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Homology modeling of allergenic cyclophilins: IgE-binding site and structural basis of cross-reactivity

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Abstract

Cross-reactivity among allergens is of considerable scientific as well as clinical interest. Proteins belonging to the allergenic cyclophilin family share a high degree of sequence homology and are cross-reactive. Until date no three-dimensional structural information is available on these proteins and the shared structural features of the epitopes which are the most important determinants of cross-reactivity. Cyclophilins are also known to bind with the immuno-suppressive drug cyclosporin. Comparative molecular modeling of these allergenic cyclophilin proteins of different sources was performed in order to investigate the structural basis of their cross-reactivity. All the proteins studied revealed a similarity in the shape of the cross-reactive epitopes with varying degrees of accessibility. Cyclosporin binding and allergenic properties of these proteins were also found to be structurally related. © 2003 Elsevier Inc. All rights reserved.

Keywords: Allergen; Cyclophilin; Cross-reactivity; Homology modeling; Solvent-accessibility; Cyclosporin

In industrialized countries, up to 20% of the population suffers from allergic disorders, which is a major health problem [1]. Moreover, the prevalence of such diseases is steadily increasing [2]. Effector phase of type I (immediate type) allergic reactions occurs when allergen molecules crosslink the allergen-specific IgE bound on the effector cell surface through the high affinity IgE receptor FcεRI [3]. Pollen grains and fungal spores contain protein and glycoprotein components that act as allergens. Various groups of highly homologous cross-reactive allergens have been reported from diverse sources and termed as pan-allergens [4]. They might play very important roles in IgE-mediated poly-sensitization [5]. Allergens that belong to the cyclophilin family are well-known pan-allergens [4]. Proteins of this group have been isolated as IgE-binding proteins from the skin-colonizing yeast *Malassezia furfur* [6], from baker's yeast *Saccharomyces cerevisiae* [7], and from the pathogenic mould *Aspergillus fumigatus* [8]. They are known to bind with the immuno-suppressive drug cy-

closporin [9,10]. Airborne fungal spores are one of the major sources of aero-allergens present in both indoor and outdoor conditions [1,11]. IgE-mediated cross-reactivity within cyclophilins of *Aspergillus* (Asp f11), *Malassezia* (Mal f6), *Saccharomyces* (Sa-CypA), and also with their human counterpart, i.e., human cyclophilin A (hu-CypA) was established by IgE-specific Western blots, extended serological investigations and intradermal skin tests [4]. Apart from the allergenic cyclophilins, plant profilins [12], and fungal MnSOD [13] and their respective human homologs are cross-reactive as well. Such cross-reactivity against human proteins is responsible for severity and perpetuation of mould associated chronic asthma or severe atopic dermatitis in the absence of exogenous allergen exposure [4].

Identification of cross-reactivity between allergens is important for the grouping of allergens into few cross-reactive clusters for simplified diagnosis and therapy. Allergenic cross-reactivity was found to be due to direct result of the conserved antigenic surface of the molecules. Substitution of residues across this surface disrupts the cross-reactivity [14]. It was also suggested that molecular modeling can help to identify

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solvent-accessible common antigenic surface shared by different allergenic cyclophilins [4].

Until date no structural information is available on these allergy-causing cyclophilins. However, it is necessary to identify the conformation of the common epitopes which is the most important determinant of cross-reactivity. The availability of the sequences encoding different cyclophilin allergens together with the solved three-dimensional structures of their human homolog (hu-CypA) allows homology modeling of IgE-reactive cyclophilins from the mould *Aspergillus fumigatus* (allergen Asp f11), from the skin colonizing yeast *Malassezia furfur* (Mal f6), and from the budding yeast *Saccharomyces cerevisiae* (Sa-CypA). IgE-binding epitopes were predicted for all four proteins. Surface accessible conserved residues were examined to determine the basis of their cross-reactivity. Our study will help the researchers to identify the contribution of each surface-displayed amino acid towards allergenicity and develop hypoallergenic derivatives with mutated B-cell epitopes, for diagnosis and therapy of the cyclophilin sensitive patients.

