Dynamic Characteristics of a Peptide-Binding Groove of Human HLA-A2 Class I MHC Molecules: Normal Mode Analysis of the Antigen Peptide–Class I MHC Complex

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Class I major histocompatibility complex (MHC) binds antigen peptides with various sequences. We performed a normal mode analysis of HLA-A2 MHC that binds three peptides with different affinity. HLA-A2 MHC has a peptide-binding groove composed of two α-helices (residue 49—84, residue 140—179). Some residues in the center of the groove showed an increase in fluctuations and some residue pairs between two helix groups showed a negative change in correlations by removing the antigen peptide. The extent of the fluctuation and correlation changes correlated well with the experimental ranking of the three peptides in binding affinity. Some definite anti-correlative motions were found between two helix groups in low frequency modes ($<50\,cm^{-1}$) by removing the antigen peptide. We propose that the above anti-correlative motions play an important role to bind the antigen peptide, especially in obtaining a "dynamic fit."

Key words major histocompatibility complex; peptide-binding groove; normal mode analysis; anti-correlative motions

Class I major histocompatibility complex (MHC) binds many short antigen peptides (9—11 amino acids) with diverse sequences derived from cytosolically degraded proteins, as part of the cytolytic immune response to defend the body against viral infection.[1—4] To bind all these peptides, class I MHC primarily interacts with the N- and C-termini.[5,6] Additionally, the peptides bound with high affinity typically have conserved amino acid side chains, termed anchors, that bind in specific pockets primarily composed of polymorphic residues in a peptide-binding groove of class I MHC.[7—11] The combination of amino acids that bind at the anchor position is known as the peptide-binding motif.[12—17] However, the above motifs are not sufficient to design a suitable peptide for binding because the binding affinity probably depends on sequence diversity.

We are interested in the dynamics of the peptide-binding groove of class I MHC. To analyze the conformational dynamics of a protein, two standard approaches are used: molecular dynamic calculations and normal mode analysis. The former is free from harmonic approximation, but is only suitable for events in a time frame of 100 ps or faster. Moreover, the larger the molecule, the more the time frame is limited. Thus, to describe the dynamics of large biomolecules, normal mode analysis has been adopted, although a harmonic surface potential is used.[18—25] In this study, to describe the dynamic characteristics of class I MHC, we performed a normal mode analysis on HLA-A2 MHC that binds three peptides with different affinity: RT (ILKEPVHGV) is derived from HIV reverse transcriptase (residue 309—317).[26] GP2 (IISAVVGIL) is derived from HER-2/neu (residue 654—662), and has a binding affinity weaker than that of RT despite the presence of peptide-binding motifs.[27] I1Y (YLKEPVHGV) has a Tyr substituted for the amino-terminal end (Ile) of RT, and higher affinity than RT.[28,29] A normal mode analysis of HLA-A2 MHC complexed with the peptide (the complexed structure) and MHC alone (the peptide-removed structure) was performed. In both structures, fluctuations and motional correlation coefficients were calculated, and the differences compared among peptide–MHC complexes.

Results

Fluctuation Changes in the Peptide-Binding Groove by Removing Antigen Peptides HLA-A2 MHC has two long α-helices (α1-helix group: residues 49—84, and α2-helix group: 140—179), which form a peptide-binding groove. The fluctuations of Cα atoms in the peptide-binding groove were compared among peptide-MHC complexes (Fig. 1). In all complexes, the profiles of fluctuations in all frequency modes were very similar to those in low frequency modes below $50\,cm^{-1}$ (data not shown). The number of low frequency modes below $50\,cm^{-1}$ ranged from 126 to 139 for both structures, and the ratio to total modes was *ca.* 7.5—8.5%.

First, fluctuation changes in α1-helix group by removing antigen peptides were compared. In RT–MHC, 16 residues (residue 57—63, 65—71, 73—74) showed an increase in fluctuations, at around P1—P4 (ILKE) and P6—P8 (VHG) of the peptide (Fig. 1a). In GP2–MHC, 18 residues (residue 53—55, 57—64, 66—70, 75—76) showed an increase in fluctuations, at around P1—P3 (IIS) and P6 (V) of the peptide (Fig. 1b). In I1Y–MHC, 18 residues (residue 60—69, 71—74, 76—77, 80—81) showed an increase in fluctuations, at around P1—P2 (IL), P4 (E) and P6—P9 (VHGV) of the peptide (Fig. 1c).

