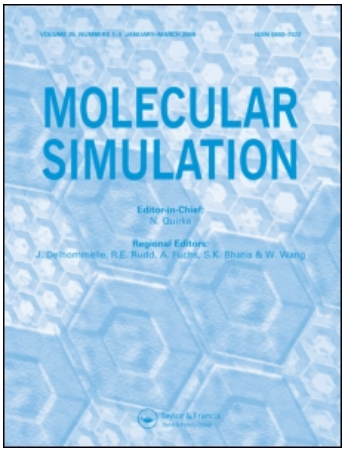

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Exploiting the Peptide — MHC Water Interface in the Computer-Aided Design of Non-Natural Peptides that Bind to the Class I MHC Molecule HLA-A2

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EXPLOITING THE PEPTIDE–MHC WATER INTERFACE IN THE COMPUTER-AIDED DESIGN OF NON-NATURAL PEPTIDES THAT BIND TO THE CLASS I MHC MOLECULE HLA-A2

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Class I major histocompatibility complex (MHC) molecules bind peptides derived from intracellular proteins and present them to cytotoxic T cells. Certain human immunological diseases are associated with errors in this process. Here we describe an approach to the design of non-natural peptides that could potentially interfere with peptide presentation associated with autoimmune diseases. We have shown previously that the interaction of the peptide GILGFVFTL with the MHC molecule HLA-A2 is mediated by a network of water molecules. In principle, the addition of hydroxyl groups to the peptide could allow for an enhanced interaction of the modified peptide with this water network. Here we illustrate this approach using a peptide having the non-natural amino acid homoserine at position 3, GIhSGFVFTL, and also peptides in which the Cα(F5)—CO—NH—Cα(V6) peptide bond is replaced by an ether, Cα(F5)—CH(X)—O—Cα(V6), to give the non-natural peptide GILGF—CH(X)—O—VFTL, where $X = CH_2OH$ or CH_3. In a 200 ps solvated molecular dynamics simulation of the HLA-A2 complexes of each peptide for GIhSGFVFTL and GILGF—$CH(CH_2OH)$—O—VFTL the peptide conformation remained essentially unchanged from that of GILGFVFTL in the X-ray structure of its complex with HLA-A2. In contrast, for GILGF—$CH(CH_3)$—O—VFTL the peptide conformation deviated from the X-ray conformation, indicating the importance of the hydroxyl group.

Keywords: MHC; non-natural peptide; water; computer-aided design; simulation

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INTRODUCTION

Class I major histocompatibility complex (MHC) molecules selectively bind peptide fragments derived from intracellular proteins and present them at the cell surface to cytotoxic $CD8^+$ T cells [1, 2]. This type of peptide presentation provides a means for the immune system to recognize and eliminate intracellular pathogens (through T-cell recognition of peptides from, for example, viral or bacterial proteins), or to eliminate cells that are malignant or transformed [3]. Normally, the T-cells are pre-selected such that they cannot recognize self-peptides (those derived from host proteins) that are bound to class I MHC molecules. It is believed that certain diseases involve the recognition (by autoreactive cytotoxic T-cells, on the surface of normal cells) of such MHC/self-peptide complexes, leading to an autoimmune response [4–7]. In such cases, a possible therapy would be to deliver peptide-based therapeutic agents that could selectively interfere with the peptide presentation process without compromising the overall ability of the immune system to eliminate pathogens.

