



Crystal structure and some properties of a major house dust mite allergen, Derf 2

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Abstract

Pyroglyphid house dust mites are a major source of allergens in house dust. Mite allergens sensitize and induce asthma, rhinitis, and eczema in a large portion of patients with allergic diseases. Here, the crystal structure of a major mite allergen, Derf 2, derived from *Dermatophagoides farinae* was solved by single isomorphous replacement method with anomalous scattering (SIRAS) at 2.1 Å resolution. The present study also demonstrated that the conformation of the allergen was critical in the determination of Th1/Th2 shift based on physicochemical and immunological analyses. This indicates that rigidly folded and singly dispersed structure is essentially required for the generation of Th2 type cells by the allergen, while conformational variant protein leads to Th1 skewing, irrespective of the same amino acid sequence. This structure/function relationship may allow us to develop a novel strategy for hyposensitization therapy in patients with allergic diseases triggered by house dust mite allergens.

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There has recently been a rise in the incidence of allergic diseases in the industrialized countries [1]. The diseases include asthma, rhinitis, hay fever, eczema, and so forth, and are characteristic for superfluous immunologic responses to innocuous and common environmental antigens, like house dust mite products and pollens. The prevalence of the diseases is considered to be attributable to increased exposure to allergens in less ventilated homes, increased air pollution, decreased exposure to pathogens, and changes in diet. Much effort has been made to develop a strategy for treatment of the diseases like administration of corticosteroids, antihistamines, and leukotriene receptor antagonists [2], while no treatments to achieve complete

cure of the diseases have been developed yet. Since evidence has been accumulating for the involvement of Th2 deviation and subsequent IgE production in the onset of the diseases [3,4], a variety of immunotherapies have been conducted to modulate Th1/Th2 balance [5,6], such as hyposensitization [7], the administration of alternative peptide ligands [8,9], polymerized allergens [10], chemically modified allergens [11–13], adjuvants [14], and humanized monoclonal antibody specific for IgE, the use of regulatory T cells, the change in allergen-administration route [15], the blockade of costimulatory signals, the use of Schiff-base forming drugs [16], and the derivation of dendritic cells [17,18]. Hyposensitization therapy is one of the oldest strategies and is likely to be promising, if properly engineered hypoallergenic derivatives are prepared using genetics and recombinant techniques.

We previously examined the immunologic properties of C8/119S [19], one of the derivatives of the major house dust

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mite allergen, Derf 2 [20], whose cystein residues at the 8th and 119th positions are replaced by serine residues, respectively. This derivative induced a potent proliferative response in allergen-specific human T cells, had a weak capability to bind Derf 2-specific human serum IgE, and exerted a deviation from Th2 to Th1 response. Although the mutant protein was demonstrated to be ideal for the use as a hypoallergenic protein in antigen-specific immunotherapy for allergic diseases, the mechanism for the Th1 skewing has not been identified yet. Since recent reports indicated that some alternative peptide ligands were potent Th1 inducers, the replacement of cystein residues with serine residues might have generated new epitopes for T cells, leading to Th1 differentiation. In addition, reduced affinity to IgE might have also affected the modulation of Th1/Th2 balance. It was, however, strongly suggested that the exposure of hydrophobic amino acid residues to the outer environment and the following molecular polymerization played a major role in the enhanced T cell stimulatory activity and the Th2 to Th1 balance deviation, implicating that the conformation of allergens primarily determined the allergenicity. In the present study, we crystallized Derf 2 and solved the crystal structure at 2.1 Å resolution. In addition, we prepared conformational derivative of the allergen, with the amino acid sequence being the same as the correctly refolded Derf 2, and determined its physicochemical properties and immunogenicity to examine the relationship between structure and function of the allergen.

Materials and methods

Preparation of Derf 2 and its conformational variant. Recombinant Derf 2 protein was prepared as described previously [19]. In brief, *Escherichia coli* Rosseta(DE3)pLys (Merck KGaA, Darmstadt, Germany) was transfected with an expression plasmid harboring cDNA for Derf 2 and induced for protein expression with 1 mM IPTG. The resultant inclusion bodies were dissolved in buffer containing 8 M urea and 1 M guanidine, and refolded in arginine-based buffer. After dialysis against 10 mM Tris–HCl buffer, pH 8.0, the protein solution was applied to Q Sepharose HP anion-exchange column chromatography and Superdex 200 pg gel filtration (Amersham Biosciences, Piscataway, NJ, USA). The correctly refolded protein was stored at –30 °C until used. For preparation of conformational variant of Derf 2, the correctly refolded Derf 2 was dissolved in 25 mM MES buffer, pH 6.0, containing 1 M guanidine hydrochloride, 8 M urea, and 50 mM DTT. The solution was diluted with 50 volumes of 25 mM MES buffer, pH 6.0, containing 1 M guanidine hydrochloride, 8 M urea, and 5 mM MgCl₂, through which O₂ gas was bubbled for 30 min. Then, the denatured protein was diluted with 20 volumes of 100 mM Tris–HCl buffer, pH 8.0, containing 0.5 M arginine and allowed to stand overnight at 4 °C with continuous stirring. After dialysis against 10 mM Tris–HCl buffer, pH 8.0, the conformational variant of Derf 2 was subjected to Q Sepharose HP anion-exchange column chromatography and Superdex 200 pg gel filtration. The protein preparation was then dialyzed against PBS and stored at –30 °C until used.

