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The Structure of the Dust Mite Allergen Der p 7 Reveals Similarities to Innate Immune Proteins

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Abstract

Background—Sensitization to house dust mite allergens is strongly correlated with asthma. Der p 7 elicits strong IgE antibody and T-cell responses in mite allergic patients. However, the structure and biological function of this important allergen are unknown. Allergen function may contribute to allergenicity as shown for the protease activity of Group 1 mite allergens and the interaction with the innate immune system by Group 2 mite allergens.

Objective—To determine the crystal structure of Der p 7 and to investigate its biological function.

Methods—X-ray crystallography was utilized to determine the Der p 7 structure. NMR analysis and biochemical assays were used to examine the binding of Der p 7 to predicted ligands.

Results—Der p 7 has an elongated structure with two 4-stranded anti-parallel β -sheets which wrap around a long C-terminal helix. The fold of Der p 7 is similar to lipopolysaccharide binding protein (LBP), which interacts with Toll-like receptors (TLRs) after binding lipopolysaccharide and other bacterially-derived lipid ligands. NMR and biochemical assays indicate that Der p 7 does not bind lipopolysaccharide but binds with weak affinity to the bacterial lipopeptide polymyxin B in the predicted binding site of Der p 7.

Conclusions—Der p 7 binds a bacterially-derived lipid product, a common feature of some allergens. The finding that the Group 7 as well as the Group 2 mite allergens are structurally similar to different proteins in the TLR pathway further strengthens the connections between dust mites, innate immunity, and allergy.

Keywords

Asthma; allergens; dust mites; Der p 7; lipopolysaccharide binding protein; TLR4; lipopeptide; innate immunity

Key Messages

1. The structure of the dust mite allergen Der p 7 reveals a distant homology to a family of proteins involved in the human innate immune recognition of bacterial lipid products.
2. Der p 7 binds specifically to a bacterially derived lipopeptide.
3. Der p 7 is the second dust mite allergen identified to be structurally related to a protein in the TLR pathway.

Introduction

The development of asthma associated with allergens is known as extrinsic asthma, which differentiates it from intrinsic asthma of unknown etiology. Primarily, extrinsic asthma is associated with exposure to indoor allergens and occasionally with exposure to outdoor allergens.¹ Among indoor allergens, the most commonly associated with asthma are those of the house dust mite.² Indeed, greater than 80% of asthma patients show immediate hypersensitivity to allergens of the house dust mite.³ A significant question remains as to why dust mite allergens are so strongly associated with the etiology of asthma. Early studies to address this question focused on the discovery that many dust mite allergens are proteolytic enzymes.⁴ Experiments showed that these proteases can damage lung epithelia, which may help explain the chronic inflammation of the lung that characterizes asthma symptoms.^{5, 6} Other studies have shown that digestion of specific proteins could encourage IgE synthesis and/or skew the immune response towards a Th2 or symptomatic response instead of tolerance.^{7, 8}

An important dust mite allergen for which there is not a known function from sequence analysis is Der p 7. The mature protein is 198 amino acids (MW= 22.2 kDa) and is estimated to be found in dust at concentrations of 2.5 µg per gram of dust.⁹ Natural Der p 7 has been described as a mixture of three molecules with molecular weights between 24 and 31 kDa. These different molecular forms could represent different levels of glycosylation or isoforms.^{10, 11} More than 50% of dust mite allergic patients react specifically to Der p 7 and some patients respond with an IgE titre as strong as that to the major allergen Der p 2.¹² Previous studies suggested that Der p 7 promotes an abnormally high reactivity in T-cell proliferation assays among allergic and non-allergic patients.^{9, 11, 13-15} In this study, the three dimensional structure of the protein was determined in order to gain insight into the abnormal patient response and th100 e natural function of the protein.

