



MECHANISM OF ALLERGENIC CROSS-REACTIONS—IV. EVIDENCE FOR PARTICIPATION OF AROMATIC RESIDUES IN THE LIGAND BINDING SITE OF TWO MULTI-SPECIFIC IgE MONOCLONAL ANTIBODIES

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Abstract—The binding sites of two IgE monoclonal antibodies (mAbs), LA2 and LB4, were examined by absorption, fluorescence spectroscopy and computer-aided molecular modeling (CAMP). Absorption spectra revealed the formation of 1:1 molecular complexes for both LA2 and LB4 with a variety of structurally different ligands. For mAb LA2, the binding constants for ligands consisting of different amino acid derivatives coupled to DNP could be divided into two groups, suggesting that certain amino acid side chains (e.g. hydrophobic) of the derivatives were a contributing feature in ligand recognition. The presence of a charge-transfer band (320–340 nm) was also observed for complexation with several different ligands, indicative of aromatic ligand interactions with mAb binding site tryptophans. CAMP studies of the Fv region for both mAb support of the empirical observations and inspection of the Fv models reveal numerous binding site aromatic residues that are likely candidates for ligand recognition and complexation. The multi-specificity of these mAbs for different ligands may be due to a multitude of interactions with aromatic residues in the binding sites.

Key words: IgE antibodies, multi-specificity, computer-aided molecular modeling, charge transfer.

INTRODUCTION

The concept of multi-specific antibodies was first stated by Talmage (1959) and has been extended by others (Richards *et al.*, 1975; Varga *et al.*, 1973). In several studies of antibody specificity for monoclonal antibodies (mAbs) it has been noted that some mAbs have a broad binding specificity and some even demonstrate a higher affinity for ligands structurally different than the original antigen (so-called “heteroclitic antibodies”, i.e. they deviate from the rules) (Imanishi and Makela, 1974). In 1981 Rudolph *et al.* (1981) made a library of 13 IgE monoclonal antibodies to the hapten trinitrophenyl (TNP) and it was observed by others that some of these were heteroclitic (Furusawa and Ovary, 1988) in that they reacted with higher affinity to dinitrophenyl (DNP).

Varga *et al.* (1991) examined two of the IgE clones (noted as LA2 and LB4) from this collection and found that they had a wide range of ligand binding specificities. They examined ligand binding with over 2000 compounds and found several families of compounds (tetracyclines, polymyxins, phenothiazines, salicylates and quinones) that had no obvious structural similarity to DNP that were highly reactive. For example, these two IgE were found to bind diaspirin, DDT (chlorophenothane), hymecronon, diamox and cycrimine, all in the μ M affinity range; these IgE also react with a house dust mite peptide allergen (μ M affinity) (Varga *et al.*, 1991). The variable (V) region genes for these two IgE mAbs were cloned, sequenced and found to have an unusual number of aromatic residues in the V-regions (Kofler *et al.*, 1992).

In general, the antigen binding site consists of an area located near the top of the Fv region and is formed by six adjacent polypeptide segments or “complementarity determining region” (CDR) loops (Wu and Kabat, 1970; Kabat *et al.*, 1977), three contributed by the L chain, L1, L2, and L3, and three from the H chain, H1, H2, H3. The CDR loops are formed by the folding of the anti-parallel β -sandwich polypeptide segments for each chain. Although no one has crystallized an IgE antibody or derived Fab fragment, X-ray diffraction studies of IgM, IgG and IgA derived Fab crystals from over 30 different myeloma proteins and mAbs have revealed that

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Abbreviations: CAMP, computer-aided molecular modeling; CDR, complementarity determining region; CT, charge transfer; Fab, fragment of immunoglobulin which binds antigen; FR, framework residues, Fv region, fragment variable of Ig consisting of variable H-chain and variable L-chain; H-chain, heavy chain of immunoglobulin; mAb, monoclonal antibody; L-chain, light chain of immunoglobulin; PBS, phosphate buffered saline, pH 7.0; V-region, variable region of H- or L-chain; VDW, van der Waals.

Table 1. Light chain and heavy chain complementarity determining region (CDR) amino acids for mAbs LA2, LB4 and the "parent" structures used in the CAMM studies^a

Antibody	L1: 26-32	L2: 50-56	L3: 89-97
LA2	SQSLLYSSNQKNY	WASTRES	QHYYSSPYT
2MCP	SQSLLNSGNQKNF	GASTRES	QNDHSYPLT
Total of nine amino acids replacements; no insertions or deletions.			
	H1: 26-35	H2: 50-65	H3: 94-102
LA2	GDSITSGYWN	FINYSGNTYYNPSLRS	RANWDVFAY
3HMF	GDSITSDYWS	YVSYSGSTYYNPSLKS	
1D13			RERDYRLDY
Total of 14 amino acid replacements; one segment replaced.			
Antibody	L1: 26-32	L2: 50-56	L3: 89-97
LB4	SQSIVHSNGNTY	KVSNRFS	FQGSHVPLT
4FAB	SQSLVHSQGNTY	KVSNRFS	SQSTHVPWT
Total of six amino acid replacements; no insertions or deletions.			
	H1: 26-35	H2: 50-65	H3: 94-102
LB4	GYTFTSYGIN	YIYIGYGYIEYNEKFKG	RWGLIFAMDY
6FAB	GYTFTSNGIN	YNNPGNGYIAYNEKFKG	
3FAB			RNLIAGCIDV
Total of 13 amino acid replacements; one segment replaced.			