Crystallization of Derf 2 and preparation of heavy atom derivatives. The properly refolded Derf 2 was dialyzed against 10 mM Hepes buffer, pH 7.0, and concentrated to 20 mg/ml. Crystallization conditions were evaluated by utilizing Crystal Screen reagents (Hampton Research, Laguna Niguel, CA, USA) with the hanging drop vapor diffusion method. The solution for crystallization drop was prepared on a silicized coverslip by mixing 4 µl of the protein solution with 4 µl of mother liquor reagent. The

crystals were soaked in a heavy atom derivative solution comprising 2 mM uranyl acetate and the mother liquor solution for crystallization for 40 min at 20 °C.

Data collection. Diffraction data for the native and derivative crystals were collected with a Bruker Hi-Star multiwire area detector at 20 °C, using CuKα radiation generated by a MAC Science M18XHF rotating anode generator, and were processed with the SADIE and SAINT software packages (Bruker, Karlsruhe, Germany) as shown in Table 1.

Phasing and refinement. The crystal structure of Derf 2 was solved by the single isomorphous replacement method with anomalous scattering (SIRAS). The site of heavy atom was determined by interpretation of the peaks in difference Patterson maps obtained at 3.0 Å resolution. Phase refinement was carried out with the program package PHASES [21]. The results of heavy atom refinement and phasing by m.i.r. at 2.8 Å resolution are presented in Table 1. The phase was improved greatly and average figures of merit increased to 0.511, 0.807, and 0.913 after SIRAS, solvent flattening [22], and averaging, respectively, with PHASE. The model was built using a program TURBO-FRODO (AFMB-CNRS, Marseille, France) on a Silicon Graphics Octane computer. Simulated annealing refinement was carried out with this model using 15–2.1 Å resolution data obtained with CNS [23]. The model was heated to 2500 K and then slowly cooled to 300 K (time-step, 0.5 fs; decrease in temperature, 25 K; number of steps at each temperature, 50), and then 150 cycles of Powell minimization were performed. $F_o - F_c$ and $2F_o - F_c$ maps were used to locate the correct model. Several rounds of positional and B-factor refinement followed by manual model building were carried out to improve the model by increasing the data to 2.1 Å resolution. Water molecules were incorporated where the difference in density was more than 3.0 σ above the mean and the $2F_o - F_c$ map showed a density of more than 1.0 σ. The stereo quality of the model was assessed using the programs, PROCHECK [24] and WHAT-CHECK [25]. The Cα trace plots were prepared using the program, MOLSCRIPT [26]. The coordinates of solution structure for Derf 2 determined by NMR were taken from the RCSB Protein Data Bank [27]. These molecular models were superimposed by means of fitting the programs RIGID and TOP included in TURBO-FRODO and CCP4, respectively.

Structure comparison and calculation. The 3-D structure comparison was performed by DALI server [28] (<http://www.ebi.ac.uk/dali/>). The cavity detection and calculation of cavity surface and volume were performed by CASTp sever [29] (<http://cast.engr.uic.edu/cast/calculation/>).

Protein Data Bank accession code. The coordinates and structure factors have been deposited with the RCSB Protein Data Bank under accession code 2F08.

Derivation of Derf 2-specific T cell lines. PBMC provided by a patient with allergic rhinitis was purified on Ficoll–Paque PLUS (Amersham Biosciences, Piscataway, NJ, USA). After washing three times with PBS, the cells, 1×10^7 , were resuspended in 6 ml of the modified Yssel's medium supplemented with 10% pooled human serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. The cell suspension, 1.5 ml, was placed in a well of 24-well plate and incubated in the presence of 1 µg/ml of the properly refolded Derf 2 allergen or artificially misfolded one at 37 °C for 2 weeks. The resultant cells were harvested and washed two times with the modified Yssel's medium. Then, 5×10^5 cells were restimulated with 1 µg/ml of the same allergen in the presence of 1.5×10^6 freshly isolated and irradiated PBMC in a well of 24-well plate. IL-2, 20 U/ml, and IL-4, 5 U/ml were added to the culture medium every 2 days and the cells were incubated for 15 days. The resultant T cells were harvested, washed two times with the modified Yssel's medium, resuspended in FCS supplemented with 10% DMSO, and frozen at –80 °C until used.