Allergens can be enzymes, structural proteins, or ligand-binding proteins that often show specific binding affinity for lipids.¹⁶ The intrinsic adjuvant activity of lipid-binding proteins and their lipid cargo may be a general mechanism underlying allergenicity.¹⁷ Supporting this hypothesis, coexposure to allergens and lipopolysaccharide (LPS) from bacteria can drive the immune response away from benign tolerance to symptomatic response.^{18, 19} Interestingly, the dust mite allergen Der p 2 can bind to LPS and initiate the signaling of the TLR4 complex in MD-2-deficient mice, thus functionally substituting for the mammalian innate immune

protein MD-2. This raises many interesting questions about how other allergens, especially the lipid-binding proteins, might interfere with normal host defense signaling.

Herein, we report that structural analysis reveals Der p 7 to be distantly related to another family of innate immune proteins that bind to various hydrophobic ligands. One such protein, LPS binding protein (LBP), is in the TLR4 pathway. To explore the possibility of immune protein mimicry analogous to the recently reported behavior of Der p 2 and MD-2, we examined the binding of LPS and select other hydrophobic compounds to Der p 7.

Materials and Methods

Chimeric ELISA for IgE antibodies to Der p 7

IgE binding to Der p 7 was analyzed by a chimeric ELISA as previously described.²⁰ The plates were coated overnight at 4°C with anti-Der p 7 mAb WH9. Plates were blocked with PBS-Tween-1%BSA, followed by an incubation with 400 ng/mL of recombinant or natural Der p 7 at room temperature. The source of natural Der p 7 was a dust mite extract from *Dermatophagoides pteronyssinus* (HollisterStier, Spokane, WA) which contained 126 µg/ml Der p 7 (30,000 AU/ml). Sera from allergic patients was purchased from PlasmaLab International (Everett, WA). Thirty-three sera were added at 1:2 and 1:10 dilutions and bound IgE was detected using biotinylated goat anti-human IgE. Anti-Der p 2 mAb αDpX, purified natural Der p 2 at 500 ng/ml, and chimeric Ab 2B12-IgE were used for the standard curve to quantify the assay. Natural allergen (nDer p 7) and rDer p 7 expressed in *Pichia pastoris*²¹ were compared to constructs of rDer p 7 expressed in bacteria, *vide infra*.

Crystallographic Studies

The best results for crystallography were obtained with a customized maltose binding protein (MBP) fusion with Der p 7. Briefly, the MBP fusion was designed with surface entropy reducing mutations in MBP to enhance crystallization.²² DNA encoding residues 18-215 of Der p 7 representing the native expressed mite protein¹⁰ was cloned into a modified fixed-arm PMAL vector between the NotI and BamHI restriction sites with MBP mutations D82A/K83A/E172A/N173A/K239A.^{23, 24} The MBP-Der p 7 fusion protein was expressed in *E. coli* Rosetta2(DE3) pLacI cells (Novagen), and purified using the amylose affinity resin in batch followed by gel-filtration utilizing a 16/60 superdex 200 column and ionic exchange chromatography step using Q sepharose. The purified protein was dialyzed against 25 mM HEPES pH 7.4, 75 mM NaCl, 5 mM maltose and concentrated to 27 mg/mL.

Crystals of the MBP-Der p 7 fusion protein were obtained at 4°C by mixing 1 µL of the protein solution with 1 µL of the reservoir consisting of 42.5 mM Tris pH 8.5, 12.75% PEG4000, 85 mM lithium sulfate, and 7.5% glycerol. The crystal used in this experiment reached a maximum size of 0.2×0.2×0.2mm after one week. Prior to data collection the crystal was transferred to 63.8mM Tris pH 8.5, 19.1% PEG4000, 128mM lithium sulfate, and 11.25% glycerol and then quickly transferred to 85 mM Tris pH 8.5, 25.5% PEG4000, 0.17 M lithium sulfate, and 15% glycerol. The crystal was flash frozen in liquid nitrogen and placed in a nitrogen gas stream cooled to -180°C. Data were collected to a resolution of 2.35Å, and were scaled and processed using HKL2000.²⁵ Molecular replacement using the MBP from the MBP-RACK1 fusion structure was used to solve the position of the three molecules of MBP in the asymmetric unit.^{24, 26} The structures of the three Der p 7 molecules were built manually by iterative cycles of model building in O²⁷ as well as density modification and refinement in CNS.²⁸ We were unable to identify a structure with sufficient homology to Der p 7 to allow solution by molecular replacement, but the coordinates from 1EWF.pdb (bactericidal/permeability increasing protein) and 3E8T.pdb (Takeout 1) were consulted for help in chain tracing and secondary structure based on structural similarity suggested by GenTHREADER.²⁹⁻³¹ Table 1 lists

important statistics for assessing the quality of the crystallographic data and the resulting structures.