^aResidue positions are numbered according to the system of Kabat *et al.* (1991). "Parent structures" are noted as the BPDB codes for different antibodies as follows: 2MCP (McPC603); 3HMF (HyHEL-10); 1D13 (D1.3); 6FAB (36-71); 4FAB (4-4-20); 3FAB (NEWM).

the six CDR loops are paramount to antigen recognition (Davies *et al.*, 1990). The canonical nature of five of the CDR segments has been previously noted and has led to successful computer-aided molecular modeling (CAMM) of antibody Fv regions (Chothia *et al.*, 1986, 1989; Chothia and Lesk, 1987; Padlan, 1990).

In the present study, we examined the binding of a variety of ligands, including drugs, aromatic compounds and amino acid derivatives of DNP, to the IgE mAbs LA2 and LB4. The binding constants obtained for different amino acid derivatives of DNP could be divided into two broad categories: (1) polar and charged DNP-amino acid ligands with low affinities; and, (2) nonpolar and hydrophobic DNP-amino acid ligands with higher affinities. In addition, we conducted CAMM studies of the mAb Fv regions to identify solvent exposed aromatic residues that might participate in ligand recognition and complexation. These observations should prove useful in identifying the interactive motifs in these IgE mAbs and subsequent site-directed mutagenesis experiments with altered Fab fragments.

Table 2. Intrinsic association constants (K_A) of mAbs LA2 and LB4 with non-DNP ligands

Ligand	K_A (Imole ⁻¹) LA2	K_A (Imole ⁻¹) LB4	CT ^a
Cyclimine	1.7×10^5	6.9×10^4	Y
Hemimellitic acid	4.5×10^4	5.9×10^4	Y
Endoiodine	3.3×10^5	4.6×10^4	N
Diaspirin	2.9×10^5	3.7×10^4	N
α -nitrilo-cinnamic acid	3.6×10^5	1.1×10^5	N
Diamox	3.2×10^4	1.0×10^5	Y

^aPresence of charge-transfer (CT) band at 320–340 nm.

Antibody binding site aromatic residues may play an important role in defining antigen specificity.

MATERIALS AND METHODS

Monoclonal IgE antibodies

The mAbs LA2 and LB4 were purified from tissue culture media by affinity chromatography using a

Table 3. The molar extinction coefficients and intrinsic association constants for mAbs LA2 and LB4 with different ligands of DNP-amino acid derivatives

Ligand	$\epsilon_{260 \text{ nm}}$ Imole ⁻¹ ($\times 10^4$)	K_A (LA2) Imole ⁻¹ ($\times 10^5$)	K_A (LB4) Imole ⁻¹ ($\times 10^5$)
DNP only	1.26	0.68	0.77
DNP-ala	1.84	5.21	6.25
DNP-arg	0.80	0.52	1.10
DNP-asn	1.85	0.32	0.83
DNP-asp	1.76	2.47	3.13
DNP-glu	1.18	0.34	0.43
DNP-gly	1.57	3.76	0.80
DNP-gln	1.90	0.18	1.20
DNP-leu	1.92	2.5	2.48
DNP-lys	1.19	1.32	0.24
DNP-met	1.97	2.48	5.30
DNP-phe	1.99	6.75	6.07
DNP-pro	1.36	4.77	1.55
DNP-ser	2.03	0.48	3.19
DNP-thr	1.89	0.42	3.10
DNP-trp	1.74	7.20	8.50
DNP-tyr	1.45	1.05	0.71
DNP-val	1.91	3.07	8.54

goat anti-mouse Ig Affigel-15 affinity column (Siorad, Richmond, CA). Purity of the mAb obtained after chromatography was tested by polyacrylamide gel electrophoresis using the Phastgel system (Pharmacia, NJ). DNP-amino acid derivatives were obtained from Sigma Chemical Co. (St Louis, MO) and were used as such. The non-DNP containing ligands were obtained from our collection at the University of Innsbruck and have been described previously (Varga *et al.*, 1991).

Absorption and fluorescence spectroscopy

The experimental techniques and procedures employed for absorption and fluorescence spectroscopy studies, as well as methods of data analyses have been described previously (Droupadi *et al.*, 1992, 1993). Briefly, absorption spectral measurements were carried out using a Shimadzu PC2101 UV-VIS double beam spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). The spectra were recorded in the wavelength range of 230–800 nm. A slit width of 2.0 nm and fast scan were employed. CT band formation was examined in the protein absorbance range of 0.3–0.5 at 280 nm. Ligand solution was added in microliter

quantities to the solution containing mAbs, starting with the lowest ligand concentration solution first. A spectral scan was carried out after 2 min; the absorbance values did not change with longer reaction time periods. An initial titration was performed to obtain 30 data points for a wide concentration range of the ligand before the data were analyzed. Titrations were repeated for 20 data points in the narrow concentration range of interest. Association constants were determined by stepwise multiple nonlinear regression analysis (Sherman *et al.*, 1986).

Fluorescence spectral measurements were made on an SLM 8000 spectrofluorimeter (SLM Instruments, Urbana, IL) equipped with a thermostatic cell holder and magnetic stirrer. The excitation wavelength employed was 280 nm and emission was monitored in the range of 310–370 nm; peak emission was usually near 335 nm. The excitation and emission slit widths were maintained at 4 and 8 nm, respectively. The fluorescence intensities were obtained as the ratio of a fluorescent beam to a straight through beam, in order to compensate for fluctuations in lamp intensity. Emission intensities at a single wavelength were also collected over a period of