PAGE. Davis' native PAGE and SDS-PAGE were carried out as described previously [19].

Circular dichroism (CD) spectroscopy. Properly refolded and artificially misfolded recombinant Derf 2 protein species were suspended in PBS at a final concentration of 60 µM. UV-CD spectra of the preparations were measured in a circular quartz cuvette of 0.02 cm path length using a spectropolarimeter (Japan Spectroscopic, Tokyo, Japan). CD spectra were recorded from 250 to 190 nm at a digital resolution of 0.1 nm with a scan speed of 10 nm/min. The scans were single-averaged, and the data sets were concatenated.

Table 1
Data collection, phasing, and refinement statistics

Data set	Native	Uranyl Acetate
X-ray source	Roating anode	Roating anode
Detector	Bruker Hi-Star	Bruker Hi-Star
Space group	C 2	C 2
<i>a</i> , <i>b</i> , <i>c</i> (Å), β (deg)	106.12, 54.33, 102.35, 91.35	65.18, 110.66, 65.20, 89.99
Wavelength (Å)	1.54	1.54
Resolution range (Å)	28.3–2.10 (2.175–2.10)	29.7–2.65 (2.715–2.621)
Total/unique reflections	110,232 (10.170)/32,658 (3009)	40,582 (1613)/16,527 (656)
Completeness (%)	95.1 (99.1)	92.7 (36.6)
<i>R</i> _{sym} (%)	5.8 (34.2)	5.1 (18.6)
Phasing power (25–2.8 Å)	—	2.45
Figure of merit (25–2.8 Å): 0.51/0.807/0.913 (MIRAS/density modification/NCS average)		
Refinement		
Program used	CNS	
Resolution range (Å)	15–2.2 (2.28–2.20)	
Used reflections	26,532 (2225)	
Completeness (%)	89.1 (75.5)	
Residues/waters/PEG	129 × 4/156/1	
Average protein <i>B</i> -factor (Å ²)	35.32	
Average waters <i>B</i> -factor (Å ²)	41.56	
Average PEG <i>B</i> -factor (Å ²)	32.26	
Bond length r.m.s. (Å)	0.0061	
Bond angles r.m.s. (deg)	1.31	
<i>R</i> _{work} (%)	20.64 (29.6)	
<i>R</i> _{free} (%)	24.47 (34.6)	

Statistics for the highest resolution shell are given in parentheses.

Proliferation assay. Derf 2-specific T cell lines, 5×10^4 cells, stimulated with either properly refolded or artificially misfolded Derf 2, were suspended in the modified Yssel's medium and incubated in 96-well round-bottomed plates in the presence of 1.5×10^5 of freshly irradiated autologous PBMC and varying concentrations of allergens at 37 °C for 36 h. Then, 2 μ Ci of [³H]thymidine was added to the cell culture. After 12 h, the cells were harvested and the incorporation of radioactivity into DNA was measured by a β -counter (Perkin–Elmer Life Sciences, Japan, Tokyo, Japan).

ELISA. Derf 2-specific T cells were cultured for 2 days as described above, and the culture supernatants were harvested and frozen at –80 °C. After thawing, the supernatants were examined for the concentration of IL-4 and IFN- γ by utilizing ELISA kits (Amersham Life Sciences, Buckinghamshire, UK). For the determination of the IgE binding capacity, ELISA was carried out as described previously [19].

Results and discussion

Crystallization and structure determination

In search of hypoallergenic variants of Derf 2 for the use in hyposensitization therapy for allergic asthma, we previously demonstrated that the structure of allergen is intrinsically involved in allergenicity. Since only preliminary X-ray data were available [30], we first attempted to prepare crystals of Derf 2 and solve the X-ray structure in the present study. As shown in Fig. 1A, Derf 2 was crystallized in a mixture consisting of 20 mg/ml Derf 2, 23% PEG4000, 0.06 M ammonium sulfate, 0.1 M sodium acetate, pH 5.2, and 10 mM Hepes, pH 7.0, at 20 °C using the hanging drop vapor diffusion method.