Materials and methods

The sequences of three fungal allergenic cyclophilins (Asp f11, Mal f6, and Sa-CypA) were taken from Fluckiger et al. [4]. The identical residues are marked in bold letters. As an attempt to construct three-dimensional models of three proteins, sequence similarity search was carried out with each of the sequence separately against proteins whose known structures have been deposited in the protein Data Bank (PDB) [15]. BLAST search algorithm [16] was used for the search. The atomic coordinates of human cyclophilin A complexed with cyclosporin (pdbcode: 2rmb) [17] were obtained from PDB. The chain Q of this X-ray structure was used to build the three-dimensional models of three proteins (Asp f11, Mal f6, and Sa-CypA). The sequences were aligned using the program ClustalW [18]. Program Modeller [19] was used to build the three-dimensional structures of the three proteins. Energy minimization was carried out by conjugate gradient using the program Discover with the Consistent Valence Force Field (CVFF) [20] until all the structures reached the final derivative of 0.001 kcal/mol. The stereochemical qualities of the models were checked using the program PROCHECK [21] and Ramachandran Plots [22] were drawn. Program Modeller and PROCHECK were run on a Silicon Graphics O2 R12000 workstation. The visualization of the above studies was done using Insight II of Molecular Simulations, San Diego, CA.

Prediction of B-cell epitopes was performed online (http://mi-foundation.org/Tools/index_frames) according to the method of Kolaskar et al. [23]. Welling's antigenicity plot [24] was prepared using the program EPIPLOT [25]. The accessible surface areas of predicted B-cell epitopes of the final structures were calculated with the program NACCESS [26]. The % accessibility is the ratio of the accessible area of an amino acid in the model to the accessible area of the amino acid in a tripeptide Gly-X-Gly in 100.

Cyclosporin-binding site was determined by superimposing the models on the crystal structure of human cyclophilin complexed with cyclosporin A. Having determined the binding site in the superimposed structure the cyclosporin was subsequently merged with the model to mimic a model-cyclosporin complex.

The molecular surface was calculated with Insight II. The solvent probe radius used for molecular surface was 1.4 Å.

Discussion

Table 1 shows the overall sequence identity among four proteins. Comparison of the three-dimensional

Table 1
Sequence identities of four cross-reactive cyclophilins (Asp f11, Mal f6, Sa-CypA, and hu-CypA)

	Asp f11	Mal f6	Sa-CypA	Hu-CypA
Asp f11	100	65	60	55
Mal f6		100	72	68
Sa-CypA			100	65
Hu-CypA				100

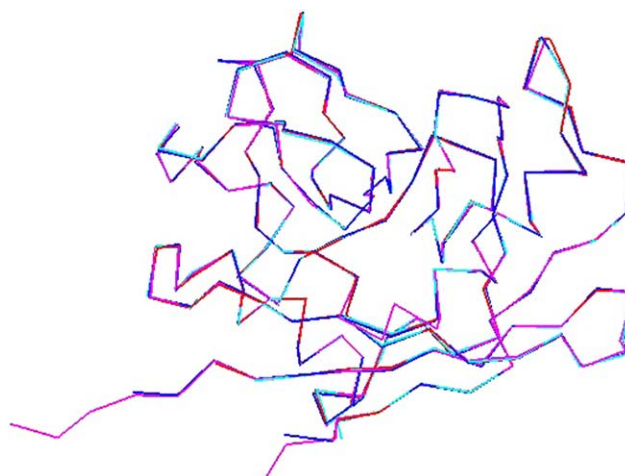


Fig. 1. Superimposition of α -carbon backbones of Asp f11, Mal f6, Sa-CypA, and hu-CypA.