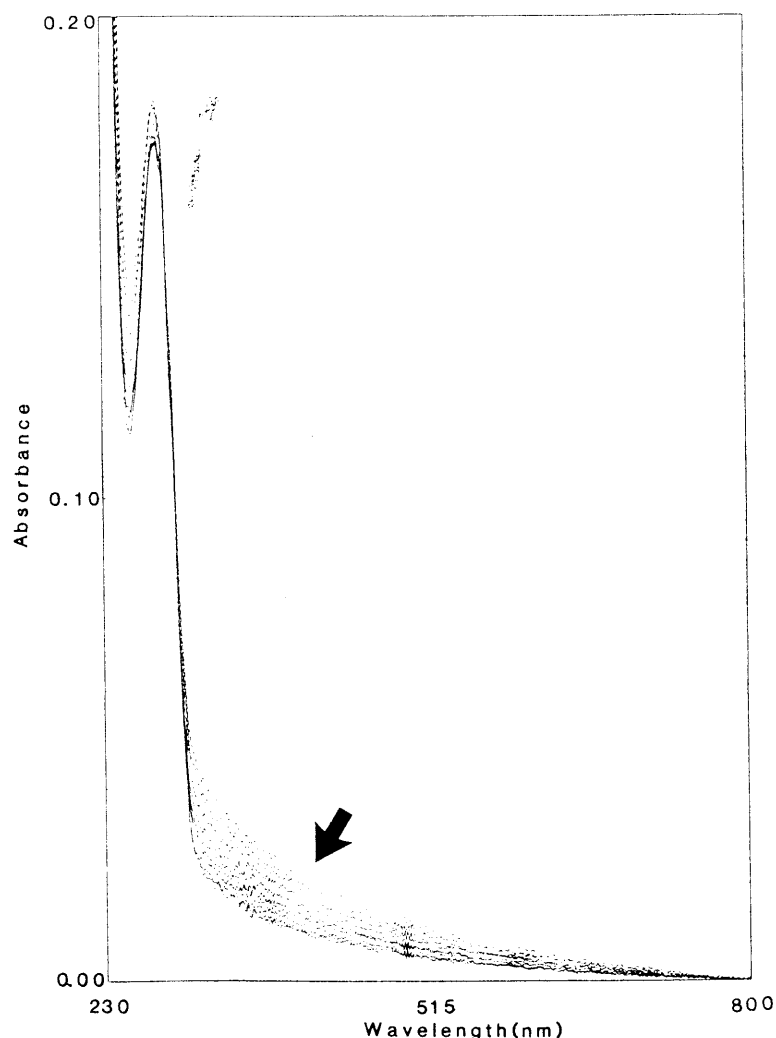


Fig. 1a. Absorption spectra of 2.0 mM of mAb LA2 in the presence of 0.0, 0.06, 0.38, 0.95, 1.76, 2.81, 4.68, 9.03, 15.80, 25.00 and 36.50 μ M of cycrimine in PBS at RT. Charge-transfer band is present in the 320–360 nm range (arrow).

10 sec. All spectral data were processed on a micro-computer dedicated to the SLM 8000. We have previously described our procedures for corrections of emission intensity due to dilution and photobleaching effects (Droupadi *et al.*, 1992, 1993).

Computer-aided molecular modeling (Camm)

A number of different computer algorithms were used in this investigation for Camm and graphics display the Fv models. The ABGEN algorithm, written in our laboratory, was used to select "parental structures" and generate a first-order Fv model; details and validation of the ABGEN algorithm, with sub-routines ABalign and ABbuild, have been presented elsewhere (Mandal *et al.*, 1993). For model refinement, the CHARMM algorithm (Brooks *et al.*, 1983) was used for potential molecular mechanics calculations. CONGEN (Brucoleri and Karplus, 1987) was used to generate the low energy conformations of the CDR loops.

Briefly, the procedure for Camm of the IgE Fv structures generally followed those described by Chothia and Lesk (1987) with modifications made by us (Mandal

et al., 1993). The program ABalign evaluates the size, sequence and location of important, canonical residues for each CDR loop and determines the degree of sequence homology with Ig loops in our library of known "parent" structures (Mandal *et al.*, 1993). The "parent" structure library consists of 20 Fab structures solved by X-ray crystallography at a resolution below 3.0 Å. We examined the entire V-region amino acid sequences for each chain of LA2 and LB4 to see that they contained residues known to create specific canonical loop conformations as identified in known structures (Chothia and Lesk, 1987).

The ABbuild program executes the construction of the H and L chain CDRs in the following order: L2, H1, L3, H2, H3, L1. This model building sequence first modifies the loops that are closest to the framework, thereby creating a stereochemical base upon which the remaining loops are modeled (Brucoleri *et al.*, 1988). The PDB files or redefined coordinate files containing the known "parent" Fab structures (Bernstein *et al.*, 1977) were edited to remove the constant domains of the H and L chain; the working file contained only the Fv structure