Second, fluctuation changes of α2-helix group by removing antigen peptides were compared. The profiles of fluctuations changes are quite different between complexes. In RT-MHC, 18 residues (residue 143, 145—157, 159—160, 169, 171) showed an increase in fluctuations, at around P1 (I), P3 (K), P5 (P) and P7—P9 (HGV) of the peptide (Fig. 1a). However, in GP2–MHC, only 7 residues (residue 147—152, 163) showed an increase in fluctuations, at around P7—P9 (GIL) of the peptide (Fig. 1b). On the other hand, in I1Y–

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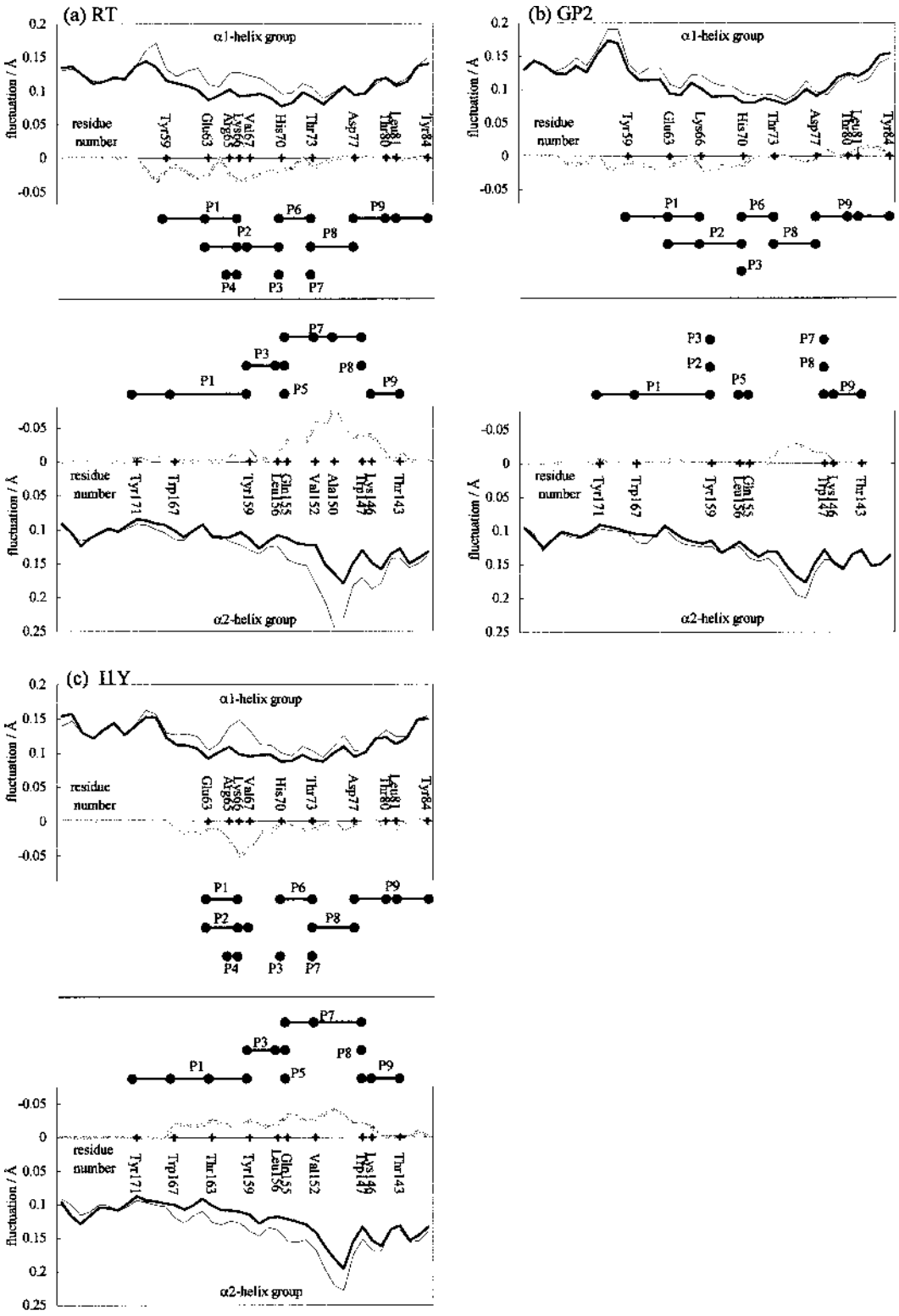