Several studies have illustrated approaches to the design of non-natural peptides that compete with natural peptides presented by class I MHC molecules [8–10]. Based on the X-ray structure of the peptide RRIKAITLK with the MHC molecule HLA-B*2705 [11], Rognan *et al.*, showed that a peptide including a single non-natural amino acid (selected to maximize enthalpic interactions between the peptide and the MHC molecule) could bind efficiently to the MHC molecule [8]. Further, it was shown that replacement of amino acids that do not directly interact with the MHC molecule with an organic spacer group did not alter binding affinity. Bouvier and Wiley [10] showed that antigenic peptides containing large polyethylene glycol loops form stable complexes with class I MHC molecules. Inclusion of the loops was aimed at blocking the T-cell recognition of the peptide/MHC complex. Guichard *et al.*, have investigated the sensitivity of the peptide/MHC interaction to modification of the peptide bonds and the formation of retro-inverso pseudopeptides (peptides in which the CO—NH bond is reversed) [9]. Using the complex of the peptide GILGFVFTL with HLA-A2 as a test case, inversal of the peptide bond between positions 1 and 2 did not diminish binding, but modification of other bonds was not tolerated [9].

The design of an inhibitor requires a complete understanding of the natural peptide–MHC interaction. Natural peptides bind to MHC molecules *via* a series of interactions mediated by the peptide N and C termini [12] and by the backbone and 'anchor' residues in the peptide [13–15]. The

anchor residues are oriented into the peptide binding groove of the MHC molecule, and largely account for the selectivity of peptide binding to a particular MHC molecule [13–15]. Reference [16] provides a recent and introductory review. However it is becoming apparent that the peptide–MHC interaction is also mediated by water [17, 18]. It has been proposed that bound water molecules facilitate different intrapeptide side chain–side chain interactions [17], which in turn allows the MHC molecule to accommodate different peptides [18].

Based on molecular dynamics simulations of the GILGFVFTL/HLA-A2 complex, we have proposed that a loosely structured network of water molecules (which are not formally bound) is located in the peptide binding groove and may contribute to the formation of the complex between the peptide and the MHC molecule [19]. This water network may play a role in determining the appropriate conformations and orientations of key protein side chains, which in turn provides a complementary binding surface for the peptide. In this paper we describe an approach to the design of non-natural peptides in which hydroxyl groups are added to the natural peptide, such that the modified peptide might interact with the water network. We illustrate this approach using hydroxylated derivatives of the peptide GILGFVFTL, and examine the binding properties of these peptides using molecular dynamics simulations.

THEORETICAL METHODS

Calculations were performed on an IBM AIX P70 or a Silicon Graphics Indigo2 workstation. Graphic display and analysis of the trajectories were performed using the molecular modeling package Quanta 4.0. Molecular dynamics simulations were performed using the AMBER4.0 all-atom force field [20]. The starting structure for all simulations was the X-ray crystal structure [21] of the class I molecule HLA-A2 complex with a peptide [22] derived from the influenza matrix protein (one letter code amino acid sequence GILGFVFTL; Brookhaven entry *1hhi* [21]). Hydrogen atoms were added to the X-ray coordinates of the GILGFVFTL/HLA-A2 complex. Only the coordinates of the peptide and the $\alpha 1$ and $\alpha 2$ domains of the MHC molecule were used in the calculations.

The HLA-A2 complexes of three non-natural peptides, based on the peptide GILGFVFTL, were examined. In the first of these, a leucine to homoserine substitution was made at position 3 (Fig. 1(a)). This peptide is henceforth referred to as GIhSGFVFTL. In the other two peptides the

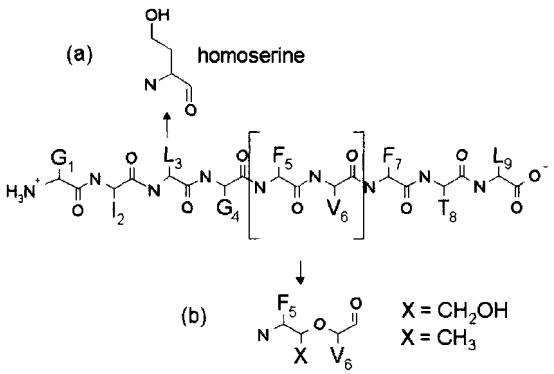

FIGURE 1 Modifications made to the peptide GILGFVFTL. In (a) a leucine to homoserine mutation at position 3 gives peptide GIhSGFVFTL. In (b) the peptide bond, Cα(F5)—CO—NH—Cα(V6), is replaced by an ether linkage, Cα(F5)—CH(X)—O—Cα(V6), where $X = CH_2OH$ or CH_3.