The results of X-ray data collection are summarized in Table 1. The crystal structure of Derf 2 was solved by

the single isomorphous replacement method with anomalous scattering (SIRAS) using uranyl acetate derivatives of Derf 2. The statistics for the heavy atom derivatives are shown in Table 2. The model included four independent Derf 2 molecules, with all 129 residues in each, 156 water molecules, and one polyethylene glycol (C₂₂O₂₂) between subunit A and D. A representative of C α traces of the crystal structures is depicted in Fig. 1B. The overall tertiary fold of Derf 2 is that of two anti-parallel β -pleated sheets overlying each other at an angle of approximately 30°. This fold is characteristic of the immunoglobulin superfamily and there are three disulfide bonds in Derf 2 forming covalent bonds between residues 8 and 119, 21 and 27, and 73 and 78, none of which is found in the typical Ig-like molecules. In contrast, the disulfide bond that connects strands B and F in many of the Ig-like molecules is absent from the Derf 2 structure.

Comparison with the solution structure determined by NMR

The quality of the protein model was assessed with the program PROCHECK [24]. As shown in the left panel of Fig. 2A, more than 90% of the residues were in the most favorable regions of the Ramachandran plot and no residue was in the disallowed regions. On the other hand, only about half of the total residues resided in the most favorable regions and several residues were located in the disallowed regions in the solution model of Derf 2 determined by NMR [27] as illustrated in the right panel of Fig. 2A. When superimposed by means of fitting program, there was a significant difference in structure between the crystal

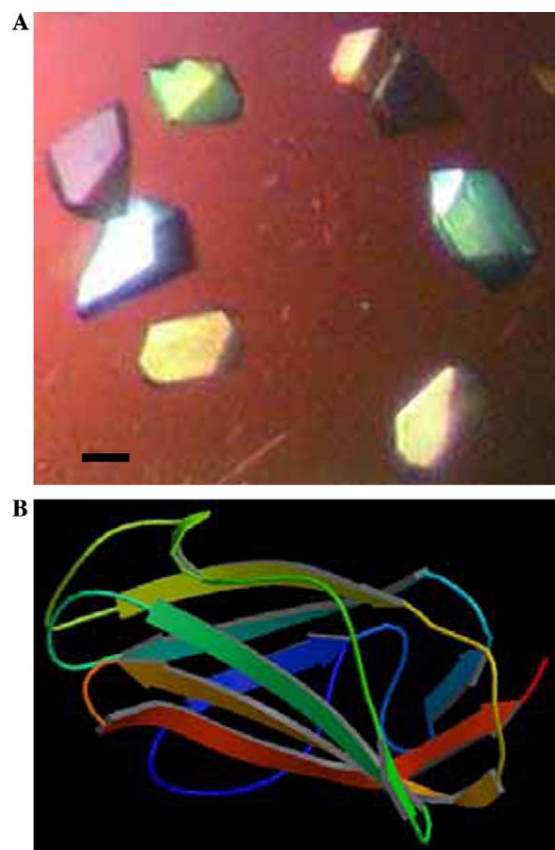


Fig. 1. Crystallization and X-ray structure of Derf 2. (A) Crystals of a major house dust mite allergen, Derf 2. Crystallization of Derf 2 was attempted by utilizing the hanging drop vapor diffusion method under the conditions with polyethylene glycol and ammonium sulfate based on the results of commercial screening kits. Sharp-edged crystals were obtained in 2 weeks at 20 °C in a droplet consisting of 20 mg/ml Derf 2, 23% PEG4000, 0.06 M ammonium sulfate, 0.1 M sodium acetate, pH 5.2, and 10 mM Hepes, pH 7.0. The scale bar is 0.1 mm long. (B) The C α trace of the X-ray structure of Derf 2. Derf 2 is composed of two anti-parallel β -pleated sheets overlying each other at an angle of approximately 30°.

and solution models of Derf 2, and the two structures did not align well (Fig. 2B).

Comparison with the other mite allergen and cholesterol binding protein

The structure comparison of a subunit model of Derf 2 with the structure-known proteins in PDB by DALI server

Table 2
Summary of statistics for heavy atom derivatives of Derf 2

Derivative	Conditions ^a	Res. (Å)	<i>R</i> -Cullis	<i>R</i> -Kraut	Phasing power	Heavy atom	Site			Occ.	<i>B</i> (Å)
							<i>x</i>	<i>y</i>	<i>z</i>		
Ac ₂ (UO ₂)	2 mM, 2 h	2.8	0.517	0.068	2.28	U-1	0.125	0.000	0.236	1.247	38.68
	2 mM, 2 h	3.0 ^b	—	0.263 ^b	2.45 ^b	U-2	0.612	0.044	0.263	0.661	35.19
						U-3	0.373	0.121	0.743	0.321	44.02
						U-1	0.140	0.076	0.238	0.359	17.18

^a Soaking solutions contain 23% PEG4000, 0.06 M (NH₄)₂SO₄, 0.1 M sodium acetate, pH 5.2, and 10 mM Hepes, pH 7.0, at 20 °C.