Fig. 1. Fluctuations of Cα Atoms of α1- and α2-Helix Groups in Low Frequency Modes below 50 cm^{-1}

The top and bottom of each figure shows fluctuations of the α1-helix group and α2-helix group, respectively. The thin black lines show fluctuations in the complexed structures and the peptide-removed, respectively. Significant differences by the Wilcoxon's rank sum test are shown by thick gray lines. A zero value indicates "no significant difference" by Wilcoxon's rank sum test. Black circles in each figure show residues of helix groups "close to" the nth residue (Pn) of the peptide (<4 Å) in the complexed structure: Upper side is residues of α1-helix group, lower side is residues of α2-helix group. (a) RT (ILKEPVHGV), (b) GP2 (IISAVVGIL), (c) I1Y (YLKEPVHGV).

MHC, 21 residues (residue 146—167) showed an increase in fluctuations, at around P1 (Y), P3 (K), P5 (P) and P7—P9 (HGV) of the peptide (Fig. 1c).

Each antigen peptide fluctuations in the complexed structure were compared among samples (Fig. 2). Each peptide had a rough common tendency that fluctuations of N-terminal (P1) and C-terminal side (P6—P9) were larger than other residues (P2—P5). However, in detail, the fluctuations of

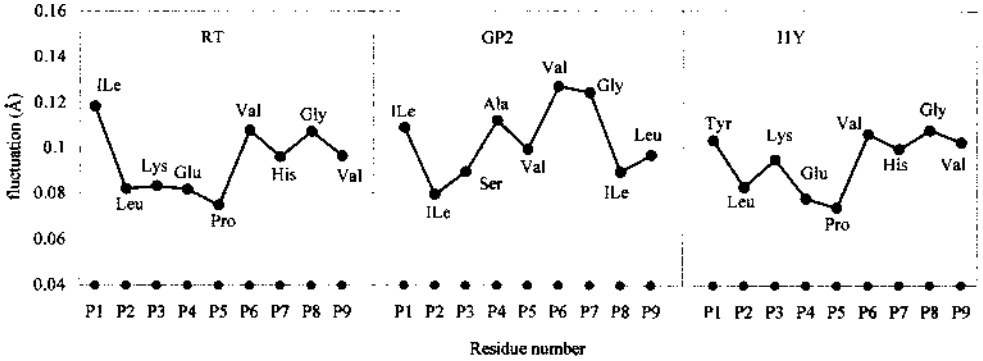

Fig. 2. Fluctuations of Cα Atoms of Antigen Peptides in All Frequency Modes in the Complexed Structure

P4—P7 (AVVG) in GP2 were markedly larger than those of P4—P7 (EPVH) in RT and I1Y, and I1Y had smaller fluctuations of P1 (Y) than P1 (I) in RT.

Motional Correlation Changes of α1- and α2-Helix Groups by Removing Antigen Peptides The motional correlation coefficients of Cα pairs between α1- and α2-helix groups were compared among the peptide–MHC complexes (Fig. 3). A positive correlation means they move roughly in the same direction, while a negative correlation means that a pair of atoms moves roughly in opposite directions. In each complex, the profiles of correlation coefficients in all frequency modes were very similar to those in low frequency modes below 50 cm^{-1} as seen for the profiles of fluctuations (data not shown). The profiles of correlative changes by removing antigen peptides were also characteristic for each peptide–MHC complex. In RT–MHC, residue pairs whose correlation coefficient changed negatively (difference >0.1) numbered 141, and were distributed around P2—P8 (LKEPVHG) (Fig. 3a). However, in GP2–MHC, they numbered only 64, and were limited to the N-terminal side of the peptide (P1—P4 (IISA)) (Fig. 3b). On the other hand, in I1Y–MHC, they totaled 185, and were distributed around P1—P8 (YLKEPVHG) of the peptide (Fig. 3c).

In all peptide-removed structures, in the center of the peptide-binding groove (α1-helix: 59—73, α2-helix: 147—159), residue pairs between the helices showed a negative correlation, and definite anti-correlative motions were found in low frequency modes below 50 cm^{-1} (Fig. 4). They appeared in all of six optimized structures about each peptide-removed structure.