peptide bond, Cα(F5)—CO—NH—Cα(V6), was replaced by an ether linkage, Cα(F5)—CH(X)—O—Cα(V6), where $X = CH_2OH$ or CH_3 (Fig. 1(b)). To obtain charges for subsequent use in the molecular dynamics simulations, AM1 [23] calculations (MOPAC 6.0) were performed on homoserine and on —Cα(F5)—CH(X)—O—Cα(V6)— containing molecules (see Appendix). The molecular electrostatic potential (m.e.p.) atomic charge distribution for each molecule were evaluated from the AM1 wave function using the program RATTLER (Oxford Molecular). The charges and all other parameters used are given in the Appendix.

For the molecular dynamics simulations, each non-natural peptide/MHC complex was first placed in a 25 Å radius TIP3P water sphere [24] centered upon the center of mass of the peptide. Any water molecule within 2.0 Å of any solute atom was discarded from the calculation. This solvent shell fully solvated all the surface residues of the binding groove to a depth of at least 20 Å. In addition, 11 water molecules were placed in the MHC peptide binding groove, based on molecular dynamics of the natural GILGFVFTL peptide complexed with HLA-A2 [19]. The groove water and the outer solvent shell were subjected to a 40 ps equilibration phase of molecular dynamics before a simulation of the whole complex was performed. Following water equilibration, each complex was subjected to a 200 ps molecular dynamics simulation, including an initial heating phase from 0 K to 298 K in 10 ps. Coordinates were saved every 0.4 ps and a residue-based non-bonded cutoff of 6 Å was used in all calculations. In these simulations the protein backbone was loosely restrained to the X-ray coordinates, and

three hydrogen bonds between terminal amino acids of the peptide and conserved residues of the MHC molecule were constrained to normal hydrogen bond distances. A more detailed description of the simulation method can be found in Meng *et al.* [19, 25]. Identical simulations of the GILGFVFTL/HLA-A2 complex have been performed and described elsewhere [19]. For comparison, some data from these simulations will be included in the current paper.

RESULTS

In Figure 2 two views of the GILGFVFTL/HLA-A2 complex are shown to illustrate two key features that may stabilize the peptide–MHC interaction. The X-ray structure conformation of the peptide [21] allows for hydrophobic packing between the amino acid side chains of L3, F5 and F7 (Fig. 2(a)), which may stabilize this particular peptide conformer. In simulations of the complex [19], we have shown that the peptide–MHC interface is mediated by a dynamic network of about eleven water molecules, as shown in Figure 2(b). In particular, the interaction between the F5 carbonyl group of the peptide and amino acid R97 of the MHC molecule is mediated by a two water molecule bridge (Fig. 2(b)). We note that these water molecules are not rigidly bound, and are in exchange with other water molecules in the network [19].

From Figure 2(b), it is apparent that the L3 side chain is spatially proximal to the water molecule network that mediates the peptide–MHC interaction. This is perhaps surprising, given the hydrophobic nature of the side chain and, in principle, one might expect a peptide having a more hydrophilic side chain at position 3 to interact more favorably with the protein. On the other hand, it is necessary for the side chain at position 3 to be somewhat hydrophobic, such that the interaction with F5 (Fig. 2(a)) will not be compromised. For this reason, we chose to replace leucine with homoserine at position 3 (Fig. 1(a)), giving peptide GIhSGFVFTL.