^b Anomalous data.

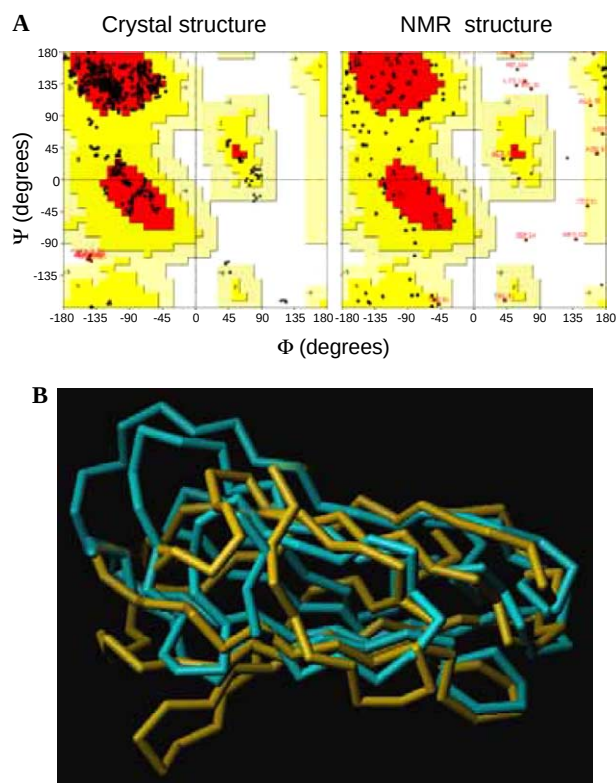


Fig. 2. Comparison of the X-ray structure of Derf 2 with the NMR structure. (A) Ramachandran plot analyses of the Φ and Ψ dihedral angles. Glycine residues are shown as triangles, and all the other residues as squares. The non-glycine residues are present in most favored regions (red) or additionally allowed regions (yellow) in the plot of the X-ray structure (left panel), while significant number of non-glycine residues are in generously allowed regions (light yellow) and in forbidden regions (white regions) in the plot of the NMR structure (right panel). The program, PROCHECK, was utilized for creation of these figures. (B) Direct comparison of the X-ray structure of Derf 2 molecule with the NMR structure. The X-ray structure of Derf 2 is depicted in yellow and NMR structure in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

[28] indicated that two proteins, a cholesterol binding protein deficient in Niemann–Pick disease type C2, NPC2 (PDB, 1NEP) [31], a mite allergen Derp 2 mutant (PDB, 1A9V, NMR data) [32] and a mite allergen Derp 2 (PDB, 1KTJ, X-ray data) [33] have high similarity with C α RMSD of 1.7, 2.6, and 1.4 Å, respectively. Fig. 3A shows the superimposition of these two proteins onto Derf 2. These three proteins have the same fold of immunoglobulin-