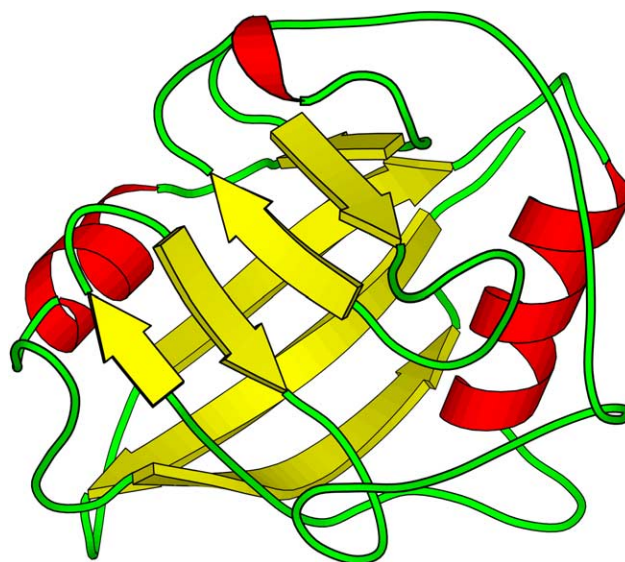


Fig. 2. Ribbon diagram of the three-dimensional model of Mal f6. β strands are shown as arrows, helices are shown as helical ribbons, and the rest are shown as loops. The structures of three modeled proteins are similar; therefore one is shown for clarity. This figure is made using the program MOLSCRIPT [33].

Table 2

Residue-wise absolute and % accessibilities of the putative B-cell epitopes of allergenic cyclophilins^{a,b}(A) *Asp f11*

1	Sequence: VFFDVEYAPVG (4–14)										
	V	F	F	D	V	E	Y	A	P	V	G
	92.1	130.7	97.8	27.2	26.3	0.3	41.3	11.8	7.5	1.0	1.1
	(5.7)	(58.5)	(0.4)	(17.5)	(16.2)	(0.2)	(17.3)	(10.1)	(5.2)	(0.6)	(1.3)
2	Sequence: VGRIVFN (20–26)										
	V	G	R	I	V	F	N				
	50.4	17.8	115.2	2.9	17.6	7.2	33.3				
	(31.1)	(20.8)	(46.2)	(1.5)	(10.8)	(3.2)	(19.7)				
3	Sequence: FRELCKR (39–45)										
	F	R	E	L	C	K	R				
	7.4	64.6	31.7	1.8	33.4	109.6	95.1				
	(3.3)	(25.9)	(17.0)	(0.9)	(2.4)	(52.8)	(38.14)				
4	Sequence: SQFFITTAVTS (113–123)										
	S	Q	F	F	I	T	T	A	V	T	S
	2.6	24.2	10.4	8.4	0.4	0.0	17.6	37.7	77.8	0.02	70.7
	(2.1)	(12.8)	(4.7)	(3.8)	(0.2)	(0.0)	(11.9)	(32.4)	(48.0)	(0.01)	(56.3)
5	Sequence: DGKHVVFG (126–133)										
	D	G	K	H	V	V	F	G			
	29.0	42.9	118.2	32.8	1.8	4.4	0.0	0.0			
	(18.7)	(23.3)	(57.0)	(16.5)	(1.1)	(2.7)	(0.0)	(0.0)			
6	Sequence: KSYSVVE (139–146)										
	K	S	Y	S	V	V	K	E			
	159.2	1.3	88.8	56.2	14.8	0.0	83.0	99.7			
	(76.7)	(1.0)	(37.0)	(44.7)	(9.1)	(0.0)	(40.0)	(114.6)			
7	Sequence: EALGSSG (148–155)										
	E	A	L	G	S	S	G				
	3.5	81.8	25.7	15.9	46.7	103.6	97.1				
	(3.6)	(70.3)	(13.0)	(18.9)	(37.2)	(82.4)	(115.8)				