In the simulation of the HLA-A2 complex with GIhSGFVFTL, the peptide backbone conformation remains close to that observed in the X-ray structure of GILGFVFTL in its complex with HLA-A2 (Fig. 3). Hence, the leucine to homoserine substitution does not exert a strong influence on the peptide conformation. A snapshot from the simulation (Fig. 4) shows that the hydroxyl group of the homoserine sidechain does interact with the water network. This interaction is not disruptive to the water-mediated F5–R97 interaction, however. We have previously shown [19] that disruption of this

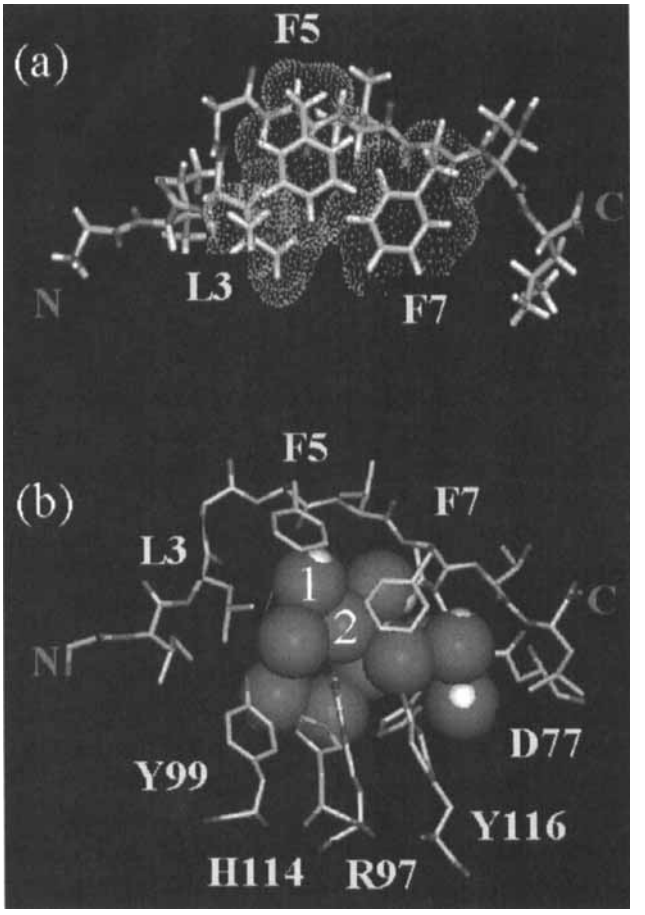

FIGURE 2 The peptide GILGFVFTL is shown in the conformation it adopts in the X-ray structure of its complex with HLA-A2 [21], with the N terminus of the peptide on the left. In (a) the hydrophobic packing between the L3, F5 and F7 side chains is evident. In (b), in which all the peptide aromatic and aliphatic hydrogen atoms (other than those of L3) are omitted, the dynamic water network that fills the interface between the peptide and the MHC molecule (and, specifically, amino acids Y99, R97, Y116, D77) is shown. It is apparent that the L3 side chain is in contact with the water molecules of this network. The F5-water–water-R97 bridge is also apparent. (See Color Plate II).

interaction results in an immediate loss of the F5 side chain conformation around the $\chi 1$ dihedral angle (N—Cα—Cβ—Cγ), but, as is apparent in Figure 5(b), this dihedral retains the *gauche*$^+$ conformation in the GIhSGFVFTL/HLA-A2 simulation. This is the same conformation that is observed in the X-ray structure and in a simulation [19] of the GILGFVFTL/HLA-A2 complex (Fig. 5(a)). Hence, the presence of