NMR Constructs and Spectroscopy

Recombinant Der p 7, residues 18-215, was subcloned and expressed in the Gateway system (Invitrogen), with a cleavable 6-His tag, in order to reduce the size of the protein for NMR spectroscopic analysis. Optimal bacterial expression of this construct was obtained in the Rosetta 2 (DE3) pLacI cell line by growing the cells at 37° C to an OD₆₀₀ of 0.6 and inducing overnight at 18° C. Stable isotopic labeling for NMR was done as described previously.³² The cells were lysed by sonication in 50 mM HEPES pH 7.4, 500 mM NaCl, and protein was found in the soluble fraction after centrifugation at >40,000g for 30 minutes. The rDer p 7 was purified with the Ni²⁺ affinity chromatography, followed by a Q-sepharose ion-exchange column. The affinity tag was cleaved with tobacco etch virus (a.k.a. TEV) protease. Subsequently, cleaved protein was passed through another Ni²⁺ column and further purified with size exclusion chromatography.

NMR backbone assignments were obtained on a U-²H, ¹³C, ¹⁵N labeled sample of 0.5 mM protein using a Varian 600 MHz spectrometer. Standard backbone assignment experiments were used to establish the connectivity of resonances through the C', CA, and CB.³² The solution conditions for all NMR studies utilized phosphate buffered saline, with 5-10% ²H₂O and 1 μM 5,5-dimethylsilapentanesulfonate (DSS) for referencing. Data were acquired at 25° C. The NMRViewJ RunAbout module was used for making the assignments.³³ Aiding the analysis were assignments from the automated algorithms, MARS and PINE.^{34, 35} The assignments of manual inspection, MARS, and PINE agree very well.

Results

IgE antibody responses to Der p 7

The prevalence of IgE ab binding to natural allergen, rDer p 7 expressed in *Pichia*, and bacterially expressed rDer p 7 constructs used for crystallography or NMR were very similar (range 43-58%). There was an excellent correlation of IgE ab binding between rDer p 7 expressed in *E. coli* with or without MBP ($r = 0.94$; $n = 18$). IgE ab binding to the rDer p 7 with cleaved affinity tag showed an excellent correlation with IgE ab binding to natural Der p 7 from mite extracts ($r = 0.99$, $n = 21$) (data not shown). Similarly, IgE ab bound to the MBP-Der p 7 fusion protein showed a high correlation with IgE ab to *Pichia*-expressed Der p 7 ($r = 0.94$; $n = 16$) and to IgE bound to the natural allergen ($r = 0.96$, $n = 21$). There was an excellent correlation between IgE antibody binding to all the rDer p 7 allergens and to the natural allergen, indicating that the recombinant proteins were suitable for structural studies. Total IgE and specific IgE antibody levels to four allergens (Der f 1, Der p 1, Der p 2, Der p 7) have been reported in Table E1 (Online Repository).

Structural Analysis

rDer p 7 without an affinity tag crystallizes readily at room temperature, although no crystals diffracted at high resolution. Therefore we utilized an alternate approach in which the Der p 7 was fused to an MBP mutant protein designed for optimal crystallization characteristics. The best Der p 7-MBP fusion protein crystals diffracted at 2.35 Å. The final model of the asymmetric unit consisted of three MBP-Der p 7 fusion proteins, shown in Figure 1a, (PDB code 3H4Z). The most extensive interactions between symmetric units are among MBP molecules, although all permutations of interactions exist, i.e. MBP-MBP, MBP-Der p 7, and Der p 7-Der p 7. The Der p 7 structure is elongated with two 4-stranded β-sheets that wrap around a long C-terminal helix (Figure 1). These two β-sheets are situated in a “head to toe” orientation appearing to form a single continuous β-sheet with a break in the middle. The N-

terminal helix forms a cleft between the adjacent β -sheet and the end of the C-terminal helix, possibly for binding an unknown substrate.