(B) *Mal f6*

1	Sequence: PLGTIKFKLFDDVVPK (residue no. 14–29)											
	P	L	G	T	I	K	F	K				
	112.4	65.4	26.6	53.5	2.3	69.6	0.6	28.4				
	(77.6)	(33.0)	(31.7)	(36.1)	(1.2)	(33.5)	(0.3)	(13.7)				
	L	F	D	D	V	V	P	K				
	1.4	66.6	62.6	100.8	37.9	0.7	67.2	73.0				
	(0.7)	(29.9)	(40.3)	(64.9)	(23.4)	(0.4)	(46.4)	(35.1)				
2	Sequence: NFRALCT (residue no. 33–39)											
	N	F	R	A	L	C	T					
	1.1	1.0	78.1	1.2	0.5	3.5	58.2					
	(0.6)	(0.4)	(31.3)	(1.7)	(0.2)	(2.5)	(39.3)					
3	Sequence: YAGSHFHRVIPD (46–57)											
	Y	A	G	S	H	F	H	F	V	I	P	D
	0.4	70.1	50.9	8.6	53.6	0.8	23.8	81.5	0.0	42.2	50.1	95.6
	(0.2)	(60.2)	(60.7)	(6.8)	(27.0)	(0.4)	(12.0)	(32.7)	(0.0)	(22.2)	(34.6)	(61.5)
4	Sequence: SQFFITTVVTS (108–118)											
	S	Q	F	F	I	T	T	V	V	T	S	
	2.2	24.2	0.0	6.8	2.3	0.01	11.4	49.5	62.8	0.5	68.9	
	(1.7)	(12.8)	(0.0)	(9.1)	(1.2)	(0.01)	(7.7)	(30.5)	(38.7)	(0.3)	(58.9)	
5	Sequence: DGKHVVFG (121–129)											
	D	G	K	H	V	V	F	G	E			
	30.2	39.2	120.8	33.8	2.4	2.8	0.0	0.9	66.9			
	(19.4)	(46.7)	(58.2)	(17.0)	(0.9)	(1.7)	(0.0)	(0.1)	(35.7)			
6	Sequence: DGMNVVKA (132–139)											
	D	G	M	N	V	V	K	A				
	91.5	17.2	66.7	113.2	18.1	0.1	122.9	31.9				
	(58.9)	(9.3)	(31.7)	(67.1)	(11.1)	(0.1)	(59.2)	(27.4)				

Table 2 (continued)