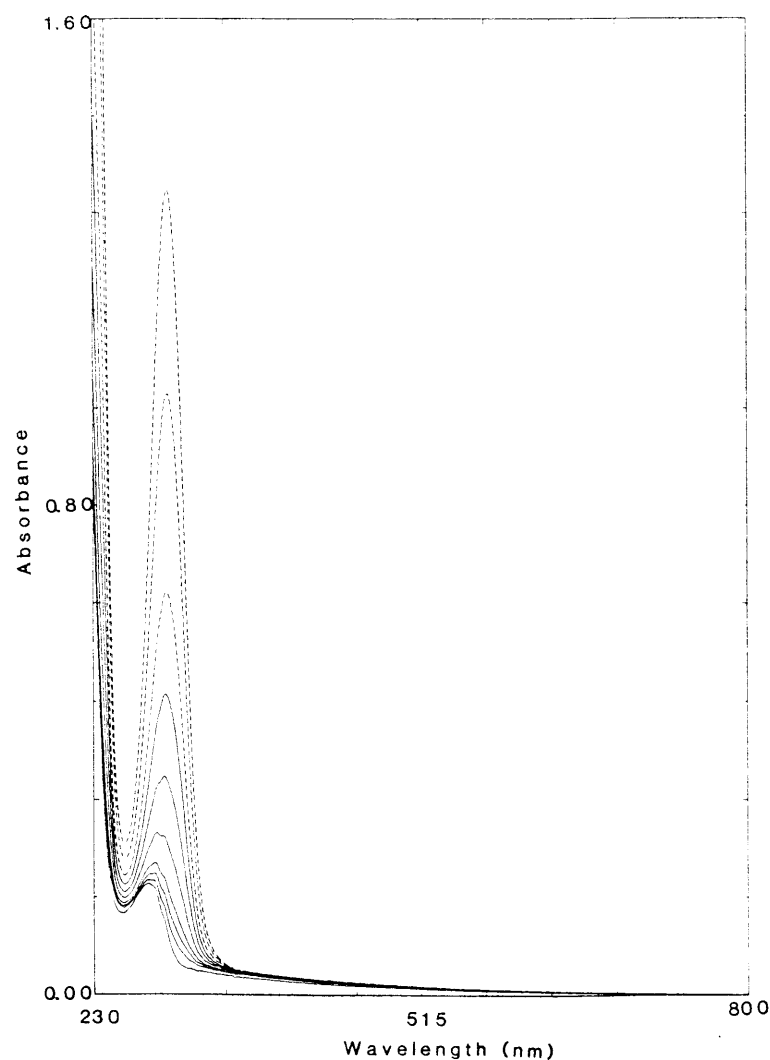


Fig. 1b. Absorption spectra of 2.0 mM of mAb LA2 in the presence of 0.0, 0.14, 0.83, 2.06, 3.38, 6.12, 10.20, 19.68, 34.50, 54.50 and 79.60 μ M of diaspirin in PBS at RT. No charge-transfer band is present in this mAb-ligand complex.

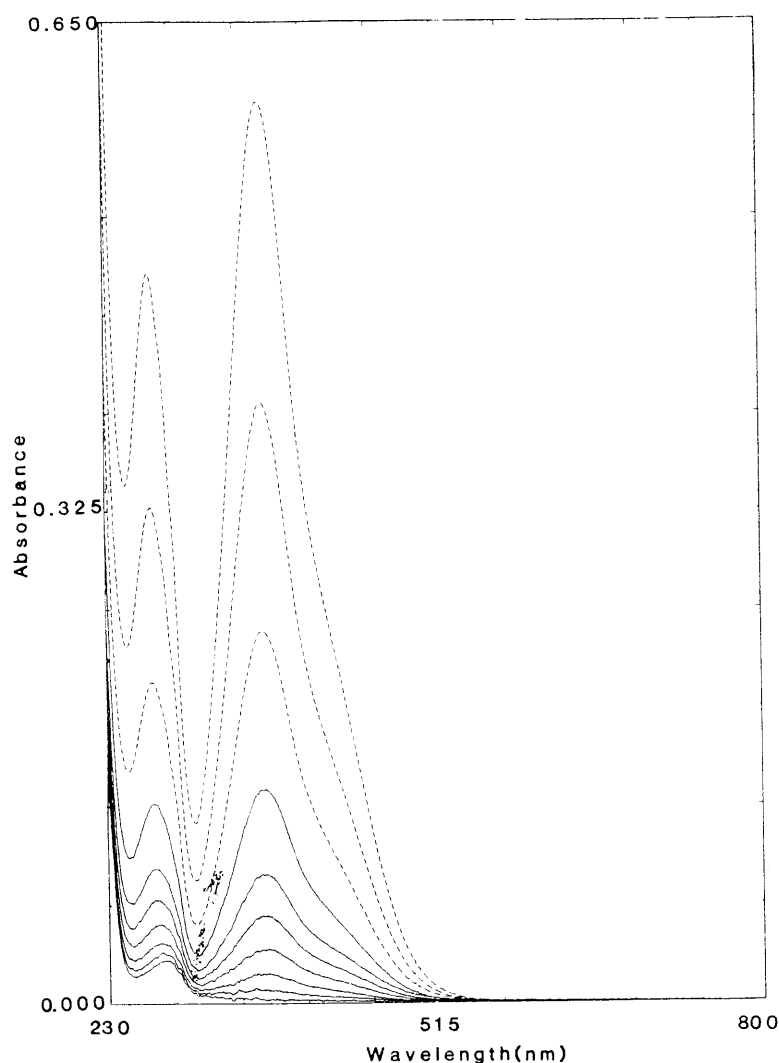


Fig. 1c. Absorption spectra of 2.0 mM mAb LB4 in the presence of increasing amounts (0.0, 0.29, 0.97, 2.00, 3.50, 5.25, 10.02, 18.6, 30.80 and 46.70 μ M) of DNP-tryptophan in PBS at RT. Increases in absorbance is due to the additional ligand. The charge-transfer bands cannot be identified due to this spectral overlap.

(V_L residues 1–107 and V_H residues 1–113). The H chain alpha carbons of the two parent structures were overlapped using a least square fitting technique. A file containing these two overlapping structures was edited to provide an Fv fragment composed of the hybrid L-chain and H-chain. This “hybrid Fv structure” served as the intermediate “parent” structure for the final Fv model building. No insertions, deletions or additions of amino acids in the parent loop structures were required for either LA2 or LB4 models (Table 1). Residue replacements were made by ABbuild and the ϕ , ψ angles were preserved at this step. After completion of each CDR loop, constraints were applied to the entire molecule, except for the side chains of the modeled loop, and this structure was subjected to CHARMM energy minimization (*vide infra*). Because polar hydrogens are not included in the PDB file, they were added to the Fv structure using the CHARMM-build hydrogen facility. Hydrogens were initially classified according to their respective environments and an optimum position was found by taking into account the energy contributed by the VDW, electrostatic and dihedral terms derived from

the full energy expression of CHARMM. The dihedral angle that yielded the lowest energy was chosen and the hydrogens were placed using this optimum dihedral angle.