Figure 1b shows the Der p 7 molecule from chain A colored by relative B-factor. Of note, the Der p 7 molecules generally showed higher B-factors than the attached MBP molecule with the exception of chain C, which had high B-factors for both the MBP and Der p 7 molecule (Table 1). The regions with very high B factors were difficult to trace, and in some cases density could not be found in all three chains. For example, all Der p 7 residues are traced in molecule A while molecule B and C are missing residues 63-67 and 59-67 respectively (see asterisk in Figure 1b). Additionally, the model of molecule C is missing residues 122-126 and the C terminal residue Q215. All three of the Der p 7 molecules superimpose well, with an RMSD of less than 0.51 Å over 197 C α atoms.

The NMR data acquired in solution is consistent with the structure determined by crystallography. The ^1H - ^{15}N heteronuclear single quantum coherence (HSQC) spectrum of Der p 7 is characteristic of a generally well folded protein, with well dispersed resonance intensities. The assignment of the backbone resonances was extremely challenging due to the high number of missing resonances in the HSQC and other 3D NMR experiments: 159 amide resonances appear out of a possible 192 backbone amides. Of those that appear, 134 could be confidently assigned with manual inspection of the spectra. The assigned residues are colored red on the structure in The Figure 1c. In general, the assigned residues correlate with regions of lower B-factors in the crystal structure. The correlation of missing assignments with high B-factors supports the contention that most of the assignment problem results from broadening due to conformational exchange. The secondary structure prediction from the assigned shifts³⁴ agrees very well with the secondary structure of the crystal structure, providing additional confidence in the assignments, and showing that the solution conformation of the protein is similar to the crystal structure.

Structural Homology

To investigate whether the structure of Der p 7 provided clues to the function of the protein in mites, or to its allergenic effect in humans, the software DALI³⁶ was used to identify related protein folds in the database. The search returned over 500 structures with marginal match scores (Z-score $\sim$ 5), and eight with very good matches (Z-score $>$ 10). Table 2 shows the top eight results as well as a few select entries that are notable because they are also allergens. For example the 37th best score is the birch pollen allergen Bet v 1. The fold superfamily of these allergens is classified as Bet v 1-like, also termed START.³⁷ The common feature of START domains is that they are known to bind hydrophobic ligands.

The best structural matches with Der p 7 include insect proteins that have very similar folds such as juvenile hormone binding protein (JHBP) and the take-out (TO) proteins, consistent with GenTHREADER predictions.^{29, 31, 38} The RMSD of the superposition for Der p 7 with JHBP is 3.6 Å over 176 C α atoms (Figure 2a). Like the TO proteins, it superimposes well over the whole molecule and shows very similar secondary structure organization. JHBP and TO proteins bind to hydrophobic ligands, similar to Bet v 1 and the other START domain-like allergens.

Another significant match to Der p 7 based on the DALI evaluation was the N terminal domain of bactericidal permeability increasing protein (BPI, pdb code 1BP1³⁰). A structural overlay is shown in Figure 2b, and has a RMSD of 3.5 Å over 174 C α atoms. BPI contains two domains, which have a very similar fold but are distantly related sequentially. The full structure of BPI is shown in Figure 2c, with the domains shaded differently and bound ligands indicated with yellow spheres. The protein family database PFAM³⁹ designates this fold as “BPI/LBP/CETP N terminal”, and the database CATH⁴⁰ classifies the architecture as a “super-roll” based on

the way the long beta strands roll around the central long helix. The fold of Der p 7 is more similar to the super-roll than to the START domains.

