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CH/π Interactions in the Crystal Structure of Class I MHC Antigens and their Complexes with Peptides

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Abstract—The crystal structure of class I major histocompatibility complex antigens (MHC) bound to their specific ligand peptides were analyzed, in the context of the CH/π interaction, with use of a computer program CHPI. A number of short CH/C_{sp}^2 distances have been shown at the boundary of the heavy chain and β2 microglobulin. These interactions are conserved between species, human versus murine. A number of contacts shorter than the conventional van der Waals distance have been disclosed between CH hydrogens and aromatic side-chain groups in the MHC/peptide complexes. The CH/π interaction has been suggested to contribute to the specificity in the complex formation of class I MHC.

Introduction

Progress in biochemistry has disclosed a number of intriguing molecular phenomena in cell biology. Recent findings which relate to the cellular immune system, the intracellular signal transduction system and the transcription regulation system, among all, are probably the most significant. The higher-order structure of proteins relevant to these findings is rapidly being elucidated at the atomic level by the X-ray and NMR techniques. Therefore, the interactions involved should be analyzed at the same level of precision, or clarity. The CH/π interaction[1] is a kind of hydrogen bond occurring between CH and π-groups. Unlike the conventional hydrogen bonds, the CH/π interaction may play its role in protic media such as water, and by implication in living systems. Here we present evidence for the role of CH/π interaction in class I major histocompatibility complex antigens (MHC) and their complexes with specific ligand peptides.

Method

The method of exploring CH/π interactions in protein crystal structures was reported earlier.[1,2] Namely, a computer program (CHPI) was written to find contacts between CH groups and π systems (Fig. 1). To participate in a CH/π interaction, the hydrogen should be positioned above the π plane though not necessarily directly above a sp^2 atom. Several kinds of distance and angle parameters were defined to cover every possibility.[1–3]

The necessary coordinates were obtained from the Brookhaven Protein Data Bank (PDB). The exact positions of hydrogen atoms are available, from neutron diffraction studies of bovine pancreatic trypsin inhibitor (BPTI), ribonuclease A (RNase A), 2-Zn insulin and sperm whale myoglobin. The PDB data, however, do not ordinarily contain hydrogen coordinates. In these cases, hydrogen were generated on heavy atoms and their positions optimized.[4] Then, the H/π interatomic distances shorter than a cut-off value with stereochemically reasonable angle factors were collected. In this paper, the distance (D_{max}, see Fig. 1) shorter than 3.05 Å [= 2.9 Å (1.2 Å for C-H plus 1.7 Å for a half thickness of the aromatic molecule)[5] × 1.05] was considered as relevant for the presence of CH/π interaction. To validate our methodology, the data obtained by use of the generated hydrogen coordinates for BPTI (PDB code 5PTI, resolution 1.0 Å for X-ray, 1.8 Å for neutron diffraction)[6] were compared with those obtained by use of the hydrogen coordinates by a neutron diffraction study. Agreement of the results obtained by the above

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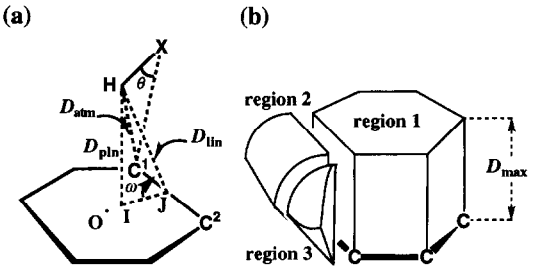

Figure 1. Method for exploring CH/π contacts in proteins. An example is given for a six-membered π-system. (a) O: centre of the plane. C^1 and C^2: nearest and second nearest sp^2-carbons, respectively, to H. ω: dihedral angle defined by C^1OC^2 and HC^1C^2 planes. θ: ∠ HXC^1. D_{pln}: H/π-plane distance (H/I). D_{atm}: interatomic distance (H/C^1). D_{lin}: distance between H and line C^1C^2 (H/J). (b) 1: region where H is above the aromatic ring. 2 and 3: regions where H is out of region 1 but may interact with π-orbitals. The program was run to search for H/π distance shorter than a cut-off value D_{max} in every region: $D_{pln} < D_{max}$, $\theta < 62.2°$, $|\omega| < 90°$ for region 1; $D_{lin} < D_{max}$, $\theta < 62.2°$, $90° < |\omega| < 127.5°$ for region 2, and $D_{atm} < D_{max}$, $\theta < 62.2°$, $\omega = 180° - \phi$ (ϕ: HC^1I), $90° < \omega < 127.5°$ for region 3.

two procedures was satisfactory,[7] at least in view of the present purpose of surveying CH/π interactions in the protein structure. A good agreement was also obtained for RNase A [5RSA[8] (2.0 Å by joint refinement of X-ray and neutron diffraction data) versus 7RSA[9] (1.26 Å by X-ray)].[10] In view of the limitation to the present methodology and the precision in the crystallographic determination of proteins, however, we do not pretend that the H/π interatomic distances thus estimated are very precise. Discussions regarding the protein structures by low-resolution studies remain qualitative.