Discussion

The above results are summarized in Table 1. In the peptide-binding groove, the number of residues whose fluctuation increased and residue pairs whose motional correlation coefficient changed negatively (difference >0.1) by removing antigen peptides correlated well with the experimental ranking of the three peptides in binding affinity.

GP2 is quite different from the other peptides for several reasons: First, in α2-helix group, residues whose fluctuation increased were limited to the C-terminal side (P7—P9 (GIL)) of the peptide. Second, the residue pairs whose correlation coefficient changed negatively were limited to the N-terminal side (P1—P4 (IISA)) of the peptide. Third, the fluctuations of P4—P7 (AVVG) in the complexed structure were markedly larger than those of P4—P7 (EPVH). Accordingly, the motional constrain of the center of GP2 (P5—P6 (VV)) with the peptide-binding groove is expected to be weaker than in the center of the others (PV). This is consistent with the result by Kuhns *et al.*[27] that the binding affinity of the GP2–MHC complex is weaker than that of the RT–MHC complex and the center of GP2 does not assume one specific conformation.

I1Y differs from RT in the following points: First, in α2-helix group, residues around P1 (Y) showed a more change in fluctuations compared with P1 (I) of RT. Second, residue pairs whose correlation coefficient changed negatively were extended around P1 (Y) of the peptide. Third, the fluctuations of P1 (Y) in the complexed structure were smaller than those of P1 (I) in RT. Accordingly, the motional constrain of P1 (Y) in I1Y is expected to be greater than that of P1 (I) in RT. This is consistent with the finding of Kirksey *et al.*[28] that the binding affinity of the I1Y–MHC complex is higher than that of the RT–MHC complex. In conclusion, the number of residues whose fluctuations increased and residue pairs whose motional correlation coefficient changed negatively by removing antigen peptides corresponds to the experimental ranking of the three peptides in terms of binding affinity.

Several motifs of peptide with a high binding affinity have been proposed. In the case of HLA-A2 MHC, P2 and P9 are considered anchor positions and certain preferred amino acids are offered at these positions.[13—16] The above motifs are based on the determined local and static crystallographic complex structures.[26,30—33] An MHC-bound peptide appears to be a highly complex function of its entire sequence, while the structural variation of MHC (especially main chain) is relatively limited.[26] However, some information about the dynamic behavior of MHC has been obtained. For example, it has been reported that the peptide-binding groove in the peptide-free state becomes unstable as the temperature increases compared with other domains of MHC.[34,35] Thus, the dynamic characteristics of MHC should be considered when designing the motifs, especially to explain the sequence variety at the center of the peptide, and the comparison between complex and peptide-removed structures is appropriate to discuss the dynamics because the fine structure of MHC (for example, the direction of side chain) is actually changed corresponding with each antigen peptide.

Generally, the dynamic characteristics of proteins are heavily influenced by low-frequency modes.[36] As shown by Fig. 4, definite anti-correlative motions in the center of the peptide-binding groove are a common characteristic of all