(C) <i>Sa-CypA</i>													
1	Sequence: PIGRVVFKLYNDIVP (13–27)												
	P	I	G	R	V	V	F	K					
	105	37.3	24.8	93.4	3.8	2.0	1.8	104.0					
	(73.1)	(19.6)	(29.6)	(37.5)	(2.4)	(1.3)	(0.8)	(50.1)					
	L	Y	N	D	I	V	P	—					
	3.4	55.9	62.5	114.2	26.8	0.6	76.5	—					
	(1.7)	(23.5)	(37.0)	(73.5)	(14.1)	(0.3)	(52.8)						
2	Sequence: YAGSPFHRVIPD (45–56)												
	Y	A	G	S	P	F	H	R	V	I	P	D	
	4.0	68.1	50.6	6.0	41.8	11.0	43.0	91.4	0.01	21.2	58.5	101.8	
	(1.7)	(58.5)	(60.3)	(4.8)	(28.8)	(0.1)	(21.6)	(36.7)	(0.01)	(11.2)	(34.9)	(65.5)	
3	Sequence: DRPGLLS (90–96)												
	D	R	P	G	L	L	S						
	78.8	147.9	25.6	1.6	15.2	0.0	0.2						
	(50.7)	(59.4)	(17.7)	(1.9)	(7.7)	(0.0)	(0.2)						
4	Sequence: SQFFITTVPCPW (107–118)												
	S	Q	F	F	I	T	T	V	P	C	P	W	
	1.9	31.6	1.3	5.7	2.9	2.1	7.1	56.5	52.2	0 (0)	78.8	133.6	
	(1.5)	(16.7)	(0.6)	(2.5)	(1.5)	(1.4)	(4.8)	(34.9)	(36.0)		(54.4)	(50.4)	
5	Sequence: DGKHVVFGEVVDGYDIVKKVESL (120–144)												
	D	G	K	H	V	V	F	G	E	V	V	D	G
	42.2	45.0	112.6	30.7	1.31	7.35	0 (0)	2.6 (3)	48.3	24.9	60 (37)	69.8	14.3
	(27.2)	(53.7)	(54.2)	(15.4)	(0.8)	(4.53)			(25.8)	(15.3)		(45)	(17)
	Y	D	I	V	K	K	V	E	S	L	G	S	
	83.3	91.31	14.32	4.31	130.7	118.9	5.1 (3)	19.9	93.3	33.3	13.2	46.0	
	(35.0)	(58.8)	(7.5)	(2.7)	(63)	(57.3)		(10.6)	(74.2)	(16.8)	(15.7)	(36.6)	
6	Sequence: KARIVVAK (150–157)												
	K	A	R	I	V	V	A	K					
	186.7	23.5	168.3	2.0	24.9	3.75	26.54	118.84					
	(90.0)	(20.2)	(67.5)	(1.0)	(15.4)	(2.31)	(22.8)	(57.3)					
(D) <i>hu-CypA</i>													
1	Sequence: TVFFDIAVDG (residue no. 4–13)												
	T	V	F	F	D	I	A	V	D	G			
	15.6	3.9	0.0	25.2	0.3	32.0	2.0	7.6	19.2	69.8			
	(10.6)	(2.4)	(0.0)	(11.3)	(0.2)	(16.9)	(1.7)	(4.7)	(12.3)	(83.1)			
2	Sequence : PLGRVSFELFADKVP (residue no. 47–59)												
	P	L	G	R	V	S	F	E	L				
	116.7	121.6	54.7	24.7	111.6	0.8	2.6	0.02	46.6				
	(80.6)	(61.4)	(65.2)	(9.9)	(68.6)	(0.6)	(1.2)	(0.01)	(23.6)				
3	Sequence: YKGSCFHRIIPGF (residue no. 47–59)												
	Y	K	G	S	C	F	H	R	I	I	P	G	F
	14.5	0.5	126.9	48.5	1.0	30.1	3.1	38.9	91.9	0 0	26.4	59.5	47.4
	(6.1)	(0.2)	(151.2)	(38.6)	(0.7)	(13.5)	(1.6)	(15.6)	(48)		(18.2)	(66.2)	(21.2)
4	Sequence: SQFFICT (residue no. 109–115)												
	S	Q	F	F	I	C	T						
	10.86	1.22	21.28	0.0	9.82	.94	0.1						
	(8.6)	(0.6)	(9.5)	(0.0)	(5.2)	(0.7)	(0.1)						
5	Sequence: DGKHVVFG (residue no. 122–129)												
	D	G	K	H	V	V	F	G					
	30.05	29.25	39.5	116.4	30.7	0.7	4.0	0 (0)					
	(19.3)	(34.7)	(19.0)	(58.6)	(18.9)	(0.5)	(1.8)						
6	Sequence: KITIADC (residue no. 154–160)												
	K	I	T	I	A	D	C						
	86.74	132.8	1.34	24.65	0.11	48.65	53.95						
	(41.8)	(7.0)	(1.0)	(13.0)	(0.1)	(31.3)	(38.1)						

^a Absolute accessibilities are in square angstrom.^b % accessibilities are shown in parentheses.

25 residues are at least 30% and 50% solvent exposed, respectively. Of the total 73 amino acids predicted as B-cell epitopes 10 are at least 30% and 29 are at least 50% solvent exposed in Sa-CypA. Ten are at least 30% and 12 thereof are at least 50% solvent exposed, out of 45 residues in case of hu-CypA. These predicted B-cell epitopes are scattered over the whole surface, indicating that the entire surface of cyclophilins is potentially antigenic. This kind of observation was reported previously for manganese superoxide dismutase (MnSoD) of *Aspergillus fumigatus* [28].