Results

The molecules analyzed here are class I MHC antigens and their complexes with ligand peptides. The MHC antigens[11] are cell surface glycoproteins which play an essential role in the cellular immune response. When a T cell receptor binds a foreign peptide complexed with a class I MHC protein on the cell surface, the cytotoxic T lymphocytes recognize this as non-self and is activated to attack the cell. Class I MHC molecules are non-covalently associated dimers of two proteins: the heavy chain (α subunit: α1, α2 and α3 domains of ca. 90 residues each) and the β2-microglobulin (β subunit: ca. 100 residues). The α1 and α2 domains form a groove at the top portion of the protein; this groove is known to be responsible for the complex formation with various peptides.[12]

Overview

The α and β subunit of a class I human leukocyte antigen HLA-A2 are consisted of 275 and 99 amino acid residues, respectively. Wiley et al. determined the crystal structure of HLA-A2[13] and examined the structural features of the domains, the ligand-binding groove and the interface between the subunits. The structures of the domains were discussed, mostly in view of conserved salt-bridges or hydrogen bonds that contribute to the stability.

Figure 2 is a global view of HLA-A2 in complex with a ligand peptide (2CLR, 2.0 Å) showing the CH/π contacts. The numbers of aromatic residues (including histidine) in HLA-A2 are 45 and 17, respectively, in subunit α and β. The numbers of short CH/Csp^2 contacts are 152 (98 within the α subunit, 35 within the β subunit and 19 intermolecular contacts) in the complex (involving 39 and 16 aromatic residues in subunit α and β, respectively). It is remarkable that almost all aromatic residues are involved in CH/π interaction. Table 1 is an output of our CHPI analysis of the HLA-A2/peptide complex (only intermolecular interactions are given).[15]

Interaction at the subunit interface

The β subunit (β2 microglobulin) of MHC has been known to stabilize the structure of class I heavy chain; human β_2m not only binds to α subunit of murine MHC, but also increases its thermal stability.[16] Importance of the nonpolar interactions at the subunit interface has been suggested. Thus Wiley et al. reported on the contribution of aromatic residues such as Tyr10, Phe56 and Trp60 in stabilizing the heterodimer, by forming 'van der Waals contacts'[17] of β2 microglobulin with the α subunit.

Table 2 summarizes the results of CHPI analyses of the α/β boundary of various class I molecules (HLA-A2, HLA-B27,[18] HLA-B8,[19] murine $H\text{-}2K^b$,[20] $H\text{-}2D^b$).[21] A number of CH/π contacts are disclosed between aromatic side-chain groups of the β subunit and CHs in the α subunit: Tyr10β/Arg234α, Tyr26β/Pro235α, His31β/Gly120α (human MHC only), Phe56β/Phe8α, Phe56β/Gln96α and Trp60β/Ala117α. The murine β2 microglobulin has ca. 70% homology in the sequence with that of human. Note that the above CH/π interactions are conserved, human as well as murine MHC, except for His31β/Gly120α. Wilson et al. reported that the residues engaged in hydrogen bondings at the boundary are not well conserved; of the 21 hydrogen bonds at the α/β interface in $H\text{-}2K^b$, only 7 were found in HLA-A2.[22]

Peptide binding

Class I human leukocyte antigen HLA-A2. Wiley et al. determined the crystal structure of HLA-A2 in complex with the N-terminal signal sequence of calreticulin

