¹ The abbreviations used are: IgE, immunoglobulin E; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; RMSD, root mean square difference.

^{15}N chemical shifts was achieved mainly by through-bond heteronuclear correlations along the backbone and side chains with the following 3D pulse sequences: HN(CO)CA, HNCA, CBCA(CO)NH, CBCANH, HBHA(CO)NH, HN(CA)HA, C(CO)NH, HC(C)H-TOCSY (17). Nuclear Overhauser effects (NOEs) were derived from three-dimensional ^{15}N -edited and ^{13}C -edited NOE spectroscopy spectra recorded with a mixing time of 75 ms. The pulse sequences of HN(CO)CA, HNCA, CBCANH, HBHA(CO)NH, HN(CA)HA, C(CO)NH and ^{15}N -edited NOE spectroscopy were modified with pulse field gradient method and sensitivity enhancement method (17).

Structure Calculations—Upper limits of distance constraints were calculated as $kI^{-1/6}$, where I is the peak intensity and k is a constant adjusted in each NOE spectroscopy spectrum and relaxed by 0.5 Å considering mobility. Lower limits of distance constraints were all 1.8 Å. The structures were calculated with the program X-PLOR ver. 3.1 (18). Initial coordinates were generated using random ϕ and ψ angles, whereas peptide bonds and side-chains took extended conformations. The macroprogram sa.inp in X-PLOR ver. 2.1 was used to carry out simulated annealing calculation. The target function that is minimized during simulated annealing comprises only potential terms for covalent geometry, experimental distance restraints, and van der Waals non-bonded repulsion. No hydrogen bonding, electrostatic, 6-12 Lennard-Jones potential or experimental torsion angle terms were present in the target function. The final structure calculations were based on 1086 interproton distance restraints (526 intra-, 258 sequential ($|i-j|=1$), 56 middle range ($2 \leq |i-j| \leq 5$), and 246 long range ($|i-j| > 5$) NOEs). A final set of 10 converged structures was selected from 20 calculations on the basis of agreement with the experimental data and van der Waals energy. A mean structure was obtained by averaging the coordinates of the structures that were superimposed in advance to the best converged structure and then minimizing under the constraints.

In Vitro Binding Assay—The *E. coli* strain C was grown to late log phase, collected by centrifugation, and suspended in 1/10 of the original volume with 0.9% NaCl. An equal volume of 20% acetic acid was added, and the bacteria were left at room temperature for 5 min. 5 volumes of 1 M Tris-HCl (pH 8.2) was added, and the bacteria were collected by centrifugation and resuspended in one-tenth of the original culture volume in 10 mM Tris-HCl (pH 8.2). 100 μg of Der f 2 were added to the bacterial suspension (0.1 ml of Der f 2 to 0.1 ml of bacteria) and incubated at room temperature for the time indicated. The suspension was centrifuged for 2 min at $10,000 \times g$, the pellet was washed twice in 200 μl of water and then suspended in 40 μl of 0.5 M ammonium formate (pH 6.4). The ammonium formate eluates were immediately adjusted to 0.1% sodium dodecyl sulfate and 10 mM dithiothreitol, heated at 70 °C for 5 min, and subjected to electrophoresis (19).

RESULTS AND DISCUSSION

Assignments and Structure Determination—Backbone sequential assignments for Der f 2 (129 amino acid residues) were obtained by a strategy using a combination of four triple resonance measurements, 3D HN(CO)CA, HNCA, CBCA(CO)NH, CBCANH, and were complete except for H^{N} and N of Asp-1 and Thr-123, and C^{α} of Glu-53. The nitrogen chemical shifts of prolines were not assigned. Side-chain assignments were obtained principally by 3D C(CO)NH, HC(C)H-TOCSY and HC(C)H-COSY, and partially extended using NOE data. All nonexchangeable resonances were assigned except for Lys-33 C^{ϵ} and Lys-126 H^{γ} , H^{δ} , and H^{ϵ} , whose assignments were not fixed owing to heavy overlaps. In addition, $\text{H}^{\epsilon 1}$ and $\text{N}^{\epsilon 1}$ of Trp-92 were not detected, possibly because of fast proton solvent exchange enhanced by interaction with added detergent molecules.