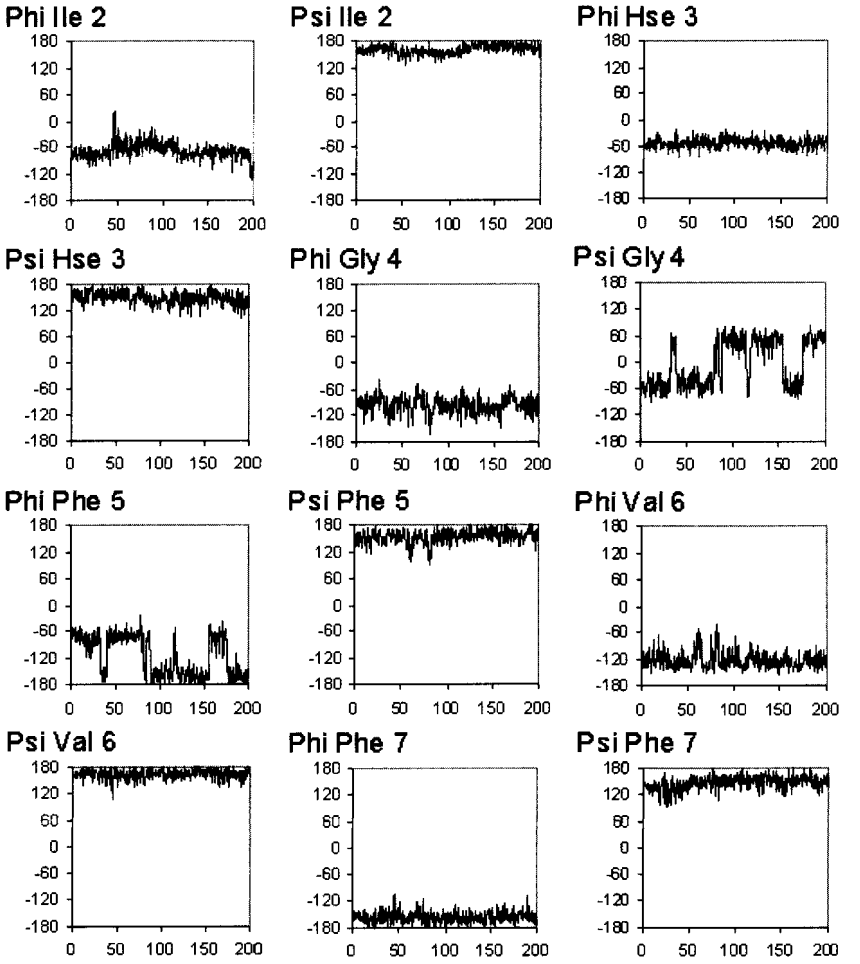

FIGURE 3 Motion of the peptide backbone in a molecular dynamics simulations of the peptide GIhSGFVFTL complexed with HLA-A2. In each plot the indicated dihedral angle is plotted (in degrees) against time for 200 ps of simulation.

homoserine at position 3 does not disrupt the key interactions between the peptide and the protein, but it does add an association between the peptide and the water network. We have subsequently shown experimentally that GIhSGFVFTL does bind to HLA-A2 with a higher affinity than GILGFVFTL [26].

In designing a second class of non-natural peptides that might interact effectively with HLA-A2, we focused on the F5(carbonyl)-water–water-R97 bridge (shown in Fig. 2(b) and, schematically, in Fig. 6(a)). The