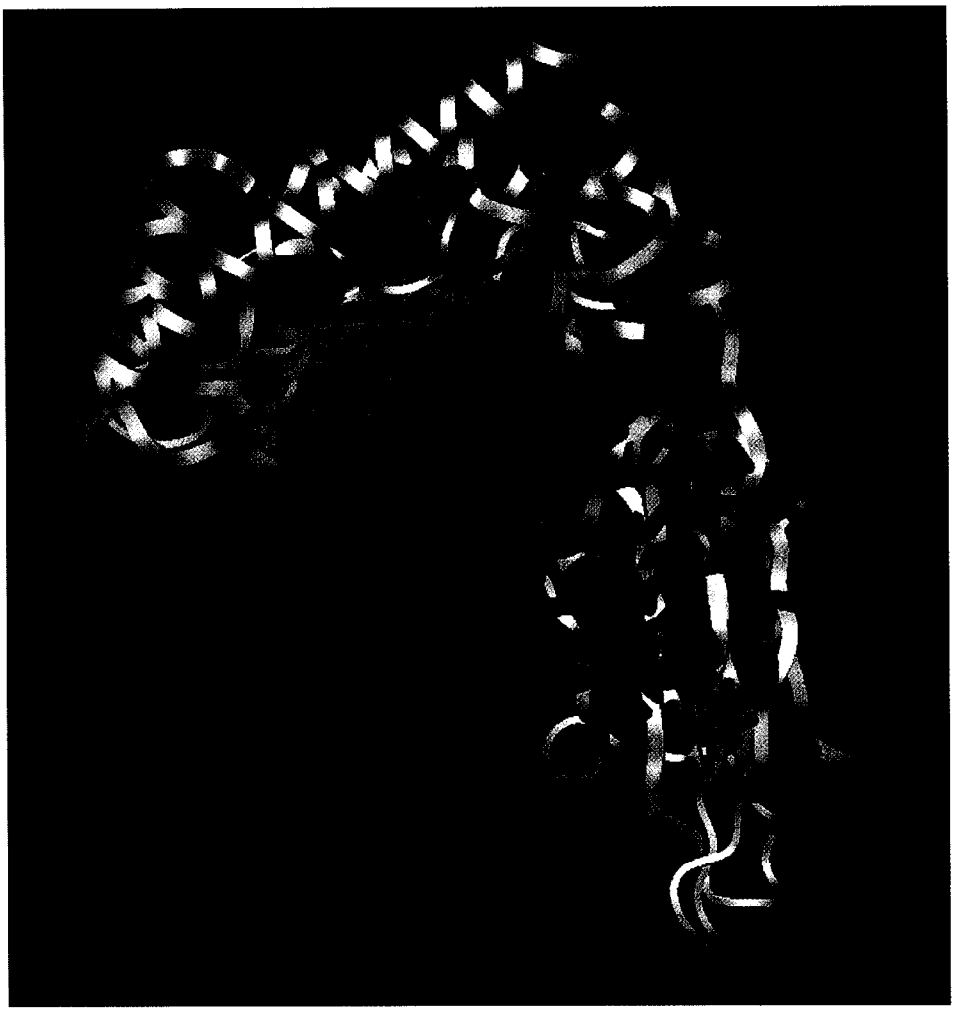

Figure 2. Global view of HLA-A2 in complex with a ligand decapeptide Met-Leu-Leu-Ser-Val-Pro-Leu-Leu-Leu-Gly (2CLR). White, purple and red ribbons indicate the heavy chain, β2-microglobulin and the ligand peptide, respectively. Yellow and green sticks indicate the intermolecular and intramolecular CH/π contacts, respectively.

MLLSVPLLLG (2CLR, 2.0 Å; M, methionine; L, leucine; S, serine; V, valine; P, proline; G, glycine). Figure 3 is a close-up view, at the entrance of the ligand-binding site, of HLA-A2 in complex with the peptide. Only three N-terminal peptide residues (P1-P3) are shown for clarity. We see eight intermolecular CH/π interactions (three×Trp167/P1-Met, Tyr7/P2-Leu, two×Tyr99/P3-Leu, two×Tyr159/P3-Leu), beside many hydrogen bonds. Intramolecular CH/π interactions are seen between Tyr59 and Glu63 (Cζ/Hγ, 2.8 Å), Trp167 and Tyr59 (Cζ3/Hε1, 3.0 Å) and Tyr171 and Trp167 (Cζ/Hβ, 3.0 Å; Cε2/Hε3, 2.6 Å). The above CH/π interactions form an extensive network, in cooperation with hydrogen bonds, to suggest the molecular basis of the strong affinity of HLA-A2 with the peptide.

Wiley et al. also determined the crystal structure of HLA-A2 in complex with various viral peptides:[23] HIV-1 reverse transcriptase residues 309-317 ILKEPVHGV (1HHJ, 2.5 Å; I, isoleucine; K, lysine; E, glutamic acid; H, histidine); HTLV-1 Tax residues 11–19 LLFGYPVYV (1HHK, 2.5 Å; F, phenylalanine; Y, tyrosine); influenza A virus matrix M1 residues 58–66 GILGFVFTL (1HHI, 2.5 Å; T, threonine); HIV-1 gp120 residues 197–205 TLTSCNTSV (1HHG, 2.6 Å; C, cysteine; N, asparagine) and hepatitis B virus nucleocapsid residues 18–27 FLPSDFFPSV (1HHH, 3.0 Å; D, aspartic acid). It was reported that interactions (nonpolar as well as hydrogen-bonding) are distributed mostly at the entrance of the groove. Thus Trp167 is placed at the entrance to accommodate the N-terminal

Table 1. Computer output of the CHPI analysis of HLA-A2 in complex with peptide MLLSVPLLLG (2CLR, 2.0 Å)[a]

H/π interaction

RES	K	L	VPI	1	2	3	4	5	6
HIS	1	1	FIV	CG	ND1	CE1	NE2	CD2	
PHE	1	1	SIX	CG	CD1	CE1	CZ	CE2	CD2
TYR	1	1	SIX	CG	CD1	CE1	CZ	CE2	CD2
TRP	1	1	FIV	CG	CD1	NE1	CE2	CD2	
TRP	1	2	SIX	CE2	CD2	CE3	CZ3	CH2	CZ2

Range	2.00	<	DMAX	<	3.05
Range	−127.50	<	OMEGA	<	127.50
Range	0.00	<	THETA	<	62.20