Harmonic constraints were applied to fix the entire protein backbone structure and the Fv fragment was then subjected to CHARMM energy minimization, which consisted of 100 steps Steepest Descents, 100 steps Conjugate Gradient and 200 steps Adopted-Basis Newton-Raphson (Anchin and Linthicum, 1992). After final CDR loop construction, constraints were applied to all modeled loops and energy minimization was performed as before on the entire molecule. The CAMM studies were carried out on Silicon Graphics IRIS 4D210GTX or Indigo workstation; QUANTA 3.3 software (Molecular Simulations, Waltham, MA) was used for graphics display.

Surface renderings (NCView) and calculations (ACCESS)

Electrostatic surface renderings of the models were generated using the NCView algorithm. NCView uses

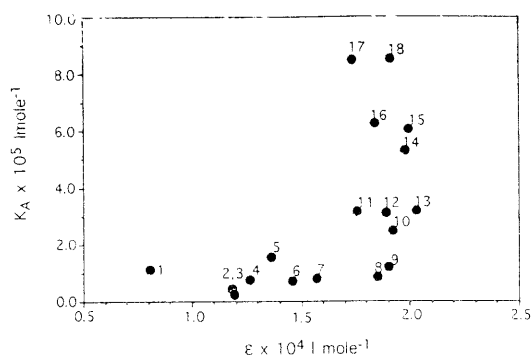


Fig. 2. A plot of K_A versus the molar extinction coefficient (ϵ) at 360 nm for binding to mAb LB4. Points 1–8 are dinitrophenol derivatives of the following L-amino acids: 1—arginine, 2—glutamic acid, 3—lysine, 4—DNP only, 5—proline, 6—tyrosine, 7—glycine, 8—asparagine, 9—glutamine, 10—leucine, 11—aspartic acid, 12—threonine, 13—serine, 14—methionine, 15—phenylalanine, 16—alanine, 17—tryptophan and 18—valine.

the van der Waals surface derived from the radii values compiled by Bondi (1964); the NCView surface contours were produced using the contour algorithm of Purvis and Culbertson (1986). The electrostatic potential was generated for each surface point using the point charge approximation. The Coulombic charges for each amino acid were obtained from a list of united-atom atomic charges for standard amino acids. The blue color represents the basic or positively charged amino acids (Lys, Arg, His) and red represents the acidic or negatively charged amino acids (Glu and Asp). Other colors (cyan, white and yellow) represent the charge gradient between these two extremes.

Solvent-accessible surface areas (SASA) were calculated according to the program ACCESS by Lee and Richards (1971), which uses a spherical probe of water ($r = 1.4 \text{ \AA}$) and calculates the contact area ($\text{\AA}^2$) on the van der Waals (VDW) surface of each atom that can be contacted by the probe.

RESULTS

The association constants (K_A) for a number of structurally different ligands was determined using spectroscopic techniques (Droupadi *et al.*, 1993). In Tables 1 and 2 the association constants for these ligands with mAbs LA2 and LB4 are presented. The association constants for most of the non-dinitrophenyl ligand were determined to be in the micromolar range, which agrees with earlier immunoassay determinations (Varga *et al.*, 1991). Three of these ligands, cycrimine, hemimellitic acid and diamox, showed a CT band at 320–340 nm upon complexation with mAb LA2. The absorption spectra for cycrimine complexed with LA2 is shown in Fig. 1a; the charge transfer band is visible in the 320–340 nm range (Fig. 1a, arrow). A similar band was observed with hemimellitic acid and diamox. In contrast, no CT band is visible for the complexation with diasprin (Fig. 1b) or endoiodine. The presence of CT spectra in anti-fluorescein antibodies has been attributed to the presence of tryptophan in the antibody binding site (Watt and Voss, 1977).

Absorption spectral changes for ligand complexation was also observed for ligands consisting of dinitrophenyl (DNP) covalently coupled to various amino acids (via the alpha or epsilon amino group to the C1 of the DNP). Changes in the absorption spectra of mAb LA2 in the presence of increasing amounts DNP–tryptophan are shown in Fig. 1c. We were unable to discern a CT band in this experiment because the 320–340 nm region is obscured by the overlapping absorption of DNP–Trp ligand. The intrinsic association constants for the interaction of mAbs LA2 and LB4 with different amino acid derivatives of DNP are listed in Table 2; the extinction coefficient at 360 nm for each ligand is also listed.

We observed that the K_A values for LB4 with different DNP–amino acid ligands could be divided into two groups, based on the molar extinction coefficient of the ligand. The intrinsic association constants for the

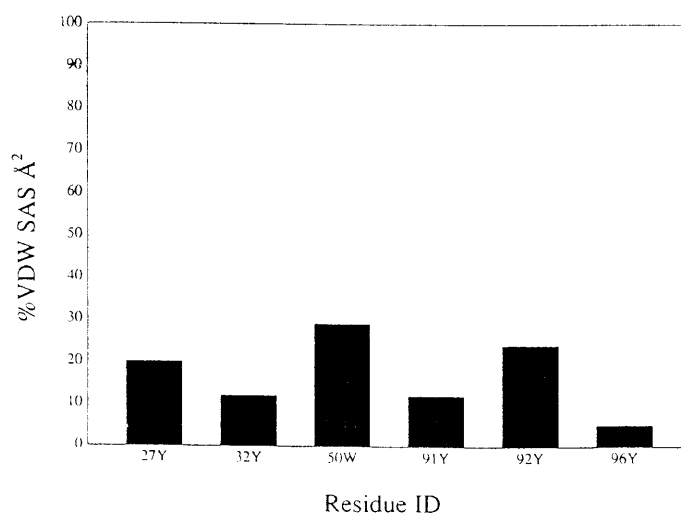


Fig. 3. The solvent accessibility of light chain aromatics for the Fv model of LA2 is calculated using the Lee and Richards algorithm ACCESS. The values shown represent the percentage of the van der Waals (VDW) solvent accessible surface area ($\text{\AA}^2$).