The secondary structure was determined as all β , using tertiary NOEs between backbone protons (Fig. 1), which had been estimated beforehand by the results of chemical shift index method (20). Using 1086 distance constraints extracted after assignments of 3D ^{15}N - and ^{13}C -edited NOE spectroscopy spectra, we obtained 10 structures from 20 calculations. A summary of the structural statistics for a set of the final structures and for the mean structure is presented in Table I. There were no violations above 0.6 Å in any of the structures, and the number of violations above 0.3 Å ranged from 4 to 13. The deviation from ideal bond lengths was 0.004 Å. These figures

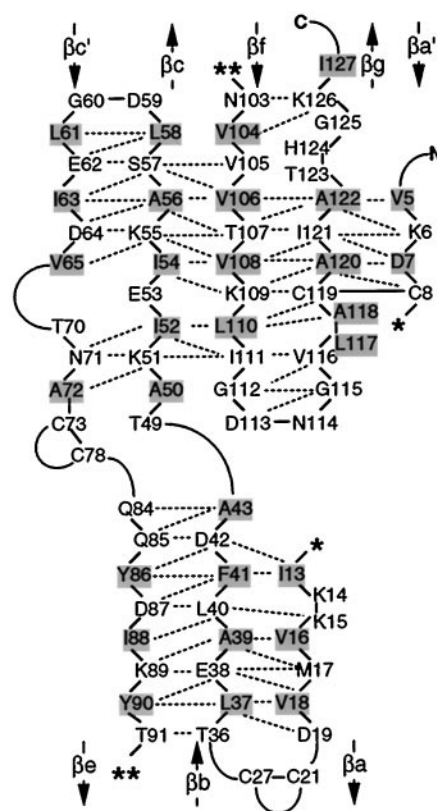


FIG. 1. Secondary structure of Der f 2. Interstrand backbone-backbone NOEs are marked by dotted lines. The residues that project their side chains below the sheet are shown in the shadowed box. The topology of the β -strands are depicted by arrows.

TABLE I
Structural statistics

(SA) refers to the final set of the simulated annealing structures and (SA)_r is the mean structure. The number of terms is given in parentheses.

Parameter	(SA)	(SA) _r
RMSDs from experimental distance constraints (Å) (1086)	0.058 ± 0.001	0.057
Number of distance constraint violations greater than 0.3 Å	4–13	9
Maximum (Å)	0.53	0.42
F_{NOE} (kcal/mol) ^a	183.6 ± 7.9	178.6
F_{repel} (kcal/mol) ^a	73.5 ± 6.1	64.0
RMSDs from idealized geometry		
Bonds (Å) (1995)	0.004 ± 0.000	0.004
Angles (degrees) (3644)	0.78 ± 0.01	0.74
Impropers (degrees) (994) ^b	0.66 ± 0.04	0.59

^a The value of the square-well NOE potential, F_{NOE} , is calculated with a force constant of 50 kcal/mol per Å². The value of F_{repel} is calculated with a force constant of 4 kcal/mol per Å² with the van der Waals radii scaled by a factor of 0.8 of the standard values used in the CHARMM empirical function.

^b The improper torsion term is used to maintain the planar geometry and chirality.

are relatively good, considering that our distance constraint set was tighter than those in the typical three-level classification. However, since the number of NOE constraints was not large, the quality of the structure should be regarded as medium.

Description and Evaluation of the Structure—Fig. 2a shows the tertiary structure determination of Der f 2. The root mean square difference (RMSD) value of the 10 final structures from the minimized averaged structure was 0.90 ± 0.15 Å for the backbone (N, C $^{\alpha}$, C $^{\gamma}$) atoms of residues 1–129 and 1.44 ± 0.17 Å for the nonhydrogen atoms of the same residue range. We also calculated local RMSD values for the backbone atoms (N,