Intermolecular H/π interactions

π						HX				Geometry					
IDRD	RES	KL	VPI	VATM	N	IDRD	RES	VATM	N	DATM	DPLN	DLIN	OMEGA	THETA	RG
A7	TYR	11	SIX	CD1	2	C2	LEU	HD2	19	2.88	[2.80]	2.85	79.41	46.44	1
A99	TYR	11	SIX	CZ	4	C3	LEU	HB	11	[2.79]	2.61	****	110.75	49.20	3
A99	TYR	11	SIX	CE1	3	C3	LEU	HB	12	2.83	2.77	[2.80]	98.31	37.07	2
A116	TYR	11	SIX	CD2	6	C9	LEU	HD2	17	2.87	[2.71]	2.81	75.05	18.88	1
A147	TRP	12	SIX	CZ2	6	C7	LEU	HD1	16	2.72	2.56	[2.67]	106.55	26.31	2
A147	TRP	11	FIV	NE1	3	C7	LEU	HD2	18	2.81	2.52	[2.81]	116.00	40.78	2
A159	TYR	11	SIX	CE1	3	C3	LEU	HA	10	2.75	2.66	[2.71]	100.23	33.08	2
A159	TYR	11	SIX	CD2	6	C3	LEU	HD1	16	2.76	2.58	[2.68]	105.91	25.29	2
A167	TRP	11	FIV	CD2	5	C1	MET	HB	14	3.05	3.01	[3.04]	98.26	35.32	2
A167	TRP	12	SIX	CZ2	6	C1	MET	HE	17	[3.01]	2.85	****	108.42	57.89	3
A167	TRP	12	SIX	CZ2	6	C1	MET	HE	19	3.01	[2.89]	2.96	77.32	58.05	1
B10	TYR	11	SIX	CE1	3	A234	ARG	HD	19	2.62	[2.60]	2.60	87.34	23.60	1
B26	TYR	11	SIX	CG	1	A235	PRO	HD	13	2.87	[2.71]	2.79	76.73	25.19	1
B31	HIS	11	FIV	CE1	3	A120	GLY	HA	7	[2.79]	2.43	****	119.22	55.78	3
B56	PHE	11	SIX	CE1	3	A8	PHE	HB	14	2.47	2.42	[2.46]	101.04	10.59	2
B56	PHE	11	SIX	CE2	5	A8	PHE	HD1	16	2.70	2.59	[2.69]	105.84	24.01	2
B56	PHE	11	SIX	CG	1	A96	GLN	HE2	16	2.46	[2.37]	2.39	84.21	30.22	1
B60	TRP	11	FIV	CE2	4	A117	ALA	HB	9	2.55	[2.45]	2.46	84.33	42.02	1
B60	TRP	12	SIX	CD2	2	A117	ALA	HB	10	2.73	[2.67]	2.69	82.64	51.09	1

[ ]:H/π distance

Number of H/π interactions: 19

[a]Only intermolecular interactions are listed. A: α chain. B: β chain. C: ligand.

peptide residue (P1). The P3 side-chain is close to two tyrosine residues, Tyr99 and Tyr159.

Table 3 summarizes the results of our CHPI analyses of 2CLR, 1HHJ, 1HHK and 1HHI.[24] Contacts Trp167/P1 (Met, Ile, Leu, Gly) and Tyr7/P1 (hydrogen bond) have been found in all complexes. Every aromatic residue reported as responsible for the ligand binding is CH/π-interacted with the side-chain of the peptides. Thus the aromatic ring of Tyr7 is in contact with CHs in the peptide residue at P2 (Leu, Leu, Leu or Ile). The aromatic rings of Tyr99 and Tyr159 are involved in CH/π interactions with the P3 residues (Leu, Lys, Phe or Leu).

In 1HHJ, 1HHK, 1HHG and 1HHH, Trp167 was reported to be in a similar geometry with respect to the P1 side-chain, whereas in 1HHI it is shifted to pack against the main chain of P1 glycine. Our CHPI analysis showed that CH/π bondings are formed in the former four complexes (P1 = Ile, Leu, Thr and Phe, respectively, for 1HHJ, 1HHK, 1HHG and 1HHH) with the use of hydrogens of the side-chain whereas in 1HHI they are replaced by interactions with Hα (CH/π) and NH (NH/π)[25] of the glycine.

HLA-B8. Jones et al.[19] reported the crystal structure of HLA-B8 in complex with residues 24–31 GGKKKYKL of the HIV-1 Gag protein p17 (1AGD, 2.05 Å) and compared this to four complexes obtained with variant epitope sequences GGKKKYRL (1AGE, 2.3 Å), GGKKKYQL (1AGC, 2.1 Å; Q, glutamine), GGKKRYKL (1AGF, 2.2 Å) and GGRKKYKL (1AGB, 2.2 Å; R, arginine).