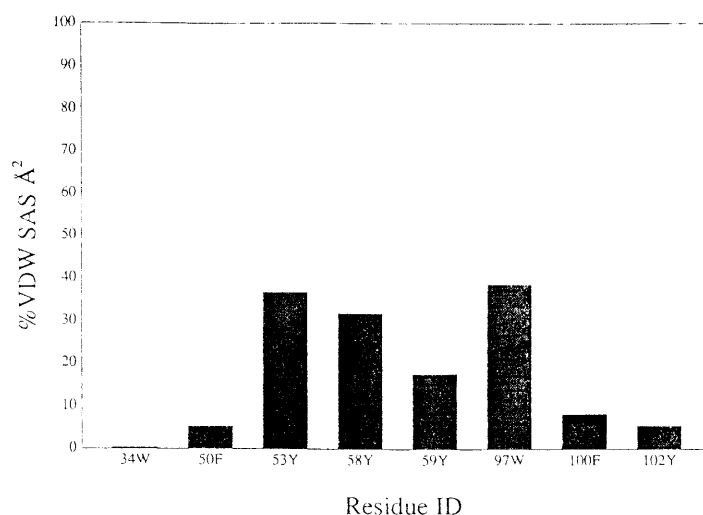


Fig. 4. Some of the heavy chain aromatics of the mAb LA2 have significant VDW solvent exposure according to the CAMM Fv model. Residues such as H:53Y, H:58Y and H:97W may participate in ligand complexation.

different ligands plotted against the molar extinction coefficients for the mAb LB4 are shown in Fig. 2. For some ligands (i.e. Nos 1–9, Fig. 2) the intrinsic association constants appears to have little correlation with increasing molar extinction coefficients. In contrast, a second set of ligands (Nos 10–18, Fig. 2) had a similar high extinction coefficient, but differed in their individual affinity values.

A number of the aromatic ligands examined in this study were found to have endogenous fluorescence properties in the 320–350 nm range and so it was impossible to examine mAb tryptophan related fluorescence quenching (330–340 range) due to the spectral overlap. However, with endoiodine, which is non-fluorescent, we found only 2% ligand-induced fluorescence quenching.

Table 1 lists the CDR residues for each mAb Fv model and the “parent” structures (known structures from the Brookhaven Protein Database) used for the CAMM. Only residue replacements were required for these Fv models. Both mAbs had very high sequence homology

with the parent structures. LA2 had 84% H-chain sequence homology with mAb 36–71 (Strong *et al.*, 1991) and 66% L-chain homology with McPC603 (Satow *et al.*, 1986). LB4 had 80% H-chain homology with HyHEL-10 (Padlan *et al.*, 1989) and 90% L-chain sequence homology with mAb 4-4-20 (Herron *et al.*, 1989). These parent structures served as the scaffolding for the FR and five CDR loops. Because the H3 loop is not canonical, we selected a CDR from different known structures, based on loop length and sequence homology. For LA2 we used the CDR H3 from D1.3 (Amit *et al.*, 1986); for LB4 the CDR H3 from 36-71 was used (Strong *et al.*, 1991).

Calculation of the theoretical van der Waals (VDW) solvent exposed surface area (SAS) as a percent of the total Å² for individual aromatic residues (W, Y and F) was completed using the ACCESS algorithm (Lee and Richards, 1971). The SAS areas of L-chain and H-chain aromatics for LA2 are shown in Figs 3 and 4. Several tyrosines and tryptophans have at least 30% of their

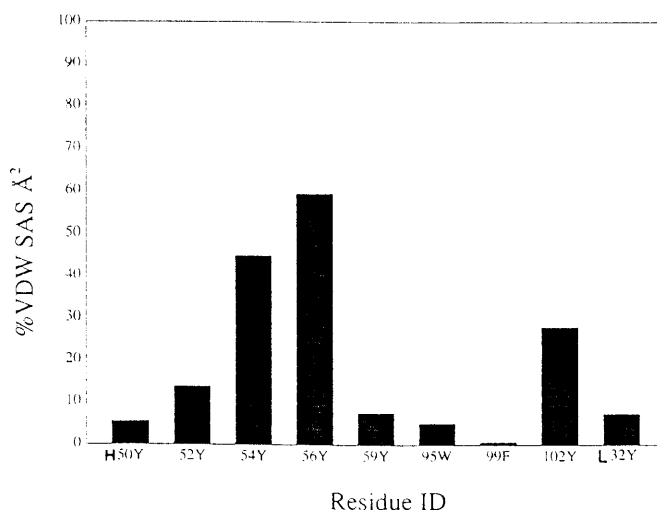


Fig. 5. The aromatics residues H:54Y, H:56Y and H:102Y of the LB4 H-chain have significant VDW solvent exposure according to the CAMM Fv model.