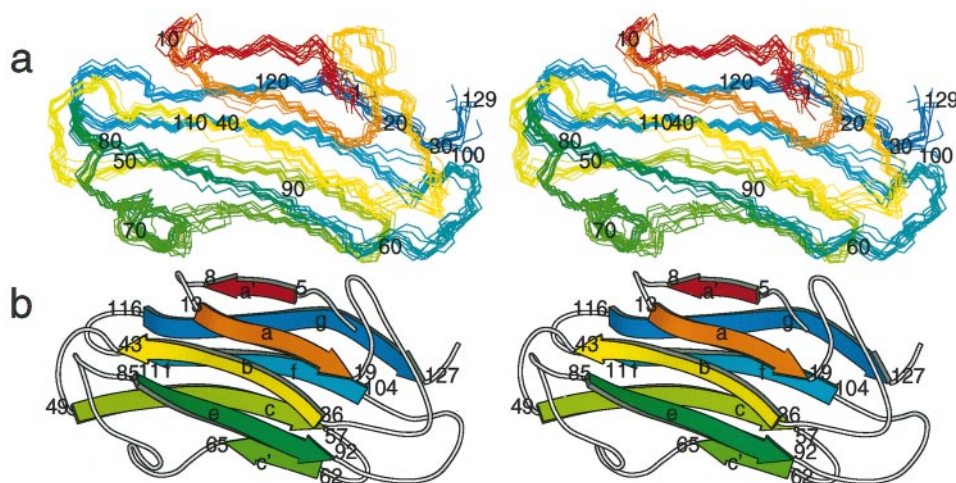


FIG. 2. **Tertiary structure of Der f 2.** *a*, Best fit superpositions of the backbone atoms (N, C α , C') of the 10 final structures of Der f 2. Amino acid residue numbers are shown every ten residues. *b*, Ribbon diagrams of the mean structure of Der f 2. The start and end residue numbers of the β -strands are also shown. Both figures were produced by the program MOLSCRIPT (44) and shown in stereo. Color changes from red (N terminus) to blue (C terminus).

C α , C') at each residue after the best fit superposition of the whole backbone (data not shown), and found that the residues of large local RMSD value were distributed around the whole sequence. Therefore the reason the structural convergence was not excellent is not due to local flexibility but to the number of NOE distance constraints.

Der f 2 has an immunoglobulin fold (Fig. 2*b*). The topology corresponds to s-type (21). One β -sheet consists of three strands of residues 13–19 (*a*), 36–43 (*b*), and 85–92 (*e*), and the other sheet is composed of four β -strands 62–65 (*c'*), 49–57 (*c*), 104–111 (*f*), and 116–127 (*g*), and an additional short strand of residues 5–8 (tentatively named as *a'*). They include three β -bulges at the positions 14–15, 117–118, and 123–124. Der f 2 has three disulfide bonds at the positions 8–119, 21–27, and 73–78 (22), but no disulfide bridge between the β -strands *b* and *f*, which is often found in immunoglobulin-fold domains.

The immunoglobulin fold is the most ubiquitous module and is distributed among many protein superfamilies of different functions (21). There are, however, few single-domain proteins with the immunoglobulin fold. Der f 2 is not membrane bound like Thy-1, a subunit in a protein complex like β_2 -microglobulin, nor does it polymerize into filaments like the major sperm protein from nematode. Therefore, this simple immunoglobulin-fold protein, Der f 2, might reflect the characteristics of the most ancient immunoglobulin-like domain, and it is of special interest to compare this structure with other domains of immunoglobulin fold, mainly distributed among vertebrate immune systems, cell surface receptors, coagulation/fibrinolysis systems, and some enzymes that bind to sugar chains.

Structural Similarity to Domains of Transglutaminase—We searched for structural similarities to the known protein folds using the program Dali (23). It listed many proteins of immunoglobulin fold, but the fourth domain of human blood coagulation factor XIII (24) was reported to be the most structurally similar to Der f 2, where the Z-score was 4.6. This value is not large, probably because of the quality of our structure determination. Because the second one, the second domain of vascular cell adhesion molecule-1 (25), had the Z-score of 4.2, it is difficult to conclude that the domain of factor XIII is the most similar.