Table 2. CH/π interactions at the subunit interface of class I MHC molecules

Residue	Atom	Residue	Atom	Distance (Å)
HLA-A2 (2CLR, 2.0 Å)				
Tyr10β	Cε1	Arg234α	Hδ	2.6
Tyr26β	Cγ	Pro235α	Hδ	2.9
His31β	Cε1	Gly120α	Hα	2.8
Phe56β	Cε1	Phe8α	Hβ	2.5
Phe56β	Cε2	Phe8α	Hδ1	2.7
Phe56β	Cγ	Gln96α	HNε2	2.5
Trp60β	Cδ2	Ala117α	Hβ	2.7
Trp60β	Cε2	Ala117α	Hβ	2.6
HLA-B27 (1HSA, 2.1 Å)				
Tyr10β	Cζ	Arg234α	Hβ	2.9
Tyr10β	Cε1	Arg234α	Hδ	2.9
Tyr26β	Cγ	Pro235α	Hδ	3.0
His31β	Nε2	Gly120α	Hα(1)	2.9
His31β	Nε2	Gly120α	Hα(2)	3.0
Phe56β	Cε2	Phe8α	Hβ	2.8
Phe56β	Cε1	Phe8α	Hδ1	2.6
Phe56β	Cζ	Gln96α	Hβ	2.9
Phe56β	Cδ2	Gln96α	HNε2	2.3
Trp60β	Cε2	Ala117α	Hβ	2.5
Trp60β	Cε3	Ala117α	Hβ	2.8
Asp98β	Hβ	His192α	Cε1	2.9
Met99β	Hα	Trp244α	Nε1	2.9
HLA-B8 (1AGD, 2.05 Å)				
Tyr10β	Cε2	Arg234α	Hγ	2.8
Tyr10β	Cε1	Arg234α	Hδ	3.0
Tyr26β	Cδ1	Pro235α	Hδ	2.8
His31β	Nε2	Gly120α	Hα	3.0
Phe56β	Cε2	Phe8α	Hβ	2.8
Phe56β	Cε1	Phe8α	Hδ2	2.7
Phe56β	Cζ	Gln96α	Hβ	2.9
Phe56β	Cδ2	Gln96α	HNε2	2.2
Trp60β	Cη2	Met98α	Hε	2.8
Trp60β	Cδ2	Ala117α	Hβ	2.8
Trp60β	Cε2	Ala117α	Hβ	2.6
H-2K^b (2VAA, 2.3 Å)				
Tyr10β	Cε1	Arg234α	Hδ	2.9
Tyr26β	Cδ2	Arg234α	Hβ	3.0
Tyr26β	Cζ	Pro235α	Hδ	2.8
His34β	Cε1	Val12α	Hγ1	2.8
Phe56β	Cζ	Phe8α	Hβ	2.7
Phe56β	Cδ2	Phe8α	Hδ2	2.6
Phe56β	Cε2	Gln96α	Hβ	2.9
Phe56β	Cδ1	Gln96α	HNε2	2.5
Trp60β	Cε3	Ala117α	Hβ	3.0
Trp60β	Cε2	Ala117α	Hβ	2.5
H-2D^b (1HOC, 2.4 Å)				
Tyr10β	Cζ	Arg234α	Hβ	2.7
Tyr10β	Cε1	Arg234α	Hδ	2.7
Tyr10β	Cδ2	Arg234α	Hδ	2.9
Tyr10β	Cγ	Arg234α	HNη1	2.3
Tyr26β	Cδ2	Pro235α	Hδ	3.0
Tyr26β	Cε2	Pro235α	Hδ	3.0
Phe56β	Cδ2	Phe8α	Hδ2	2.8
Phe56β	Cζ	Gln96α	Hβ	2.9
Phe56β	Cζ	Gln96α	HNε2	2.9
Trp60β	Cε3	Ala117α	Hβ	2.7
Trp60β	Cε2	Ala117α	Hβ	2.5
Asp98β	Hβ	His192α	Nε2	2.8
Met99β	Hα	Trp244α	Nε1	3.0

The results of CHPI analyses are listed in Table 4. The above five peptides have similar affinity with HLA-B8. In consistent with this observation, little difference has been noted, in the context of the CH/π interaction, on substitution of the residue at P3, P5 and P7. One of the glycine residues at P1 and P2 is CH/π-bonded either with Trp167 or Phe67 (Phe67 is replaced by valine in HLA-A2). Lysine or arginine side-chain at P3 is in extensive CH/π contact with Tyr99 and Tyr159. This is consistent with our earlier finding that lysine and arginine are effective in forming CH/π interaction with aromatic groups (lysine CH/π interaction).[1,2] Val152 seems to be effective in binding to aromatic residues (tyrosine in the above cases) at P6 with the use of its geminal methyl groups. This type of interaction is also found in 1HHJ (Val152/P7-His) and 1HHI (Val152/P7-Phe). Leucine at P8 effectively binds to the two tyrosine side-chains (Tyr116 and Tyr123) by use of its geminal methyl groups. Interactions Tyr116/P8-Leu and Tyr123/P8-Leu may be specific to HLA-B8 but this type interaction is found also in 2CLR (Tyr116/P9-Leu).