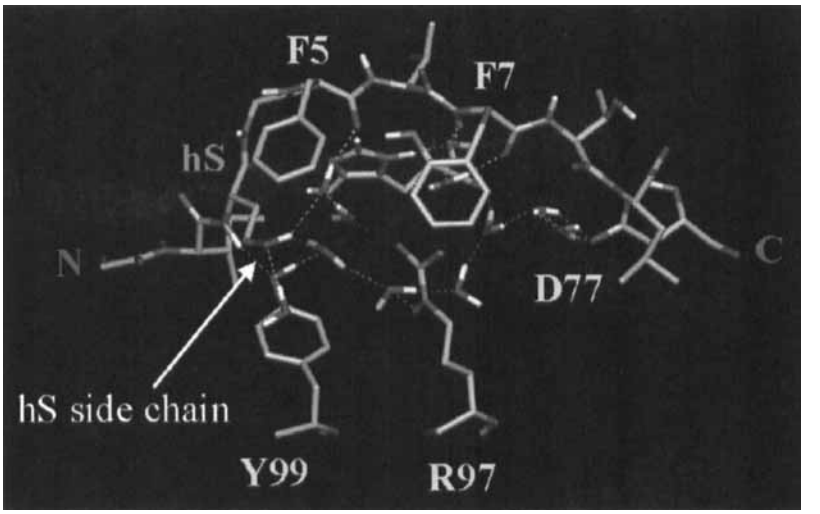

FIGURE 4 A structure of the GIhSGFVFTL/HLA-A2 complex taken from the molecular dynamics simulation. The complex is viewed from the side showing only the peptide, amino acids Y99, R97, Y116 and D77 of the MHC molecule, and the water network between the peptide and the protein. The homoserine sidechain is identified, and it is apparent that the hydroxyl group of the sidechain interacts with the water molecules at the peptide–MHC interface. (See Color Plate III).

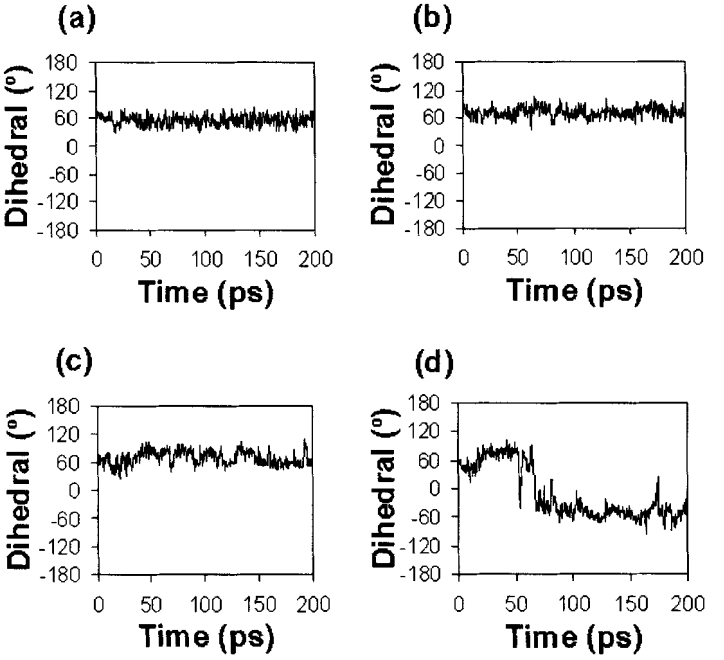

FIGURE 5 Motion of the $\chi 1$ torsion angle of the F5 side chain (N—Cα—Cβ—Cγ) in molecular dynamics simulations of the HLA-A2 complexes of (a) GILGFVFTL, (b) GIhSGFVFTL, (c) GILGF—CH(CH_2OH)—O—VFTL and (d) GILGF—CH(CH_3)—O—VFTL.

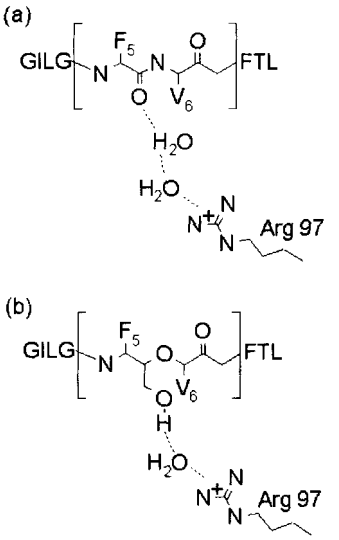

FIGURE 6 A schematic diagram of water-mediated peptide interactions with the side chain R97 of HLA-A2. (a) A two water bridge between R97 and F5 is required in the HLA-A2 complex of the peptide GILGFVFTL. (b) Only one water molecule is necessary to bridge between R97 and the hydroxyl group of the non-natural peptide GILGF—CH(CH_2OH)—O—VFTL.

goal in the design is to include a hydroxyl group that could substitute directly for one of the water molecules in the two water bridge (Fig. 6(b)). To do this, one approach is to derivatize the carbonyl group of F5 of the peptide, and change simultaneously the F5-V6 peptide linkage into an ether (Fig. 1(b)), giving a non-natural peptide which will be referred to as GILGF—CH(X)—O—VFTL, where $X = CH_2OH$ or CH_3. For $X = CH_2OH$ the hydroxyl group is positioned such that it could 'replace' one water molecule in the F5-water–water-R97 bridge found in the GILGFVFTL/HLA-A2 complex. The peptide in which $X = CH_3$ was included as a control.