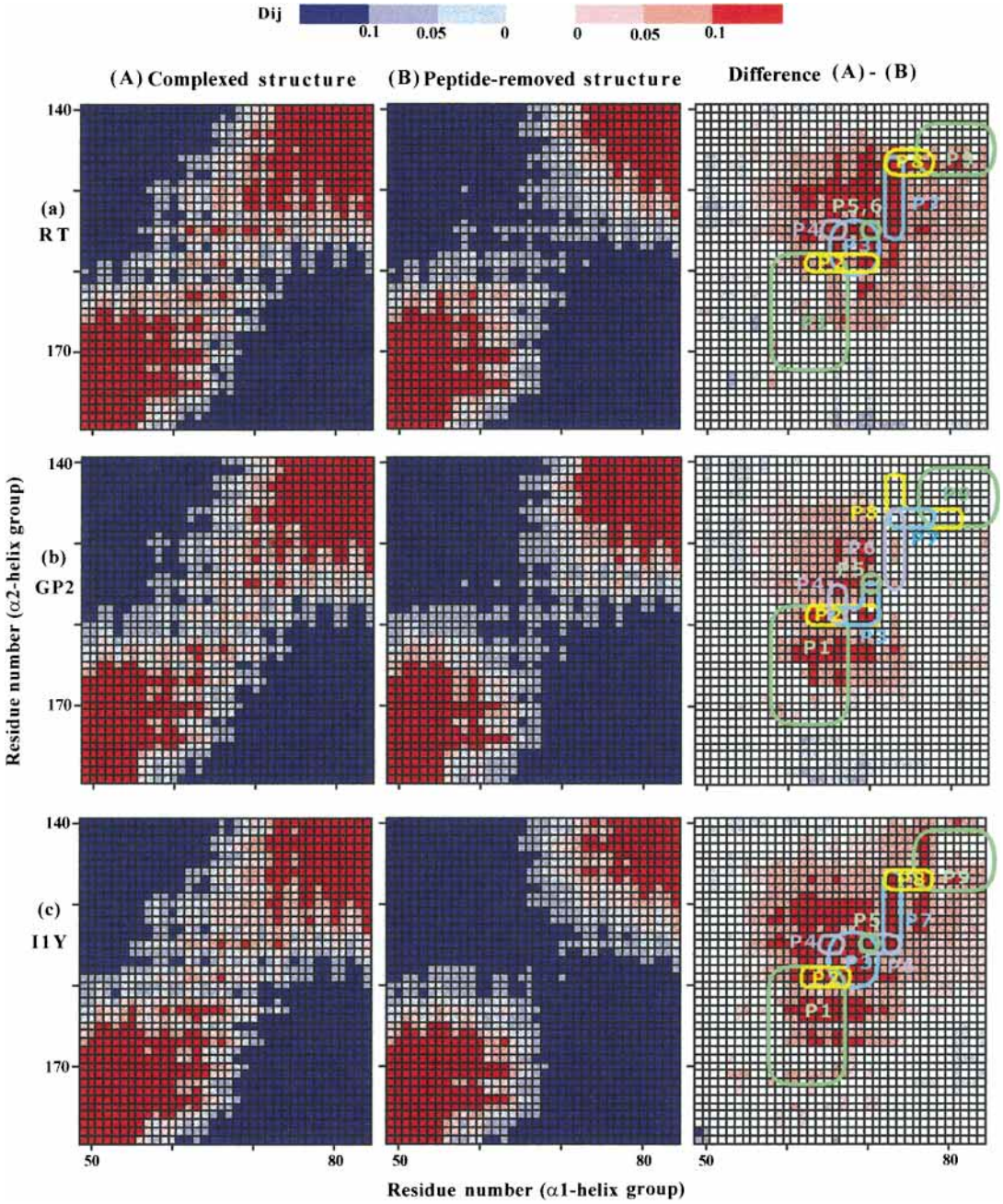

Fig. 3. Maps of Motional Correlation Coefficients of Cα Pairs between the α1- and α2-Helix Groups in the RT–MHC Complex in Low Frequency Modes below 50 cm^{-1}

Left (A) and center (B) lines show the complexed structure and the peptide-removed structure, respectively: In both lines, positive (Dij>0) and negative (Dij<0) correlation coefficients are shown in red and blue, respectively. The deeper the colors, the higher the absolute values. The right line shows significant differences between the structures. If Dij in the complexed structure is significantly different from Dij in the peptide-removed structure by Wilcoxon's rank sum test, the matrix is in color. Red shows the difference (Dij(A)−(B)) is positive (>0) and blue shows the difference is negative (<0), and no color means the difference is not significant by Wilcoxon's rank sum test. The top line (a) is RT, middle line (b) is GP2, and bottom line (c) is I1Y. Green, yellow, blue and purple circles in the right lines show the region including residue pairs "close to" Pn (n=1—9) of the peptide in the complexed structure. Here, "close to" means that the average of Pn—a residue (in α1-helix group) and Pn—a residue (in α2-helix group) is shorter than 4 Å except in the following case: the average of P5(Pro) and P6(Val) in RT is shorter than 5 and 4.5 Å, respectively, the average of both P5(Val) and P6(Val) in GP2 is shorter than 5 and 4.5 Å, respectively, and the average of P5(Pro) and P6(Val) in I1Y are shorter than 4.5 Å.

peptide-removed structures, and appeared in low-frequency modes below 50 cm^{-1}. There are total 195 residue pairs (15×13) in the center (α1: 63—77, α2: 147—159) of the peptide-binding groove. The above motions have negatively correlated (correlation coefficient <-0.5) residue pairs over 77% to the above total residue pairs. The correlation coefficients as shown by the complexed structure and the peptide-removed structure of Fig. 3 are average of all frequency modes below 50 cm^{-1}, therefore the threshold of the differences between both structures is small (0.1). However the definite anti-correlative motions as shown by Fig. 4 are influenced by removing antigen peptides, that is, in the peptide-removed structure the definite anti-correlative motions are increased and the definite correlative motions are decreased in low-frequency modes below 50 cm^{-1} (Fig. 5). This phenomenon is very similar to the report of Radkiewicz & Brooks that

the strong-coupled motions of DHFR disappeared by changing co-enzyme.[37] This also indicates that the definite anti-correlative motions are decreased by a "dynamic fit" of divers sequence of the peptide to the moving peptide-binding groove. In the complexed structure, GP2–MHC may have more definite anti-correlative motions in the center of the peptide-binding groove than other complexes because the sequence of the center of GP2 does not give a good dynamic fit.