Transglutaminase, including factor XIII, is composed of four domains, and the third and fourth domains have the s-type immunoglobulin fold. We superimposed the Der f 2 structure onto the third and fourth domains of factor XIII using 62 C α pairs (Fig. 3*a*) and obtained RMSD values 2.77 Å in both cases.

Then we aligned the primary sequences of these factor XIII domains (26) to those of Der f 2 (2) and homologous proteins (3–7) on the basis of tertiary structures (Fig. 3*b*). Although the sequences in each group are highly variable, we could find identical amino acid residues shared by both groups at many positions. The numbers of such residues were 32 both in the third and fourth domains of factor XIII, which is much larger than the number in the second domain of vascular cell adhesion molecule, which is 22. Phe-41, which is located at the center of domains, is perfectly conserved among the two groups shown in Fig. 3*b*. In particular, the factor XIII fourth domain is well aligned to the group-2 mite allergens without large insertions or deletions except for three disulfide bond-related segments.

Intriguingly, Der f 1, the other major mite allergen, is a cysteine protease, and this protease family has been shown to be evolutionally related to the second domain of factor XIII (27). In addition, Tyr-372 (Fig. 3*a*, red) of factor XIII, neighboring the catalytic triad (orange), corresponds to the tyrosine that has been recognized as unique to the mite allergen cysteine proteases (8). The fact that Der f 2 inhibited guinea pig liver transglutaminase² is also interesting as it relates to the structural similarity described above.

Implications for Innate Functions in Mites—Although the biological function of Der f 2 is unknown, the above findings reminded us of the innate immune system of invertebrates (28). In invertebrates that do not have immunoglobulins, the coagulation system is prominent as an antimicrobial response. Two types of coagulation mechanisms have been reported, each of which are associated with transglutaminase and a cascade of serine proteases, respectively. Der f 3, a minor mite allergen, is a serine protease that has a similar substrate specificity to blood coagulation factor XII and is reported to activate the human serine protease cascade (29).

We found that Der f 2 binds the surface of *E. coli* cells (Fig. 4) in a manner similar to hemolin, a bacteria-binding protein from moths composed of four immunoglobulin-like domains (30). Der f 2 has a cluster of nine basic amino acids (Fig. 3*a*), which implies a negatively charged target surface. Preliminary results show that Der f 2 does not bind to strains K12 or BL21. All of the three types of mite allergens mentioned are localized in the gastrointestinal tract, mouth region and feces (31, 32), and the feces, which are suggested to cause allergic symptoms

² S. Kojima, unpublished observation.

FIG. 3. Relationship between Der f 2 and factor XIII. *a*, Superpositions of the mean structures of Der f 2 (black) and human coagulation factor XIII (green; Ref. 24). The regions used for superposition are shown in **bold lines**. A cluster of nine basic amino acids in Der f 2, His-22, His-30, Arg-31, Lys-33, Lys-96, Lys-100, His-124, Lys-126, and Arg-128, is shown in blue. Domain 2 of factor XIII is discriminated by yellowish green. The catalytic triad residues Cys-314, His-373 and Asp-396 are shown in orange, and Tyr-372 is shown in red. *b*, Sequence alignments of the factor XIII third and fourth domains (26) and Der f 2 (2) and homologous proteins Der p 2, Lep d 1, HE1, EPV20, and esr16 (3–7). The conserved cysteines among Der f 2 homologs are shown in cyan. Identical amino acid residues shared by both groups are in red. β -strands are underlined. The numbers above the sequences correspond to Der f 2.