Class I murine MHC H-2K^b. The crystal structure was determined for a murine class I MHC molecule H-2K^b in complex with residues 52–59 RGYVYQGL of the vesicular stomatitis virus nucleocapsid protein (2VAA, 2.3 Å, 2MHA, 2.8 Å[26]), residues 324–332 FAPGNYPAL of the Sendai virus nucleoprotein (2VAB, 2.5 Å)[27] and residues 257–264 SIINFGKL of the ovalbumin (1VAC, 2.5 Å).[28] Table 5 lists CH/π interactions disclosed in the groove of H-2K^b. Interactions Trp167/P1, Tyr7/P2 and Tyr159/P3 are found in every complex. Tyr99 in HLA is replaced by serine in H-2K^b. Presence of aromatic residues (Tyr or Phe) at P3 and P5, and a bulky nonpolar residue (Leu) at P8 (or P9) was reported to be essential for the effective binding.[29] This preference was illustrated by a study where substitution by alanine at any of the above positions significantly reduced the binding ability.[30] In agreement with this observation, P3-Tyr and P5-Tyr are in contact with a terminal methyl group of Leu156 and Val9, respectively. The preference of leucine for P8 may be explained in terms of the interaction with Tyr116.

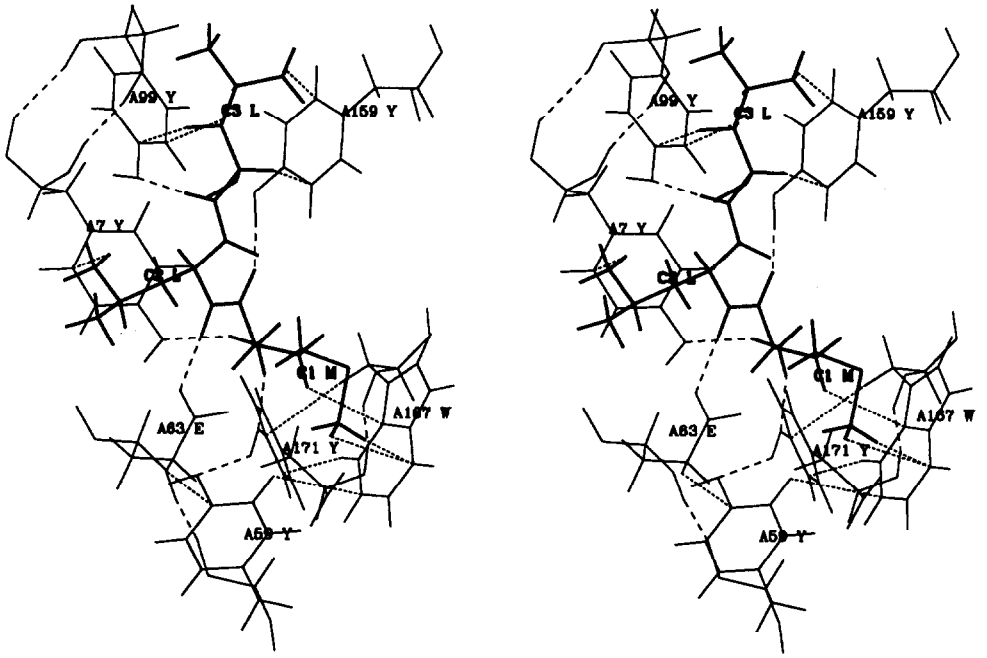

Figure 3. Close-up view of HLA-A2 in complex with a peptide MLLSVPLLLG (2CLR) at the entrance of the ligand-binding site (stereo). Y, tyrosine; L, leucine; M, methionine; E, glutamic acid; W, tryptophane. Thick lines: ligand (C1–C3). Thin lines: protein. Dashed and dotted lines indicate hydrogen-bonds and CH/π contacts, respectively.

Discussion

The CH/π interactions at the α/β interface involving aromatic residues (Tyr10β, Tyr26β, Phe56β and Trp60β) are conserved between the human and murine MHC molecules. It is interesting that residues involved at the boundary of the heterodimer are π-accepting in the α subunit, while in the β subunit they are aromatic.[31] As for the binding to peptides, the aromatic side-chain of Trp167 and Tyr7 have been shown to be in CH/π contact with the P1 and P2 residue, respectively. Interactions Tyr99/P3 and Tyr159/P3 are conserved throughout the class I HLA complexes, suggesting that they are common binders to the ligands.[32] The other CH/π interactions may be specific to the peptides.

Conclusion

To summarize, CH/π interaction has been shown to be important in a wide range of protein/ligand and protein/protein interactions.[33] These include the MHC molecules presently studied, haemoglobin,[34] enzymes and enzyme inhibitors,[35] neocarzinostatin,[36] G proteins[2] and SH2 domains.[2] The CH groups involved are α-CH, aliphatic CHs such as those in the side-chain group of alanine, valine, leucine, etc., lysine and arginine (lysine CH/π interaction), as well as aromatic CHs in phenylalanine, tyrosine and tryptophane (aromatic CH/π interaction). In other words, virtually every kind of CH group may participate.