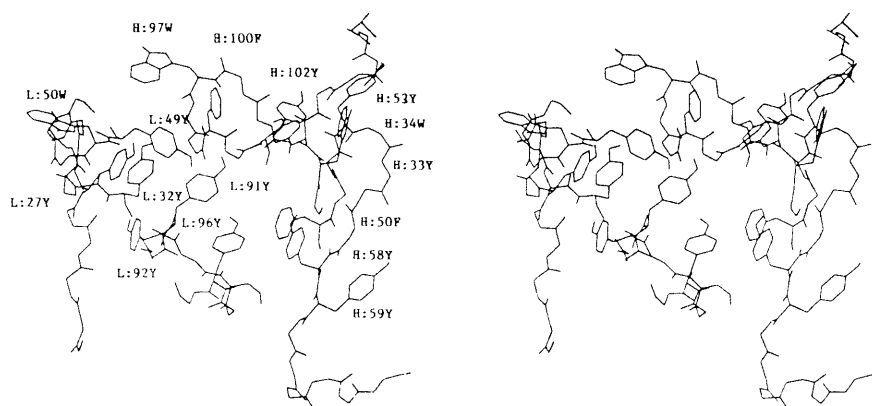


Fig. 6. Stereo view of the peptide backbone and key aromatic residues for the mAb LA2 Fv loop regions. Most of these aromatics are in the CDR.

surface solvent exposed in this model. In the model of LB4 (Fig. 5) two tyrosines (H:54 and H:56) have more than 45% solvent exposure. In several known antibody structures (e.g. HyHEL-10 and D1.3) residues in positions H:54 and H:56 are known to contact antigen (Amit *et al.*, 1986; Padlan *et al.*, 1989). It is also known, however, that other aromatics, such as H:33W are in contact with antigen (e.g. HyHEL-5 and HyHEL-10) and yet this residue is deep in the binding site and lacks solvent exposure (Sheriff *et al.*, 1987).

Figure 6 shows a stereo drawing of the peptide backbone (*en face* view) with aromatic residues in the binding site of mAb LA2. There are three W, two F and 11 Y shown. Although several of these are considered FR residues (e.g. L:32Y, L:49Y, H33), some of these positions contact antigen in known structures (e.g. L:49Y in D1.3). Figures 7a and b depict *en face* stereo views of the Fv model VDW surface for the LA2 and LB4 models with a color rendering that identifies the local charged surface areas. Blue colors represent residues with positive charge (e.g. R, H or K), while red areas represent residues with a negative charge (e.g. D or E). Note the absence of electrostatic charge in the binding site for LA2 (Fig. 7a, white colored area, center). The model of LA2 has less surface charge in and around the binding pocket than that calculated in the model of LB4; this is probably due to the presence of glutamic acids (H:58 and H:61) in CDR H:2 for LB4 (Fig. 7b, shown in red). The aromatic residues do not register a color coding in this algorithm and therefore, their surface area is rendered white. These models and calculations suggest that there are a number of specific aromatic residues in the binding site that may participate in ligand recognition and complexation.

DISCUSSION

According to current hypotheses, the molecular complexation between IgE and antigen (allergen) usually demonstrates exquisite stereospecificity. The geometry of the IgE-antigen complex at equilibrium is primarily dictated by non-covalent interactions with a precise set of amino acid residues located in the antibody CDR

segments (Novotny *et al.*, 1983, 1989). These interactions are determined by contributions from different components of the total interaction energy (Morakuma, 1977). Certain types of side chains in the CDR segments, such as those capable of π - π electronic interactions (e.g. F, Y and W) or ionic interactions (e.g. D, E, R, K and H), provides highly favorable motifs for complexation with complementary sites of the antigen. The propensity for such amino acids to be located in antibody CDRs has been previously noted (Padlan, 1990; Mian *et al.*, 1991). In addition, some FR residues are known to participate in antigen recognition, but this usually involves contact with large protein antigens (Davies *et al.*, 1990).

The interactions between aromatic amino acid side chains in mAb binding sites and ligands can be examined using sensitive spectroscopic techniques (Lakowicz, 1991; Droupadi *et al.*, 1993). In the study presented here, we examined the binding interactions between structurally different ligands and two anti-trinitrophenyl IgE mAbs in order to understand the "multi-specificity" of these particular mAbs. Using spectroscopic techniques we were able to derive binding constants in the micromolar range, similar to those obtained by immunoassay techniques (Varga *et al.*, 1991). In addition, the spectroscopic data provided some insight into the nature of the ligand-mAb complexation.

For some ligands, we observed the presence of a CT band upon ligand complexation, suggesting participation of a mAb binding site tryptophan in ligand recognition. For example, a CT band was observed for the complex of LA2-cycrimine and it was different from the absorption spectra for LA2 or cycrimine alone. We have verified that the new absorption band is not an artifact due to light scattering. Antibody LB4 exhibited a similar absorption spectra upon complexation with cycrimine.

With the ligand endoiodine, which is non-fluorescent, we found only 2% ligand-induced fluorescence quenching, suggesting that this ligand does not interact with any mAb combining site tryptophan residues. In addition, the absence of a CT band in the absorption spectra for this complex supports this conclusion for the binding of this particular ligand. For the mAb NC10.8, which binds