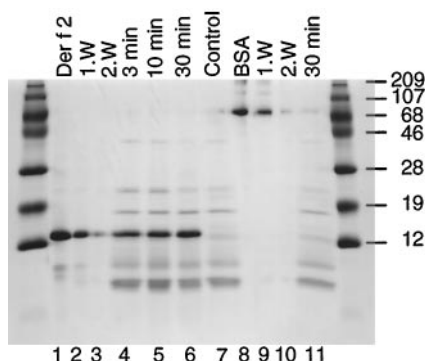
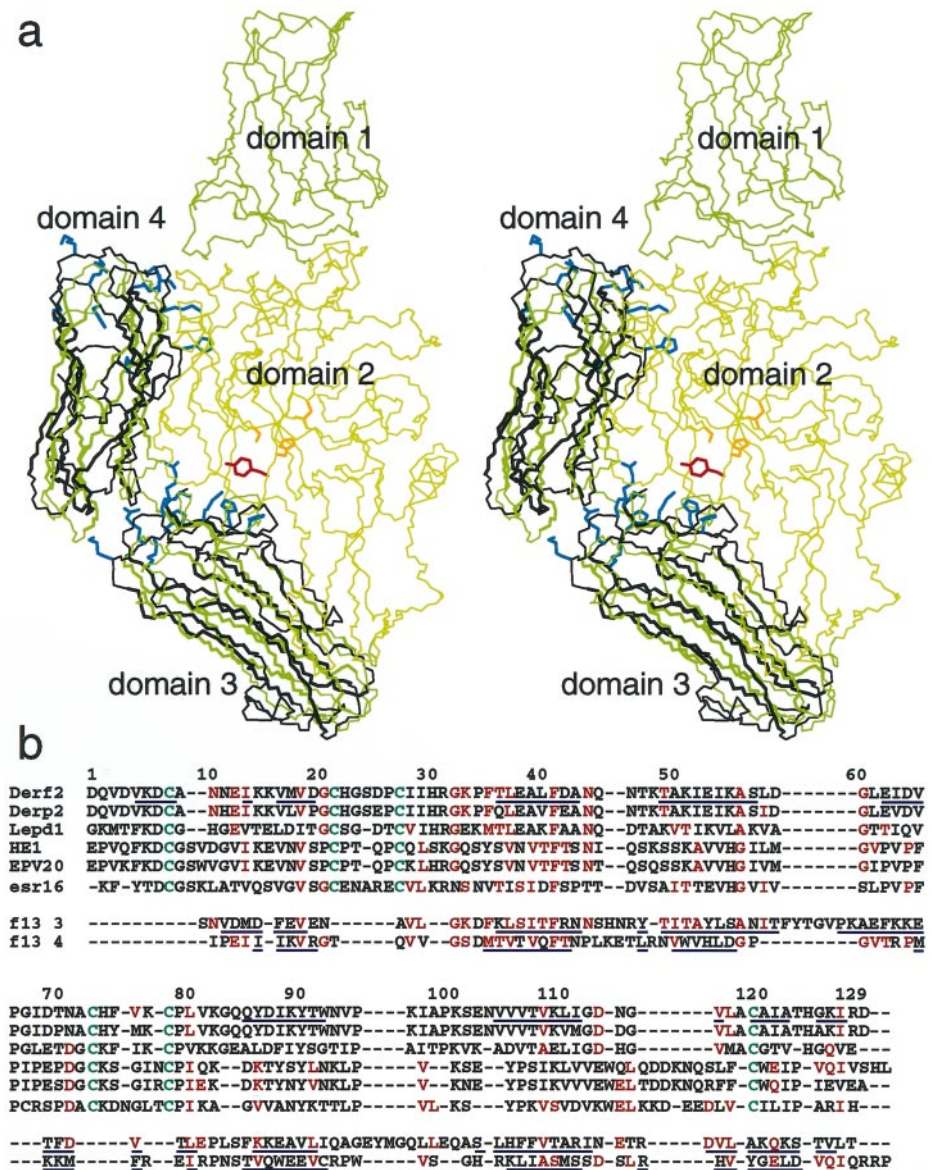


FIG. 4. In vitro binding assay. The gels were stained with Coomassie Brilliant Blue after separation of the following: first washing, 12 μ l (lane 2); second washing, 12 μ l (lane 3); ammonium formate eluate after 3 min, 10 min, and 30 min of incubation, 12 μ l (lanes 4, 5, and 6, respectively); and ammonium formate eluate from untreated bacteria (lane 7). In lanes 9–11, bovine serum albumin was used instead of Der f 2 for comparison. 1 μ g of Der f 2 and bovine serum albumin were loaded in lanes 1 and 8.