Importance of the CH/π interaction has been suggested only recently.[37] The enthalpy of a one unit CH/π interaction is small. However, a unique feature of this force is that many CH groups may participate simultaneously in the interaction with a π-base. Total energy of the interaction will increase by organizing CHs and/or π-groups into a favorable chemical structure.[38] Another point, which is important when considering biochemical processes, is that the CH/π interaction can play its role in aqueous media as well as in nonpolar media. The ordinary hydrogen bond and Coulombic force tend to be weakened in a manner that is inversely proportional to the dielectric constant of the medium and are obscured in polar solvents. The groups participating in CH/π interaction are not dipolar; this type of interaction persists even in polar protic media such as water.[39] In view of this, we conclude that a considerable part of the nonpolar interactions, broadly attributed in the past to lipophilic forces such as the van der Waals interaction or the so-called 'hydrophobic effect' should now be re-examined in light of this new paradigm.

Table 3. CH/π interactions in class I human leukocyte antigen HLA-A2 complexed with ligand peptides

Residue	Atom	Residue	Atom	Distance (Å)
HLA-A2/MLLSVPLLLG (2CLR, 2.0 Å)				
Trp167	Cδ2	P1-Met	Hβ	3.1
Trp167	Cζ2	P1-Met	Hε(1)	3.0
Trp167	Cζ2	P1-Met	Hε(2)	3.0
Tyr7	Cδ1	P2-Leu	Hδ2	2.9
Tyr99	Cε1	P3-Leu	Hβ	2.8
Tyr99	Cζ	P3-Leu	Hβ	2.8
Tyr159	Cε1	P3-Leu	Hα	2.8
Tyr159	Cδ2	P3-Leu	Hδ1	2.8
Trp147	Cζ2	P7-Leu	Hδ1	2.7
Trp147	Nε1	P7-Leu	Hδ2	2.8
Tyr116	Cδ2	P9-Leu	Hδ2	2.9
HLA-A2/ILKEPVHGV (1HHJ, 2.5 Å)				
Tyr59	Cε2	P1-Ile	Hγ2	2.7
Trp167	Cδ1	P1-Ile	Hγ1	2.7
Trp167	Cε3	P1-Ile	Hγ2	2.8
Tyr7	Cδ1	P2-Leu	Hδ2	3.0
His70	Nε2	P2-Leu	Hδ2	2.8
Tyr99	Cζ	P3-Lys	Hβ	3.0
Tyr159	Cε2	P3-Lys	Hβ	2.9
His70	Cε1	P6-Val	Hγ2	2.5
Ala150	Hβ	P7-His	Nδ1	3.0
Val152	Hγ2	P7-His	Cγ	2.8
Tyr116	Cε1	P9-Val	Hδ1	3.1
HLA-A2/LLFGYPVYV (1HHK, 2.5 Å)				
Trp167	Cδ2	P1-Leu	Hβ	2.7
Trp167	Cη2	P1-Leu	Hδ1	2.8
Trp167	Nε1	P1-Leu	Hδ2	2.6
Tyr7	Cγ	P2-Leu	Hδ2	3.0
Tyr99	Cζ	P3-Phe	Hβ	2.8
Tyr159	Cε1	P3-Phe	Hα	2.9
Tyr159	Cε2	P3-Phe	Hβ	3.0
Tyr159	Cγ	P3-Phe	Hδ1	2.8
Leu156	Hδ2	P3-Phe	Cζ	3.2
Thr73	Hα	P8-Tyr	Cε2	2.8
Thr73	Hγ2	P8-Tyr	Cζ	2.7
HLA-A2/GILGFVFTL (1HHI, 2.5 Å)				
Trp167	Cδ1	P1-Gly	HN	2.6
Trp167	Cε2	P1-Gly	Hα	2.8
Tyr7	Cε1	P2-Ile	Hγ2	2.8
Tyr7	Cδ1	P2-Ile	Hγ2	2.8
His70	Cε1	P2-Ile	Hδ1	2.8
Tyr99	Cζ	P3-Leu	Hδ1	2.8
Tyr159	Cε2	P3-Leu	Hγ	2.9
Gln155	Hβ(1)	P5-Phe	Cζ	2.9
Gln155	Hβ(2)	P5-Phe	Cζ	3.0
Trp147	Cζ2	P7-Phe	Hδ1	2.7
Trp147	Cη2	P7-Phe	Hε1	3.0
Val152	Hγ1	P7-Phe	Cε2	3.0
Val152	Hγ1	P7-Phe	Cδ1	3.1
Leu156	Hδ1	P7-Phe	Cζ	3.0

Table 4. CH/π interactions in class I human leukocyte antigen HLA-B8 complexed with ligand peptides