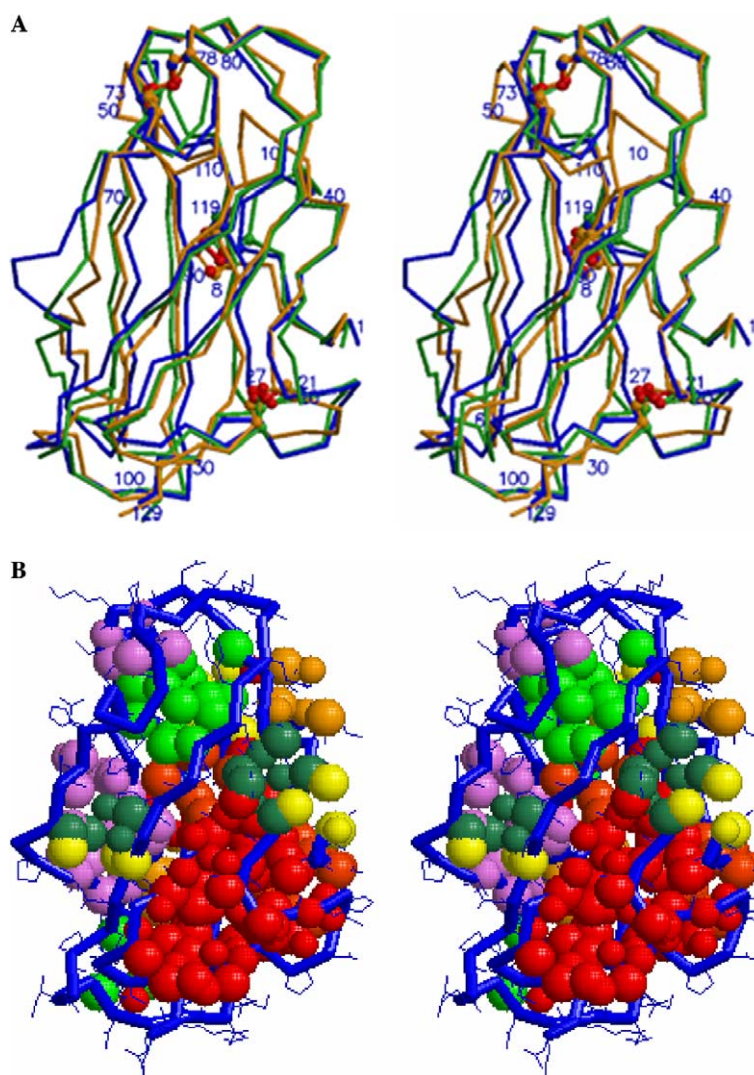


Fig. 3. Structural similarities with other molecules. (A) Comparison of main-chain structures with other structurally related proteins (stereo drawing). The α -traces of Derf 2 (blue), Derp 2 (green), and a cholesterol-binding protein (orange) were depicted after they were superimposed by a fitting program implemented in Turbo-Frodo. The positions of disulfide bridges are shown with ball-and-stick model of red sulfur atoms. (B) The cavities in Derf 2 (stereo drawing). The cavities in Derf 2 are shown in different colors. The red color shows the largest cavity with molecular surface and volume of $413 \text{ \AA}^2$ and $348 \text{ \AA}^3$, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

lin-like β -sandwich and classified as “E set domains” superfamily and ML domain family according to SCOP data base [34]. The positions of three disulfide bridges (C8/119, C21/27, and C73/78 in Derf 2) are well conserved in these proteins. It has been suggested that Derf 2 and Derp 2 can bind non-polar compounds inside the protein cavity [35]. In this connection, we have compared the cavity surface and volume of Derf 2 with those of Derp 2 and NPC2. The largest cavity of Derf 2 is formed in the region surrounded by the side-chains of V3, V5, I13, V16, V18, C21, H22, G23, D25, C27, I29, F35, L37, A39, V106, V108, A120, A122, T123, H124, and G125 as shown in Fig. 3B. The molecular surface and volume of the cavity were calculated to be $413 \text{ \AA}^2$ and $348 \text{ \AA}^3$, respectively. These values are comparable with the largest cavity found in Derp 2 ($1151 \text{ \AA}^2$ and $1509 \text{ \AA}^3$) and NPC2 ($103 \text{ \AA}^2$ and $84 \text{ \AA}^3$). The cavity of NPC2 contains more aromatic resi-

dues than Derf 2 and Derp 2. The larger cavity volume of Derp 2 than Derf 2 corresponds to the wider frame of the main-chain (Fig. 3A), as they have 86% sequence homology. Taken together, Derf 2 is likely to have a specific preference for binding non-polar substrates, and the conserved conformation of allergens might be one of the most crucial factors which determine the allergenicity.

Th2 to Th1 balance deviation elicited by conformational variant of Derf 2

Although it was previously demonstrated that C8/119S derivative of Derf 2 had no apparent secondary structure and facilitated Th2 to Th1 balance shift, the factors for causing the deviation remained enigmatic. In the present study, we examined whether or not the conformational factor is attributed to the Th skewing using a conformational