(33), have microbial degradation activities (34). HE1, EPV20, and esr16, the Der f 2 homologs from human, cow, and moth, respectively, are included in epithelial mucosae, where anti-

bacterial proteins are excreted (35–37). These observations imply that Der f 2 is a component of the antibacterial defense system in mites. A survey of recent literature data (38–40) made us realize that Bet v 1, conalbumin and lactoferrin, which show the highest positive rate for sera from allergic patients of birch pollen, hen egg, and cow milk, respectively, are components of antimicrobial host defense systems.

IgE Epitopes—Since the cloning of the group-2 allergens, IgE and T-cell epitopes have been intensely studied. The experiments using 14 synthetic peptides of 15 residues in length spanning the entire sequence of Der p 2 showed that IgE antisera do not bind to most peptides; the peptide comprising residues 65–78 bound IgE, but its activity was extremely weak (41). Therefore recognition by IgE depends strongly on the conformation of Der p 2, and considering the intense homology, it is probably also the case for Der f 2. Truncation of N- or C-terminal short sequences (42) or destruction of the disulfide bond 8–119 (43) reduced IgE-binding activity severely, which corresponds to our result that Der f 2 is composed of only one domain.

Nishiyama *et al.* (42) made site-directed mutants at residues 1–21, 70–81, and 114–129 of Der f 2, which were selected considering the studies mentioned above, and measured their

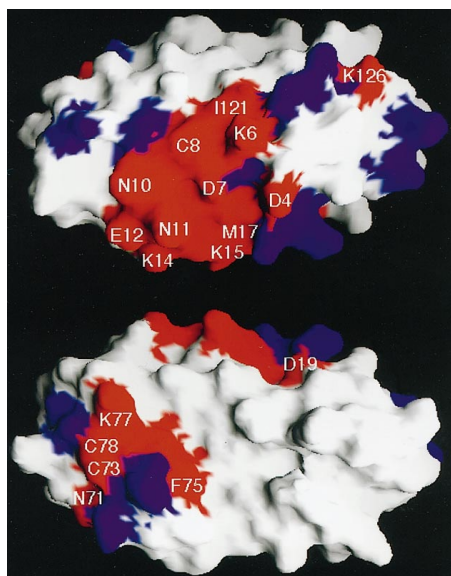


FIG. 5. **Partial characterization of IgE epitope areas.** The molecular surface of Der f 2 was produced by the program GRASP (45) and colored according to the results from the site-directed mutagenesis experiments (42). Red, residues whose substitution decreased IgE binding; blue, residues whose substitution did not decrease IgE binding; white, residues that were not tested. The top figure can be related to Fig. 2 by 105° α rotation, and the bottom can be related to the top by 180° α rotation.

IgE-binding activities. Using their results, we mapped the molecular surface for those substitutions that decreased IgE binding (Fig. 5). This figure suggests that two IgE epitope areas on the surface. One epitope area includes Asp-7, Asn-10 and Lys-15, and the second one includes Cys-73, Phe-75, Lys-77, and Cys-78. However, the borders of these areas are distorted at the residues Asp-19 and Asn-71. Experiments that can judge whether each decrease of IgE binding is due to global destabilization or local effects on the allergen-antibody interface might improve epitope definition. Although additional amino acid substitutions are desired to clarify the borders and judge the existence of other epitope areas, our structure suggests mosaic distribution of IgE epitopes and provides strategies for their complete characterization.

The engineered Der f 2 has already begun to be applied for immunotherapy strategies. For example, the mutated allergen in which the disulfide bond 8–119 was disrupted retained complete activity to stimulate T-cell proliferation (43). However, developments of monovalent ligands to allergen-specific IgE are also desired to suppress the symptoms by blocking IgE receptor aggregation. Since the protein HE1 is a human homolog of Der f 2, chimeric proteins of HE1, and Der f 2 would be candidates of monovalent IgE ligands that induce no additional responses of antibodies. The tertiary structure of Der f 2 will provide the basis of such strategies and any other drug design processes.