Lipid Binding Assays

Based on the structural similarities to other lipid binding proteins the lipid binding potential of Der p 7 was examined. Since BPI and the related LBP bind to LPS we first tested LPS binding to Der p 7. Biochemical assays indicated that Der p 7 does not bind tightly to LPS (See Figure E1 in the Online Repository). Other potential Der p 7 ligands for evaluation were identified by comparison to BPI and LBP. One type of potential Der p 7 substrate is distearoyl phosphatidyl choline (DSPC), which is found complexed to BPI in the crystal structure (Figure 2c).³⁰ Lyso-palmitoyl PC (LPPC) was tested for interactions with Der p 7 due to its similar structure to DSPC and much higher solubility. A sample containing 50 μ M U-[¹⁵N] labeled Der p 7 was titrated with increasing amounts of LPPC and two dimensional ¹H-¹⁵N HSQC spectra showed very few changes. Similar negative results were obtained upon addition of SDS and N-octyl-glucoside. Figure 3a shows an example of an NMR spectrum showing minor perturbations that were obtained upon addition of LPS. In order to see if the small changes due to LPS could be reversed, polymyxin B (PB) was added, which is known to sequester LPS.⁴¹ Surprisingly, the addition of PB produced much more significant spectral changes (data not shown). To insure that the changes were due to PB, Figure 3b shows the changes in the NMR HSQC spectra of Der p 7 upon addition of PB alone. This result was followed up with a saturation transfer difference experiment⁴² (STD) that demonstrated that Der p 7 and PB interact. Figure 4 shows the ¹H spectra of PB alone (Panel a) and the results of the STD experiment with PB and Der p 7 (Panel b). The same resonances appearing in panels a and b provide conclusive evidence of a binding interaction between PB and Der p 7.

To show that the binding of PB was a specific interaction, the assignments of the resonances in the HSQC spectra were determined to map the site of the interaction (See Figure E2 in the Online Repository). Figure 5a shows the residues involved in the interaction colored red. Many of the significant shift changes cluster near a cleft between the N and C terminus of Der p 7. However, because of missing assignments we are being cautious in our interpretation of the shift changes, as it is possible that additional residues are involved. Figure 5b shows the N terminal domain of the structure of BPI with the DSPC bound. BPI is aligned in a similar orientation to Der p 7. This figure shows the DSPC molecule bound in a cleft similar in location to the cleft in the Der p 7 molecule. Hence PB interactions with Der p 7 occur near the expected binding site.

Discussion

Der p 7 appears closely related to several proteins known to bind hydrophobic compounds, including the Take-out proteins and BPI and LBP. The TO proteins are involved in signaling in a wide variety of biological functions of insects.⁴³ Since the class *Insecta* and the class *Arachnida* (mites) are both in the phylum *Arthropoda* this may provide some clues as to the natural function of Der p 7 in mites, but there is not a good enough match in the literature with a known natural ligand. Der p 7 does not appear to bind to LPS like BPI or LBP, but exhibits affinity for PB, which is a nine residue cyclic peptide with a lipid tail from the Gram-positive bacteria *Bacillus polymyxa*. The weak binding of Polymyxin B to Der p 7 does not imply that PB is the natural substrate. Future studies to identify the natural ligand(s) will require screening of a broader spectrum of potential ligands, as well as a determination of the corresponding dissociation constants. However, the mapping of the interaction site to a similar position in homologous proteins validates the conclusion that Der p 7 has affinity for a lipid substrate, in this case a bacterially-derived lipopeptide product. The importance of this is that lipid binding

is a common feature of some allergens⁴⁴ and natural lipid adjuvants are suspected to be important in sensitizing patients to allergens.¹⁷

LBP has been shown to interact with di- and tri-acylated lipopeptides, as well as LPS.^{45, 46} Bacterial and mycobacterial lipopeptides can induce the release of cytokines through interactions with TLR2 in cooperation with TLR1 or TLR6. Several TLRs may be involved in stimulating the allergic response through promotion of Th2 immunity.⁴⁴ Indeed stimulation of TLR2 in the presence of allergen enhances Th2 responses,^{47, 48} as does TLR5 stimulation.⁴⁹ We speculate that if Der p 7 can bind bacterial lipopeptides other than PB as its natural ligand, it may promote Th2 immunity (to itself) through co-stimulation of TLR2 pathways.