In this study, we derived the correlation between the number of residues whose fluctuations increased and residue pairs whose motional correlation coefficient changed negatively (difference >0.1) by removing antigen peptides and the experimental ranking of three peptides in terms of binding affinity. To confirm this correlation, the examination of more diverse sequences, such as other peptides and mutants may be needed, but we propose that the anti-correlative motions play an important role to bind the antigen peptide, especially in obtaining a "dynamic fit" with the sequence of the peptide. The sequence diversity of the antigen peptide may be due to the dynamic flexibility of the peptide-binding groove. The peptide-binding motifs reported previously are not sufficient to rank the binding affinity of all peptides bound to MHC, but a more suitable peptide may be designed by evaluating the dynamics of the peptide-binding groove for the peptide-MHC complex.

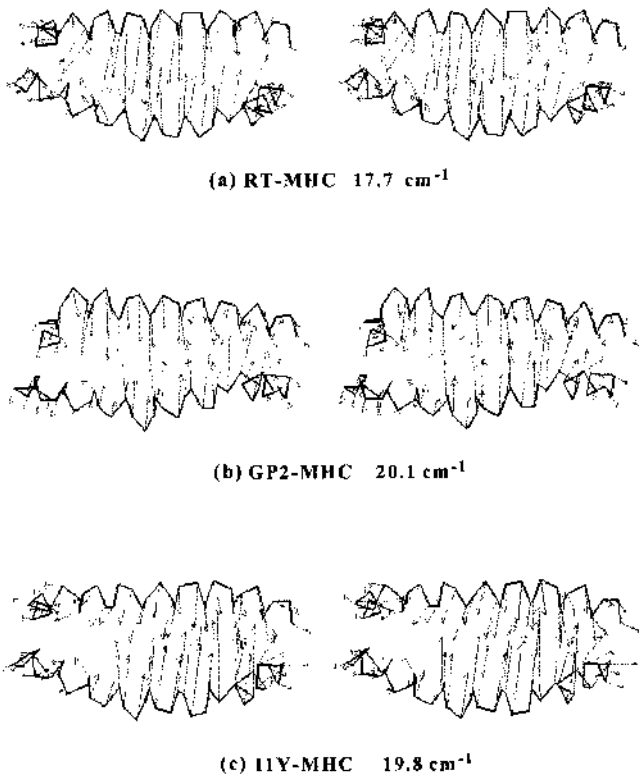

Fig. 4. Stereo Drawing of Cα Atomic Displacement Vectors in the Peptide-Removed Structure of Each Peptide–MHC Complex

Top and bottom show α1-helix group and α2-helix group, respectively. The displacement vectors are multiplied by 1000.

Experimental

Protein Structures X-Ray structures of HLA-A2 human class I MHC complexed with RT, GP2, and I1Y were obtained from the Brookhaven Protein Data Bank (PDB; PDB code 1HHJ,[26] 1QP1[27] and 1I1Y[28]). The A-chain (heavy chain: 275 residues), B-chain (β_2 microglobulin: 100 residues)

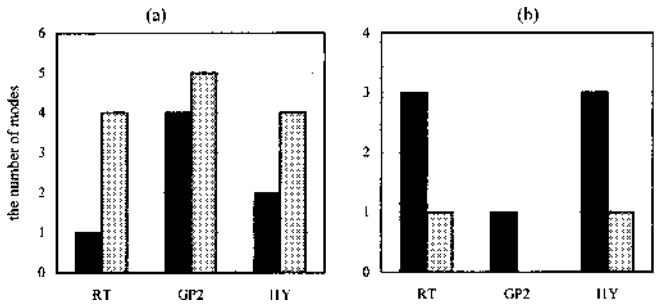

Fig. 5. Changes of Correlative and Anti-correlative Motions by Removing Antigen Peptides

(a) Shows the number of the mode having the anti-correlative motion in a local energy-minimum state. The anti-correlative motion have negatively correlated (correlation coefficient <-0.5) residue pairs over 77% to the total 195 residue pairs (15×13) in the center (α1: 63—77, α2: 147—159) of the peptide-binding groove. (b) Shows the number of the mode having the correlative motion in a local energy-minimum state. The correlative motion has positively correlated (correlation coefficient <0.5) residue pairs over 77% to the above total 195 residue pairs. Black and gray bars show the complexed structure and the peptide-removed structure, respectively.