As mentioned above, the motion of the F5 $\chi 1$ dihedral angle is sensitive to the maintenance of the interaction between the F5 carbonyl group and R97 of the MHC molecule [19]. For GILGF—CH(CH_2OH)—O—VFTL, $\chi 1$(F5) retained the *gauche*$^+$ conformation (Fig. 5(c)) seen in the GILGFVFTL/HLA-A2 X-ray structure, and, in general, the peptide conformation remained essentially unchanged throughout the simulation. In contrast, for GILGF—CH(CH_3)—O—VFTL $\chi 1$(F5) rotated from a *gauche*$^+$ to a *gauche*$^-$ conformation (Fig. 5(d)). This is caused by the absence of a hydroxyl group, which disrupts the peptide interaction with R97.

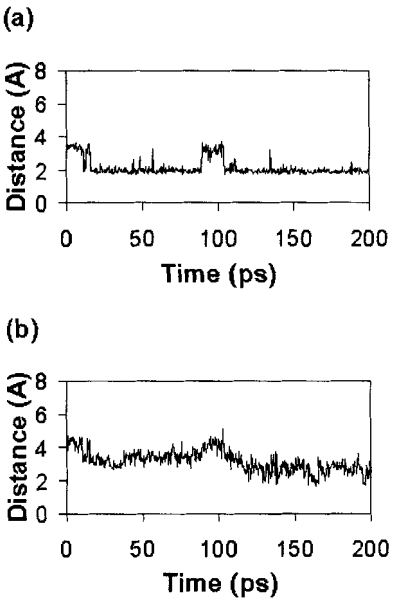

FIGURE 7 Hydrogen bonding interactions observed in the molecular dynamics simulation of the GILGF—$CH(CH_2OH)$—O—VFTL/HLA-A2 complex. Distances between (a) the hydroxyl group of the modified peptide and a water molecule in the binding groove and (b) the same water molecule and the side chain of R97 (the distance shown is that between the water oxygen and a guanidinium nitrogen of R97).

To determine if the hydroxyl group of the non-natural peptide GILGF—$CH(CH_2OH)$—O—VFTL served as a direct replacement of a water molecule in the F5–R97 bridge, we examined the hydrogen bonding interactions between the peptide, the water network and the protein. In Figure 6 we show that the same water molecule that forms a hydrogen bond with the pendant hydroxyl group of GILGF—$CH(CH_2OH)$—O—VFTL (Fig. 7(a)) is also closely associated with the R97 side chain throughout the simulation (Fig. 7(b)). Hence, the two water bridge present in the GILGFVFTL/HLA-A2 complex has been replaced by a single water bridge between the non-natural peptide and the MHC molecule.

DISCUSSION

Water mediation of ligand-receptor interactions is a well known phenomena, and disordered water molecules within proteins have been identified (see, for example, Ref. [27]). Water can play a fundamental role in the interactions of proteins with other molecules, and increasingly this is being

recognized in a number of protein–DNA complexes [28–31]. Water molecules can be displaced from a protein binding site by the interacting molecule, or be trapped at the protein-ligand interface following binding [32–34]. In each case, the water molecules contribute (either entropically or enthalpically) to the ligand binding free energy. In the case of the peptide–MHC complex, it appears that water molecules are 'trapped' at the peptide–MHC interface. Hence, the affinity of ligands that could either interact with, or replace, these water molecules might be increased over that of the natural peptide.