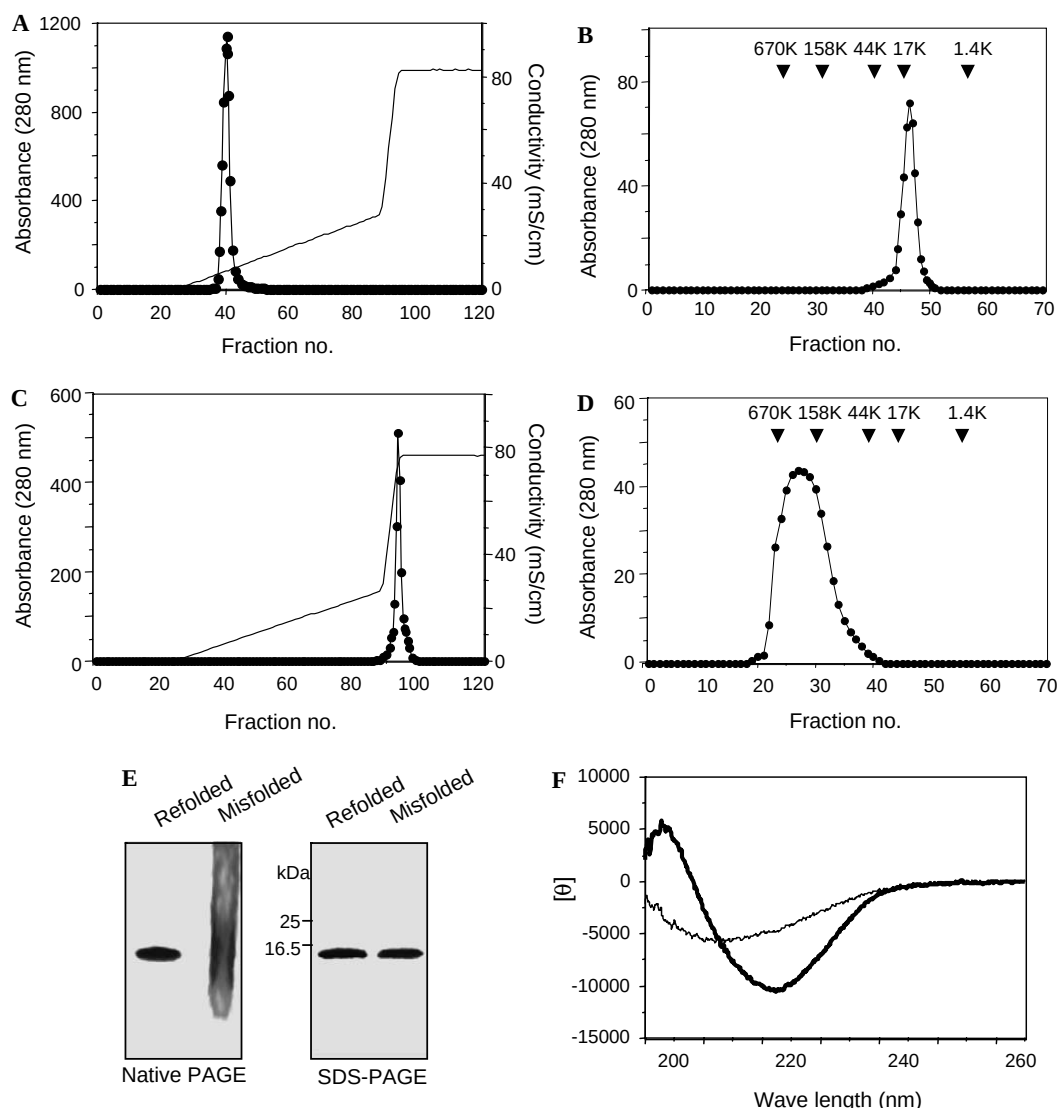


Fig. 4. Chromatographic and physicochemical comparison of properly refolded and artificially misfolded Derf 2. (A) Elution pattern of properly refolded Derf 2 on anion-exchange column chromatography. Refolded Derf 2 was applied to a column of Q Sepharose HP 200 pg anion-exchanger, $16\phi \times 100$ cm, and eluted with a linear gradient of 0–1 M of NaCl in 10 mM Tris–HCl buffer, pH 8.0. The protein was eluted at the NaCl concentration of 60 mM. (B) Elution pattern of refolded Derf 2 on gel filtration. Refolded Derf 2 was put on a column of Superdex 200 pg, $1.6\phi \times 60$ cm, and eluted with 20 mM Tris–HCl buffer, pH 8.0, with 300 mM NaCl. The protein was eluted at the fraction number of 47, which indicates the protein species had an apparent molecular weight of 15 kDa. (C) Elution pattern of artificially misfolded Derf 2 on anion-exchange column chromatography. Conformational variant of Derf 2 was subjected to the same anion-exchange column chromatography. The artificially misfolded Derf 2 was eluted at 900 mM NaCl. (D) Elution pattern of misfolded Derf 2 on gel filtration. The conformational variant of Derf 2 was loaded on the same gel filtration column. (E) Native and SDS–PAGE analyses of refolded and misfolded Derf 2. Native PAGE was carried out at pH 8.0 to examine the overall conformation of each preparation. To determine the molecular weight, both protein species were subjected to SDS–PAGE. (F) CD spectral analysis of refolded and misfolded Derf 2 proteins. Properly refolded and artificially misfolded Derf 2 protein samples were dialyzed against PBS and subjected to CD spectral analysis. CD spectra were measured from 250 nm through 190 nm with 10 nm/min scanning speed. The average signals of three scans were concatenated. Bold line: refolded Derf 2, and thin line: misfolded Derf 2.

derivative of Derf 2. As shown in Fig. 4A, properly refolded Derf 2 was eluted at the NaCl concentration of 60 mM on Q Sepharose HP anion-exchange column chromatography. On gel filtration, the protein was eluted at a retention volume corresponding to a molecular weight of 15 kDa (Fig. 4B). In contrast, the misfolded Derf 2 was eluted with Tris–HCl buffer containing 900 mM NaCl as shown in Fig. 4C. In addition, the molecules seemed to polymerize significantly on the basis of gel filtration analysis (Fig. 4D).