Table 1. Summary of the Flutuation and Motional Correlation Changes by Removing Antigen Peptides

Peptide	Motion	Number[d]	Residue in the peptide related with motional changes of the peptide-binding groove[e]									Ranking[f]
			P1	P2	P3	P4	P5	P6	P7	P8	P9	
RT	Fluct.-α1[a]	17	I	L	K	E	—	V	H	(G)	—	
	Fluct.-α2[b]	17	(I)	—	K	—	P	—	H	G	V	Standard
	Corr.[c]	141	(I)	L	K	E	P	V	H	G	V	
GP2	Fluct.-α1	17	I	I	S	—	—	(V)	—	—	—	Lower than RT
	Fluct.-α2	7	—	—	—	—	—	—	G	I	L	(Kuhns *et al.* 1999)
	Corr.	64	I	I	S	A	—	—	—	—	—	
I1Y	Fluct.-α1	18	Y	L	—	E	—	(V)	H	G	V	Higher than RT
	Fluct.-α2	21	Y	—	K	—	P	—	H	G	V	(Kirksey *et al.* 1999)
	Corr.	185	Y	L	K	E	P	V	H	G	(V)	

a) Fluctuation of the α1-helix group. *b*) Fluctuation of the α2-helix group. *c*) Motional correlation coefficient. *d*) The number of residues (or residue pairs) whose fluctuations (or motional correlation coefficient) increased (or changed negatively (diference >0.1)) by removing antigen peptides. *e*) "Residue" is noted in the following conditions: (1) "P*n*" is close (<4.0 Å) to residues in the peptide-binding groove and (2) most of their fluctuations (or motional correlation coefficients) were increased (or changed negatively (diference >0.1)) by removing antigen peptides. "(residue)" is noted in the following conditions: (1) and (3) a part of their fluctuations (or motional correlation coefficients) were increased (or changed negatively) by removing antigen peptides. "—" is noted except for conditions (1), (2) or (3). *f*) Experimental ranking of three peptide in binding affinity.

and C-chain (peptide: 9 residues) of each PDB were used in the calculations for the complexed structure. The A and B chains of each PDB were taken as the MHC in the peptide-removed structure, and were used in these calculations.

Normal Mode Analysis The energy optimization was performed with a slightly modified force field of AMBER, and the normal mode analysis was done with torsion angles. For our calculation, we assumed that the molecules were *in vacuo*, but a distance-dependent dielectric constant (r/Å) for electrostatic energy was maintained.[38] The electrostatic potential and the van der Waals potential were cut off at 9.0 Å and were switched smoothly and continuously to a value of zero at 10.0 Å. Bond energy was imposed on the X-ray structure in the geometric optimization process and the restriction was gradually relaxed so that the normal mode analysis was executed near experimental coordinates including water molecules. A threshold of 0.04 kcal/mol Å for the maximal component of gradients of atoms was used. The structure is in a local energy-minimum state near the X-ray structure in the energy landscape. Six energetically optimized structures in both the peptide-removed and the complexed structures were obtained under various restriction conditions about each peptide-MHC complex. The normal mode analysis was carried out for these optimized structures. Fluctuations of Cα atoms and motional correlation coefficients between Cα pairs were calculated using our program assuming a temperature of 300 K.[39—41] The fluctuations and correlation coefficients in the peptide-removed and in the complexed structures were determined by averaging those of six optimized structures. Under Eckart's conditions,[42] the fluctuations and correlation coefficients of the MHC inner motions in the complexed structure were compared with those in the peptide-removed structure, and under the same conditions, the fluctuations of the peptide inner motions in the complexed structure were compared among peptides. To subtract the fluctuations and correlation coefficients in the peptide-removed structure from those in the complexed structure, significant differences were estimated by Wilcoxon's rank sum test, a nonparametric test.

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