Acknowledgements—We thank Dr. Mark B. Swindells of Helix Research Institute, Dr. Daisuke Kohda of Biomolecular Engineering Research Institute, Prof. Hikoichi Sakai of Japan Women's University, Dr. Hajime Karasuyama of Tokyo Metropolitan Institute of Medical Science, Dr. Koji Nagata of Tokyo University, and our colleagues in the laboratories of Tokyo Metropolitan Institute of Medical Science and Asahi Breweries, Ltd. for stimulating discussions and advice.

REFERENCES

- Heymann, P. W., Chapman, M. D., Aalberse, R. C., Fox, J. W., and Platts-Mills, T. A. E. (1989) *J. Allergy Clin. Immunol.* **83**, 1055–1067
- Yuuki, T., Okumura, Y., Ando, T., Yamakawa, H., Suko, M., Haida, M., and Okudaira, H. (1991) *Agric. Biol. Chem.* **55**, 1233–1238
- Chua, K. Y., Doyle, C. R., Simpson, R. J., Turner, K. J., Stewart, G. A., and Thomas, W. R. (1990) *Int. Arch. Allergy Appl. Immunol.* **91**, 118–123
- Varela, J., Ventas, P., Carreira, J., Barbas, J. A., Gimenez-Gallego, G., and Polo, F. (1994) *Eur. J. Biochem.* **225**, 93–98
- Kirchhoff, C., Osterhoff, C., and Young, L. (1996) *Biol. Reprod.* **54**, 847–856
- Larsen, L. B., Ravn, P., Boisen, A., Berglund, L., and Petersen, T. E. (1997) *Eur. J. Biochem.* **243**, 437–441
- Mészáros, M., and Morton, D. B. (1996) *Insect Biochem. Mol. Biol.* **26**, 7–11
- Robinson, C., Kalsheker, N. A., Srinivasan, N., King, C. M., Garrod, D. R., Thompson, P. J., and Stewart, G. A. (1997) *Clin. Exp. Allergy* **27**, 10–21
- Metzger, H., Alcaraz, G., Hohman, R., Kinet, J., Pribluda, V., and Quarto, R. (1986) *Annu. Rev. Immunol.* **4**, 419–470
- Metzler, W. J., Valentine, K., Roebber, M., Friedrichs, M. S., Marsh, D. G., and Mueller, L. (1992) *Biochemistry* **31**, 5117–5127
- Metzler, W. J., Valentine, K., Roebber, M., Marsh, D. G., and Mueller, L. (1992) *Biochemistry* **31**, 8697–8705
- Gajhede, M., Osmark, P., Poulsen, F. M., Ipsen, H., Larsen, J. N., Joost van Neerven, R. J., Schou, C., Løwenstein, H., and Spangfort, M. D. (1996) *Nat. Struct. Biol.* **3**, 1040–1045
- Thorn, K. S., Christensen, H. E., Shigeta, R., Huddler, D., Shalaby, L., Lindberg, U., Chua, N. H., and Schutt, C. E. (1997) *Structure (Lond.)* **5**, 19–32
- Fedorov, A. A., Ball, T., Mahoney, N. M., Valenta, R., and Almo, S. C. (1997) *Structure (Lond.)* **5**, 33–45
- Nishiyama, C., Yuuki, T., Usui, Y., Iwamoto, N., Okumura, Y., and Okudaira, H. (1994) *Int. Arch. Allergy Immunol.* **105**, 62–69
- Iwamoto, N., Nishiyama, C., Yasuhara, T., Saito, A., Yuuki, T., Okumura, Y., and Okudaira, H. (1996) *Int. Arch. Allergy Immunol.* **109**, 356–361
- Cavanagh, J., Fairbrother, W. J., Palmer, A. G., and Skelton, N. J. (1996) *Protein NMR Spectroscopy. Principles and Practice*, Academic Press, San Diego, CA
- Brünger, A. T. (1993) *X-PLOR Version 3.1: A System for X-ray Crystallography and NMR*, Yale University Press, New Haven, CT