In addition to understanding allergen function, structural characterization of allergens can provide detailed knowledge of potential B-cell epitopes and aid in mapping antibody interactions. We previously used hydrogen exchange NMR to map monoclonal antibody binding sites on Der p 2 and NMR techniques have recently been used to define a mAb epitope on *Blomia tropicalis* allergen Blo t 5.^{50, 51} Several X-ray crystal structures of allergen/antibody complexes have also been reported including Bet v 1, β -lactoglobulin and Bla g 2.⁵²⁻⁵⁴ Modification of structure has been an effective way to design recombinant hypoallergenic variants of allergens, which have potential clinical application for immunotherapy in mite allergy.^{55, 56} Our IgE binding data confirms that Der p 7 is an important allergen and shows that the prevalence of IgE ab to rDer p 7 is comparable to that of the natural allergen.¹² The purified rDer p 7 was of sufficiently high quality for crystallographic studies. These observations suggest that rDer p 7 could be used in a cocktail of recombinant mite allergens for use in subcutaneous immunotherapy and for rational design of hypoallergenic variants. A cocktail of five recombinant timothy pollen allergens was successfully used in a placebo controlled trial and resulted in a 39% reduction in symptom scores and medication use.⁵⁷ Clinical improvement was associated with a strong increase in allergen-specific IgG₄ antibody production, reduced levels of IgE ab and induction of IL-10 producing regulatory T-cells. Current data suggests that recombinant Der p 1, Der p 2 and Der p 7 are important immunologic targets and should be included in similar vaccine trials for mite allergen immunotherapy. The structure presented here will be useful in the rational design of hypoallergenic mutant proteins, and to understand future studies of the cross reactivity between the Group 7 mite allergens.

Knowledge of allergen function has contributed to an improved understanding of allergenicity. This was demonstrated for the protease activity of Group 1 mite allergens on the adaptive immune system^{7, 8} and the interaction with the innate immune system by Group 2 mit337 e allergens.¹⁷ We suggest that there may be similarities between the biological functions of the group 7 and group 2 mite allergens that involve binding of lipid substrates and that both allergens may co-opt innate immune responses into promoting allergenicity. Further studies are needed to investigate this hypothesis. The structure of Der p 7 presented here will contribute to an improved understanding of the complex interactions between the innate and adaptive immune systems in the development of allergy.

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Abbreviations Used

LBP	lipopolysaccharide binding protein
TLR	Toll-like receptors
PB	polymyxin B

BPI	bactericidal/permeability increasing protein
LPS	lipopolysaccharide (LPS)
MBP	maltose binding protein (MBP)
STD	saturation transfer difference
DSPC	distearoyl phosphatidyl choline
DSS	5,5-dimethylsilapentanesulfonate
HSQC	heteronuclear single quantum coherence

Figure 1.

Der p 7 structural analysis. Panel a shows the three molecules in the crystallographic asymmetric unit, colored green (chain A), blue (chain B) and red (chain C). The Der p 7 sequence is rendered in ribbon style while the MBP sequence is represented with a solid surface. Panel b shows the Der p 7 molecule from chain A colored by relative B factor where red is high (greater than 80) and blue is low (less than 30). In Panel c, residues that could be assigned by NMR are colored red, and unassigned residues are grey. The N and C terminus are annotated in panel b. The asterisk indicates a loop with variable density in chains A, B, and C of the asymmetric unit.

Figure 2.

Examples of structurally similar proteins to Der p 7. Der p 7 is shown in green in all panels. Panel a is the best alignment with JHBP (cyan). Panel b shows the best alignment of Der p 7 with the N terminal domain of BPI shaded magenta, while the C terminal domain of BPI is shaded pale pink. Panel c shows BPI with two bound DSPC molecules (yellow spheres).

Figure 3.