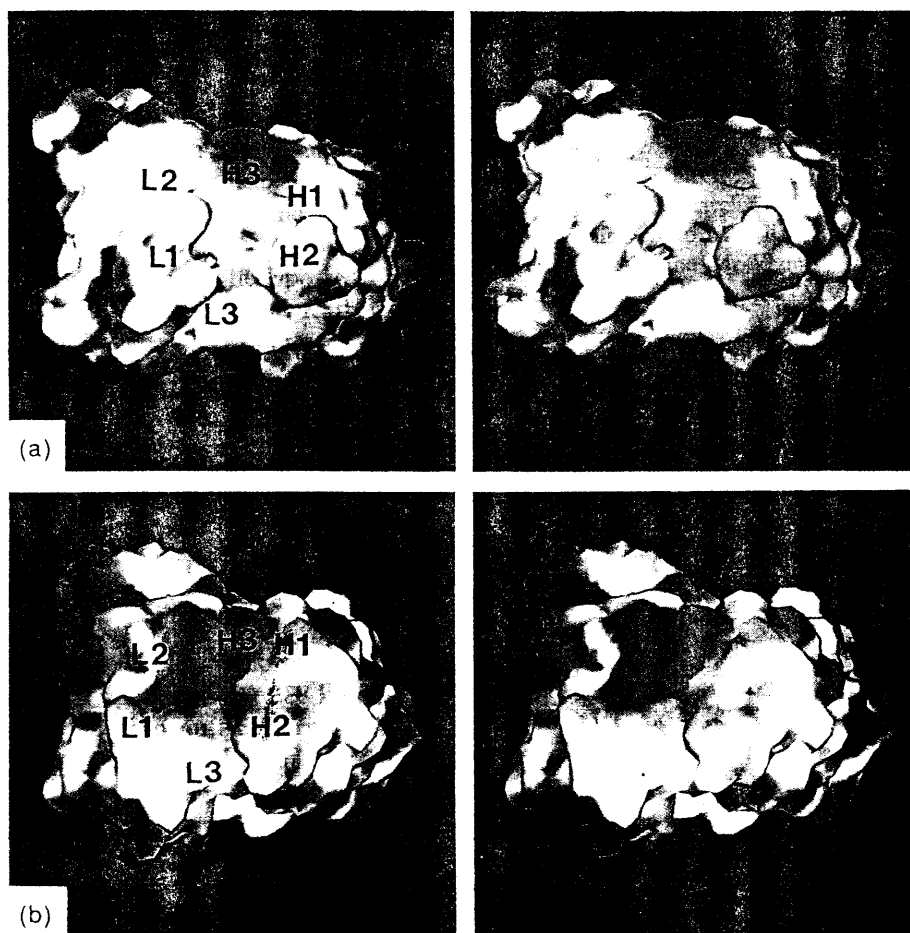


Fig. 7a. The *en face* stereo view of the VDW surface for the Fv model of LA2 is shown with an electrostatic rendering according to the Coulombic charge at the surface. The blue color represents the basic or positively charged amino acids (Lys, Arg, His) and red represents the acidic or negatively charged amino acids (Glu and Asp). Other colors (cyan, white and yellow) represent the charge gradient between these two extremes. The CDR surfaces are labeled as CDR H1, H2, H3, L1, L2 and L3.

Fig. 7b. Electrostatic surface rendering shows the charge at the VDW surface for the *en face* view of the Fv model of LB4. The color scheme and orientation are the same as in Fig. 7a.

a trisubstituted guanidinium, we have identified that the presence of a CT band was due to the π - π stacking of the aromatic ligand with a tryptophan in the light chain (L:96) as determined by site-directed mutagenesis of the Fab (Anchin and Linthicum, unpublished observations).

The interactions of LA2 and LB4 with several amino acid derivatives of DNP was examined and it was determined that ligands such as DNP-glutamic acid, DNP-asparagine, DNP-glutamine, DNP-tyrosine, DNP-arginine and DNP-lysine, which possess either polar or charged side groups, had lower K_A values as compared to more hydrophobic ligands, such as DNP-valine or DNP-phenylalanine. These observations suggest that the binding sites for these mAbs favor hydrophobic-ligand interactions.

The two IgE antibodies studied here possess an unusually high number of aromatic residues in their binding sites. For example, LA2 has two phenylalanines, three tryptophans and 11 tyrosines in the CDR and adjacent FR segments. The presence of these aromatics appears to contribute to their multi-specific ligand interactions. Binding interactions between different aromatic ligands and the W-Y-F-rich binding sites in these antibodies would be favorable due to multiple interactions between aromatics (*vide infra*).

In several families of binding proteins (e.g. immunoglobulins, MHC class I, streptavidin), there is a preponderance of solvent exposed aromatics that are involved in ligand recognition. Examination of 88 known antigen contacts in antibody Fab-antigen crystal structures reveals that 22 are tyrosine and eight are tryptophan (Mian *et al.*, 1991). In the HLA class I antigen, 50% of the residues that line the peptide recognition cleft are aromatic (Bjorkman *et al.*, 1987). High affinity ligation of biotin to streptavidin is due to a tryosine rich binding site (Weber *et al.*, 1989).

Aromatics such as tryptophan and tyrosine are favorable for ligand recognition because they are weakly polar, present large surface areas, participate in H-bonding, and can have electrostatic interactions such as those described above. In terms of ligand interaction, aromatics lose little conformational entropy (0.6 kcal at 300°K for every side chain torsion that is fixed) due to only one torsion angle (Atkins, 1978); aliphatics, on the other hand, have a negative entropy contribution to ligand binding. Aromatics (especially tryptophan) are capable of charge-transfer interactions, π - π stacking, H-bonding, large van der Waals contacts, and lose little entropy when fixed due to only one torsional angle (Cilento and Zinner, 1968). These aspects make aromatics highly favorable for multiple binding interactions.

Patients with IgE-mediated hypersensitivity reactions frequently display multiple sensitivities to a variety of related and apparently "unrelated" allergens (Moller and Dreborg, 1986; Barnett *et al.*, 1987). Such highly prevalent "multiple allergies" are paralleled by a high incidence of immunochemical cross-reactivity between structurally related and unrelated allergens (Urisu *et al.*, 1991). The cross-reactions may be explainable by: (a) the presence of common allergens in different allergen

preparations; (b) the shared antigenic or allergenic determinants on different allergens; (c) multiple sensitizations with different allergens giving rise to multiple sets of IgE antibodies; or (d) particular IgE antibodies that possess multi-specific allergen binding properties. Elucidation of the physicochemical nature of multi-specific IgE mAb may aid in our development of new diagnostic strategies for multiple allergic reactions and unanticipated anaphylactic reactions to drugs upon first exposure.

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