- Laemmli, U. K. (1970) *Nature* **227**, 680–685
- Wishart, D. S., and Sykes, B. D. (1994) *J. Biomol. NMR* **4**, 171–180
- Bork, P., Holm, L., and Sander, C. (1994) *J. Mol. Biol.* **242**, 309–320
- Nishiyama, C., Yuuki, T., Takai, T., Okumura, Y., and Okudaira, H. (1993) *Int. Arch. Allergy Immunol.* **101**, 159–166
- Holm, L., and Sander, C. (1993) *J. Mol. Biol.* **233**, 123–138
- Yee, V. C., Pedersen, L. C., Trong, I. L., Bishop, P. D., Stenkamp, R. E., and Teller, D. C. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 7296–7300
- Jones, E. Y., Harlos, K., Bottomley, M. J., Robinson, R. C., Driscoll, P. C., Edwards, R. M., Clements, J. M., Dudgeon, T. J., and Stuart, D. I. (1995) *Nature* **373**, 539–544
- Ichinose, A., Hendrickson, L. E., Fujikawa, K., and Davie, E. W. (1986) *Biochemistry* **25**, 6900–6906
- Pedersen, L. C., Yee, V. C., Bishop, P. D., Trong, I. L., Teller, D. C., and Stenkamp, R. E. (1994) *Protein Sci.* **3**, 1131–1135
- Muta, T., and Iwanaga, S. (1996) *Opin. Immunol.* **8**, 41–47
- Takahashi, K., Aoki, T., Kohmoto, S., Nishimura, H., Kodera, Y., Matsushima, A., and Inada, Y. (1990) *Int. Arch. Allergy Appl. Immunol.* **91**, 80–85
- Sun, S., Lindström, I., Boman, H. G., Faye, I., and Schmidt, O. (1990) *Science* **250**, 1729–1732
- van Hage-Hamsten, M., Olsson, S., Emilson, A., Härfast, B., Svensson, A., and Scheynius, A. (1995) *Clin. Exp. Allergy* **25**, 536–542
- Stewart, G. A., Bird, C. H., Krska, K. D., Colloff, M. J., and Thompson, P. J. (1992) *Exp. Appl. Acarol.* **16**, 165–180
- Tovey, E. R., Chapman, M. D., and Platts-Mills, T. A. E. (1981) *Nature* **289**, 592–593
- Stewart, G. A., Bird, C. H., and Thompson, P. J. (1992) *J. Allergy Clin. Immunol.* **90**, 141–142
- Reddy, E. S. P., and Bhargava, P. M. (1979) *Nature* **279**, 725–728
- Reiter, B., and Oram, J. D. (1967) *Nature* **216**, 328–330
- Ganz, T., and Lehrer, R. I. (1994) *Curr. Opin. Immunol.* **6**, 584–589
- Breiteneder, H., Pettenburger, K., Bito, A., Valenta, R., Kraft, D., Rumpold, H., Scheiner, O., and Breitenbach, M. (1989) *EMBO J.* **8**, 1935–1938
- Aabin, B., Poulsen, L. K., Ebbelhøj, K., Nørgaard, A., Frøkiær, H., Bindsløv-Jensen, C., and Barkholt, V. (1996) *Int. Arch. Allergy Immunol.* **109**, 50–57
- Atkinson, H. A. C., and Miller, K. (1994) *Toxicology* **91**, 281–288
- van't Hof, W., Driedijk, P. C., van den Berg, M., Beck-Sickinger, A. G., Jung, G., and Aalberse, R. C. (1991) *Mol. Immunol.* **28**, 1225–1232
- Nishiyama, C., Fukada, M., Usui, Y., Iwamoto, N., Yuuki, T., Okumura, Y., and Okudaira, H. (1995) *Mol. Immunol.* **32**, 1021–1029
- Takai, T., Yokota, T., Yasue, M., Nishiyama, C., Yuuki, T., Mori, A., Okudaira, H., and Okumura, Y. (1997) *Nat. Biotechnol.* **15**, 754–758
- Kraulis, P. J. (1991) *J. Appl. Crystallogr.* **24**, 946–950
- Nicholls, A., Sharp, K. A., and Honig, B. (1991) *Proteins* **11**, 281–296