The design of non-natural mimics of a peptide ligand requires that the new ligand adopt a similar conformation to the natural peptides when bound to the receptor. Most computer-aided design approaches therefore use the bound conformation of the natural peptide as the starting point for the design. An inherent assumption in this process is that the synthetic ligand will not bind to the receptor in a different conformation. With increasing modification of a nonamer peptide, it is possible that an alternative binding conformation in the MHC binding groove could occur. However, with stepwise modification, and the retention (in some form) of key peptide–MHC contacts, we believe that the design of a completely non-natural mimic of the bound peptide is possible. The results shown here represent a step towards the design of peptide-based small molecules as specific inhibitors of peptide presentation by class I MHC molecules.

Acknowledgments

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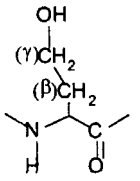
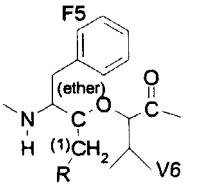
SPECIAL APPENDIX

All the molecular dynamics simulations were performed using the AMBER4.0 all-atom force field [20]. Standard bond, angle and torsional angle parameters and atomic point charges [20] were used for all atoms of the MHC molecule and the natural amino acids of the peptides. The following Tables I, II and III give the atomic charge distributions, the

TABLE I Atomic charges (millielectrons) for fragments of the non-natural peptides





Atom	*Homoserine*	*Atom*[a]	*R = OH*	*R = H*
N(H)	−463(252)	N(F5)(H)	−463(252)	−463(252)
Cα(Hα)	35(48)	Cα(F5)(Hα)	155(37)	155(37)
Cβ(Hβ)	−76(38)	C(ether)(H)	−237(193)	55(60)
Cγ(Hγ)	18(119)	O(ether)	−347	−398
O(H)	−550(310)	C(1)(H)	201(49)	−243(76)
C(=O)	616(−504)	O(H)	−534(327)	
		Cα(V6)(Hα)	149(25)	149(25)
		C(V6)(=O)	616(−504)	616(−504)

[a]Charges for F5 and V6 side chains were unchanged from those in AMBER4.0. The total charge on each side chain in AMBER4.0 is 16 millielectrons.

TABLE II Atom types for fragments of the non-natural peptides. The atom names refer to the figures shown in Table I

Atom	*Homoserine*	*Atom*[a]	*R = OH*	*R = H*
Cα(Hα)	CT(HC)	Cα(F5)(Hα)	CT(HC)	CT(HC)
Cβ(Hβ)	CT(HC)	C(ether)(H)	CT(HC)	CT(HC)
Cγ(Hγ)	CT(HC)	O(ether)	O< [a]	O< [a]
O(H)	OH(HO)	Cα(V6)(Hα)	CT(HC)	CT(HC)
		C(1)(H)	CT(HC)	CT(HC)
		O(H)	OH(HO)	

[a]Non-standard atom type, with parameters as defined in Table III.

TABLE III Forcefield parameters for the ether linkage in peptides GILGF—CH(R)—O—VFTL[a,b]

Parameter	*Force constant*	*Equilibrium value*
CT—O<	320 kcal/mol/Å	1.41 Å
CT—O<—CT	63 kcal/mol/°	120.0°
O<—CT—C	63 kcal/mol/°	109.5°
O<—CT—CT	50 kcal/mol/°	109.5°
O<—CT—HC	50 kcal/mol/°	109.5°

[a]Bond stretch and angle deformation in AMBER4.0 are included as harmonic functions. The equilibrium distance and angles given in the table were based on AM1 calculations and the force constants were chosen by analogy to those already implemented in the AMBER4.0 force field.
[b]Free rotation was assumed around the CT—O< bond (a dihedral barrier height of zero). The O< atom was assigned non-bonded parameters R^* and ε (see Ref. [20]) of 1.65 and 0.15, respectively.

AMBER atom types, and the 'non-standard' internal coordinate parameters, respectively, that were used in the simulations in the current work.

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