Sequence alignment shows that there are several individual or stretches of identical amino acids present among four proteins (Fig. 3) which are marked in bold letters. Of these identical residues we focussed on two stretches marked as II and III [SMANAGPNTN GSQFFI and WLDGKHVVFG] in Fig. 3 for cross-reactivity. Similarly, these stretches (II and III) (Table 2) were also predicted as putative B-cell epitopes by Kolasker et al. [23] and therefore were chosen over other identical residues. Region II contains 17 residues and region III contains 10 residues, respectively. These regions mainly consist of exposed loops and strands of β sheets (Fig. 3). It has been known for a long time that Ab-mediated cross-reactivity is dependent on shared structural features and conserved amino acids must be involved in the binding of IgE [28,29]. In addition, at least six residues (6–10) are required in order to be a B-cell epitope [30]. Moreover, these sites are sufficiently exposed on the surface of the proteins (Table 2). This

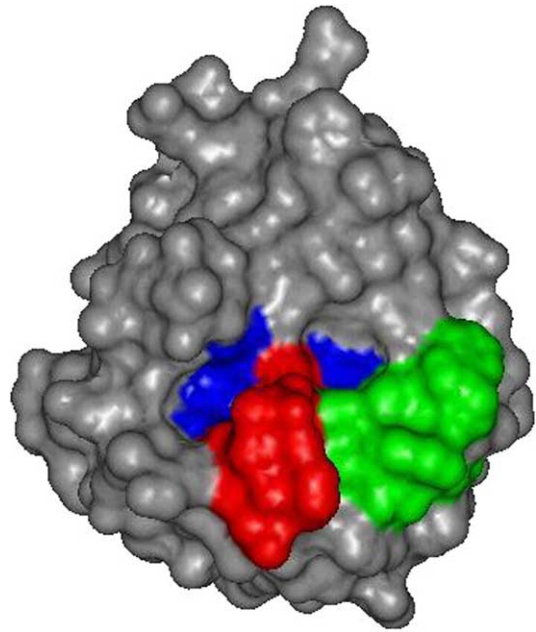


Fig. 4. Molecular surface of Mal f6.

suggests that amino-acid polypeptide sequences included in II and III (Fig. 3) might be involved in cross-reactivity. The region I contains six residues of which three are totally buried; moreover this site is not predicted as B-cell epitope by Kolaskar et al. [23]. Therefore, region I is a less likely candidate for cross-reactivity among cyclophilins. In Western blots, the antibodies