Residue	Atom	Residue	Atom	Distance (Å)
HLA-B8/GGKKKYKL (1AGD, 2.05 Å)				
Phe67	Cζ	P2-Gly	Hα	2.9
Tyr99	Cε1	P3-Lys	Hβ	2.7
Tyr159	Cζ	P3-Lys	Hβ	2.6
Tyr159	Cδ2	P3-Lys	Hδ	2.8
Val152	Hγ1	P6-Tyr	Cδ1	3.0
Tyr116	Cε2	P8-Leu	Hδ1	2.7
Tyr123	Cε2	P8-Leu	Hδ2	2.6
HLA-B8/GGKKKYRL (1AGE, 2.3 Å)				
Phe67	Cζ	P2-Gly	Hα	2.8
Tyr99	Cε1	P3-Lys	Hβ	2.8
Tyr159	Cε2	P3-Lys	Hβ	2.7
Tyr159	Cδ2	P3-Lys	Hδ	2.8
Val152	Hγ1	P6-Tyr	Cδ1	2.7
Val152	Hγ2	P6-Tyr	Cδ2	3.0
Tyr116	Cεε	P8-Leu	Hδ1	2.8
Tyr123	Cε2	P8-Leu	Hδ2	2.8
HLA-B8/GGKKKYQL (1AGC, 2.1 Å)				
Trp167	Cδ1	P1-Gly	Hα	2.9
Tyr99	Cε1	P3-Lys	Hβ	2.9
Tyr99	Cε1	P3-Lys	Hβ	2.8
Tyr159	Cε2	P3-Lys	Hβ	2.7
Tyr159	Cγ	P3-Lys	Hγ	2.9
Tyr159	Cδ2	P3-Lys	Hδ	3.0
Val152	Hγ1	P6-Tyr	Cδ1	2.8
Val152	Hγ2	P6-Tyr	Cδ2	3.0
Tyr116	Cε2	P8-Leu	Hδ1	2.7
Tyr123	Cε2	P8-Leu	Hδ2	2.7
HLA-B8/GGKKRYKL (1AGF, 2.2 Å)				
Trp167	Cγ	P1-Gly	HN	2.8
Trp167	Nε1	P1-Gly	Hα	3.0
Tyr7	Cε2	P2-Gly	Hα	3.0
Tyr99	Cε2	P3-Lys	Hγ	2.9
Tyr159	Cε1	P3-Lys	Hβ	2.8
Tyr159	Cδ2	P3-Lys	Hδ	2.9
Tyr99	Cε1	P5-Arg	Hδ	2.8
Val152	Hγ1	P6-Tyr	Cδ1	2.9
Val152	Hγ2	P6-Tyr	Cδ2	2.9
Tyr116	Cδ2	P8-Leu	Hδ1	3.0
Tyr123	Cε2	P8-Leu	Hδ2	2.8
HLA-B8/GGRKKYKL (1AGB, 2.2 Å)				
Trp167	Cγ	P1-Gly	HN	3.0
Trp167	Nε1	P1-Gly	Hα	2.6
Tyr99	Cδ2	P3-Arg	Hδ	2.7
Tyr99	Cδ2	P3-Arg	HNη1	2.7
Tyr159	Cδ1	P3-Arg	Hβ	2.7
Tyr159	Cδ2	P3-Arg	Hδ	2.5
Val152	Hγ1	P6-Tyr	Cδ2	2.9
Tyr116	Cε2	P8-Leu	Hδ1	2.6
Tyr123	Cε2	P8-Leu	δ2	2.7

Table 5. CH/π interactions in class I murine MHC H-2K^b complexed with ligand peptides

Residue	Atom	Residue	Atom	Distance (Å)
H-2K^b/RGYVYQGL (2VAA, 2.3 Å)				
Trp167	Cγ	P1-Arg	Hβ	2.8
Trp167	Nε1	P1-Arg	Hδ	2.3
Tyr7	Cε2	P2-Gly	Hα	2.9
Tyr159	Cε2	P3-Tyr	Hβ	2.9
Tyr159	Cγ	P3-Tyr	Hδ1	2.7
Leu156	Hα	P3-Tyr	Cε1	3.0
Leu156	Hδ1	P3-Tyr	Cε2	3.0
Val9	Hγ2	P5-Tyr	Cζ	3.0
Phe74	Hε1	P5-Tyr	Cδ1	2.9
Trp147	Cδ1	P6-Gln	Hγ1	3.1
Tyr116	Cε2	P8-Leu	Hδ2	2.7
H-2K^b/FAPGNYPAL (2VAB, 2.5 Å)				
Trp167	Cγ	P1-Phe	Hβ	2.7
Lys66	Hζ	P1-Phe	Cε2	2.7
Tyr7	Cζ	P2-Ala	Hα	3.0
Tyr7	Cε2	P2-Ala	Hβ	2.7
Tyr159	Cε2	P3-Pro	Hβ	2.9
Trp147	Nε1	P7-Pro	Hβ	2.6
Trp147	Cζ2	P7-Pro	Hγ	2.7
Tyr116	Cε2	P9-Leu	Hδ2	2.9
H-2K^b/SIINFGKL (1VAC, 2.5 Å)				
Trp167	Cε2	P1-Ser	Hβ(1)	2.9
Trp167	Cδ1	P1-Ser	Hβ(2)	2.8
Tyr7	Cδ2	P2-Ile	Hβ	2.6
Tyr159	Cζ	P3-Ile	Hβ	2.9
Tyr159	Cγ	P3-Ile	Hδ1	2.9
Trp147	Cζ2	P8-Leu	Hδ2	2.6

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