Changes in the ^{15}N - ^1H HSQC spectra of Der p 7 upon addition of compounds. Panel a shows very little change in the HSQC spectra of 50 μM Der p 7 going from apo (green) to 100 μM (pink) to 200 μM (purple) LPS. In contrast, addition of polymyxin B (PB) caused dramatic changes, shown in panel b. Der p 7 apo spectra is black, and upon addition PB the spectra is colored orange.

Figure 4. ^1H NMR of PB. Panel a shows the ^1H NMR spectrum of 1 mM Polymyxin B, in $^2\text{H}_2\text{O}$ with 10 μM DSS (at 0 ppm) for chemical shift referencing. Panel b shows the result of the STD experiment⁴² with 1 mM PB and 50 μM Der p 7

Figure 5.

Proposed binding site of PB in Der p 7. The significant chemical shift changes in Figure 3b correspond to the residues colored red in Panel a. The residues map to a cleft between the N and C terminus, labeled in Panel a. This location is similar to a cleft in the N terminal domain of BPI that contains DSPC in the crystal structure, see Panel b.

Table 1

Crystallographic data statistics	
data set	MBP-derp7(18-215)
unit cell	a=193.35Å, b=117.90Å, c=92.44Å; $\alpha=\gamma=90^\circ$, $\beta=114.06^\circ$
Space Group	C2
Resolution (Å)	50.0 -2.35
# of observations	407,861
unique reflections	76,800
Rsym(%) (last shell) ¹	6.3 (35.5)
I/ σ I (last shell)	28.4 (2.5)
Mosaicity range	1.1-1.4
completeness(%) (last shell)	97.8 (97.2)
<u>Refinement statistics</u>	
Rcryst(%) ²	24.9
Rfree(%) ³	29.3
# of waters	322
Overall Mean B value (Å ²)	50.4
Average for molecule A	43.99
molecule B	47.49
molecule C	61.46
<u>r.m.s. deviation from ideal values</u>	
bond length (Å)	0.004
bond angle (°)	1.0
dihedral angle (°)	22.5
improper angle (°)	0.68
<u>Ramachandran Statistics⁴</u>	
residues in:	
favored (98%) regions (%)	92.5
allowed (>99.8%) regions (%)	99.5

Crystallographic data statistics

PDB I.D. code

3H4Z

¹ $R_{\text{sym}} = \sum (|I_i - \langle I \rangle|) / \sum(I_i)$ where I_i is the intensity of the i th observation and $\langle I \rangle$ is the mean intensity of the reflection.

² $R_{\text{cryst}} = \sum ||F_o| - |F_c|| / \sum |F_o|$ calculated from working data set.

³ R_{free} was calculated from 5% of data randomly chosen not to be included in refinement.

⁴ Ramachandran results were determined by MolProbity.

Table 2

No	PDB & Chain	Z-score	RMSD	Length Align	No. Residues	% Identity	Name
1	2RCK-B	12.8	3.7	175	208	13	Juvenile Hormone Binding Protein
2	1BP1-A	12.8	3.5	177	456	14	Bactericidal/Permeability-Increasing Protein
3	2RCK-A	12.7	3.6	174	221	13	Juvenile Hormone Binding Protein
4	1BP1	12.6	3.6	174	456	14	Bactericidal/Permeability-Increasing Protein
5	1EWF	11.0	3.3	174	456	15	Bactericidal/Permeability-Increasing Protein
6	3E8W	10.7	4.4	171	219	11	Takeout-Like Protein 1
7	3E8T	10.6	4.5	172	296	10	Takeout-Like Protein 1
8	2OBD-A	10.4	3.5	169	427	12	Cholesteryl Ester Transfer Protein
37	1VJH-B	4.5	3.7	99	120	11	Bet v 1 Allergen family
290	2VJG-D	3.4	4.1	109	152	9	Major Allergen Dau c 1
453	1H ₂ O-A	2.5	4.0	92	159	10	Major Allergen Pru av 1
531	2BK0-A	2.0	3.7	89	153	12	Major Allergen Api g 1