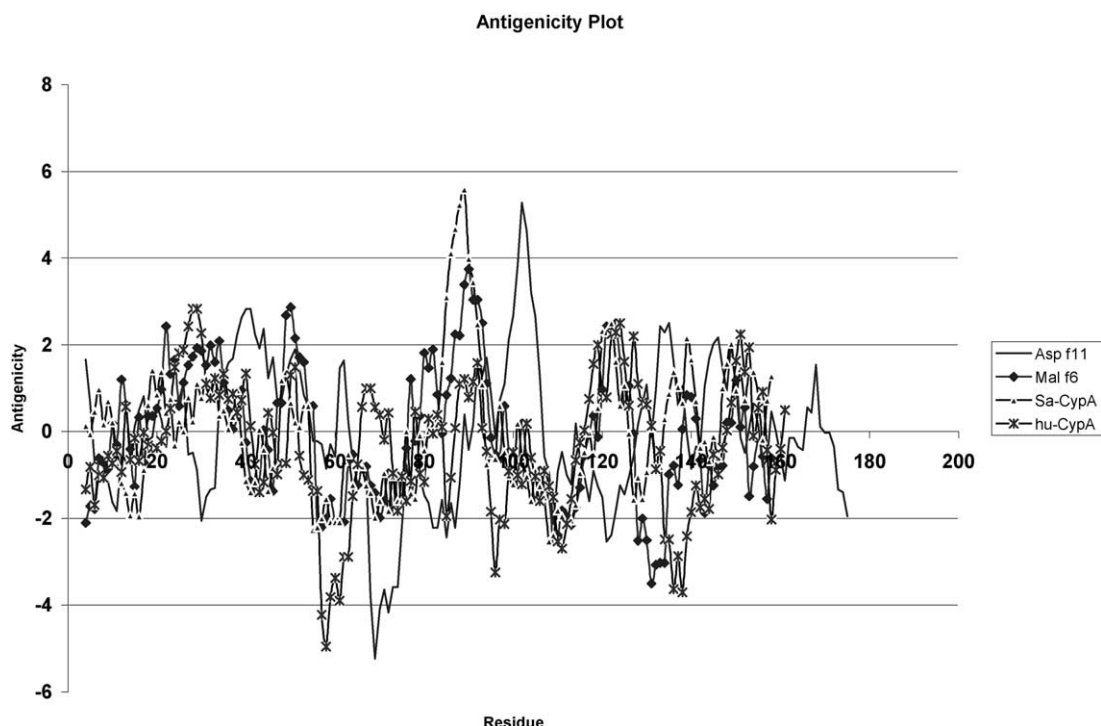


Fig. 5. Welling's antigenicity plots of the four cross-reactive cyclophilins (Asp f11, Mal f6, Sa-CypA, and hu-CypA).

recognize epitopes of denatured proteins. In contrast, clinically observed cross-reactivity is due to the similar antigenic surfaces of the native proteins. So, in our study we have determined the solvent-accessibility of the identical amino-acid patches (II and III) that occurred on the surface of the native modeled proteins (Table 2). We found that residues belonging to regions II and III are sufficiently solvent-accessible and thus substantially contribute to the extensive cross-reactivity among these proteins.

In addition to the prediction method proposed by Kolaskar et al. [23] we considered another well-established B-cell epitope prediction method by Welling [24] implemented in the program Epiplot [25]. Fig. 4 shows the antigenicity plots of four IgE-reactive cyclophilins. It also suggested the presence of antigenic determinants in the similar regions, except 92–98 (SRKHDKK), 87–93 (QLKHNP), 86–92 (KKHHDRP), and 80–86 (EK-FEDEN) in Asp f11, Mal f6, Sa-CypA, and hu-CypA, respectively. All these amino-acid stretches fall into the same loop region connecting β 1 and β 2 and exposed to the solvent (Fig. 3). Interestingly enough the first three stretches of amino acids consist of a centrally located conserved histidine residue flanked on either side by the accessible amino acids. This type of sequence arrangement was found in the IgE epitopes of cysteine proteases where the core of the epitope is distributed equally around a conserved Tyr residue [30]. In case of hu-CypA the corresponding region does not contain the His residue but it is closely placed to the proposed antigenic regions found in other three proteins as evident from our modeling studies.

Sequence comparison of cyclophilin A from different sources revealed a region with high homology, which is proposed to be involved in cyclosporin A (CsA) binding [31]. This region is marked in Fig. 3. We also identify the cyclosporin-binding site for our three modeled proteins (see Materials and methods). Cyclosporin A (CsA) binds to a groove formed by exposed loops at both sides on the surface of the protein. Incidentally, some of the predicted B-cell epitopes are localized in one of these loop regions on the surface of the protein.

Fig. 5 shows the solvent-accessible surface of Mal f6. Patches of conserved residues (II and III) among the allergens are shown to be surface exposed. Moreover, regions II and III were found to be close in the native conformation. The exposed residues in region II overlap with the residues involved in cyclosporin binding. This suggests that the allergenic properties might be structurally associated to the cyclosporin-binding properties of allergenic cyclophilin family proteins, which corroborates with the reported study that allergenicity and biological properties were found to be associated in many allergen families [32].

We have considered the 3D structures of four cross-reactive cyclophilins. Conserved antigenic surfaces and

solvent-accessibility of their putative epitopes were studied to predict the molecular basis of their cross-reactivity. Sequential site-directed mutagenesis of conserved, surface-displayed amino-acid residues identified in our study will allow experimental determination of the contribution of each single residue to the clinically observed cross-reactivity.

Acknowledgments

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