To confirm the conformational change of the protein, refolded Derf 2 and misfolded Derf 2 were applied to native and SDS–PAGE. As demonstrated in Fig. 4E, both refolded and misfolded species showed the identical molecular weight on SDS–PAGE, while misfolded protein exhibited a smear band on native PAGE, indicating a nonstructural feature of the protein. CD spectral analysis further showed the structural difference between the two molecular species. The refolded Derf 2 exhibited a typical

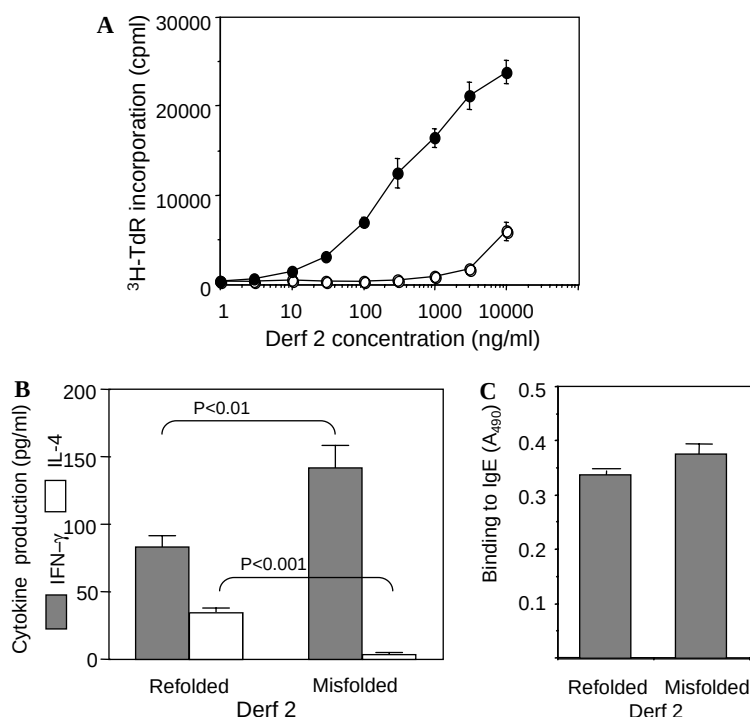


Fig. 5. Effect of conformational change of Derf 2 on proliferative responses and cytokine production. (A) Proliferative responses of Derf 2-specific T cells to refolded and misfolded Derf 2. Newly established Derf 2-specific $\alpha\beta$ T cells were incubated with varying concentrations of refolded (open circle) or misfolded Derf 2 (closed circle) prepared as shown in Fig. 3 in the presence of 30 Gy irradiated autologous PBMC as antigen-presenting cells. (B) Th2 to Th1 balance deviation caused by conformational variant of Derf 2 protein. PBMC derived from a patient with allergic rhinitis were sensitized with either properly refolded or artificially misfolded Derf 2 proteins, and challenged by C8/119S Derf 2 variant. Then, the productions of IFN- γ (filled column) and IL-4 (open column) were analyzed by the use of ELISA kit. (C) IgE binding capability of refolded and misfolded Derf 2. The degrees of IgE binding of refolded and misfolded Derf 2 were determined by ELISA.

β -strand structure, while the misfolded Derf 2 had no explicit secondary structure as illustrated in Fig. 4F.

We then analyzed the effects of conformational change of Derf 2 on immune responses in humans. On analysis of proliferative responses, the conformational variant of Derf 2 exhibited a potent stimulatory activity in Derf 2-specific T cells, compared to refolded Derf 2 as shown in Fig. 5A. It was also noteworthy that the conformational change of Derf 2 elicited Th2 to Th1 balance shift as demonstrated in Fig. 5B, even though the degree of IgE binding capacity was not significantly different between the two molecular species (Fig. 5C). One possible explanation for the alteration of immunogenicity is that the exposure of hydrophobic amino acid residues is sensed by scavenger-like receptors, through which Th1 deviation-inducing signals are delivered into antigen-presenting cells. It is also possible that the conformational variant is recognized by different types of antigen-presenting cells, like Th1-inducing dendritic cells, and so forth. In any case, it has become evident that the conformational change of the allergen leads to alteration of immune responses like immunogenicity and Th1/Th2 balance. Based on these findings, it is postulated that conformational variants of allergens can be utilized for hyposensitization therapy in general. The strategy described in this work including reduction and oxidation of disulfide bonds using DTT and O_2 gas and the

control of polymerization with arginine may be generally applicable to other major allergens.

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