The Structure and Stability of an HLA-A*0201/Octameric Tax Peptide Complex with an Empty Conserved Peptide-N-Terminal Binding Site

Amir R. Khan, Brian M. Baker, Partho Ghosh, William E. Biddison and Don C. Wiley

http://www.jimmunol.org/content/164/12/6398

**References**

This article cites 53 articles, 13 of which you can access for free at: http://www.jimmunol.org/content/164/12/6398.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
The Structure and Stability of an HLA-A*0201/Octameric Tax Peptide Complex with an Empty Conserved Peptide-N-Terminus Binding Site

Amir R. Khan,* Brian M. Baker,* Partho Ghosh, ‡ William E. Biddison, § and Don C. Wiley2*†

The crystal structure of the human class I MHC molecule HLA-A2 complexed with an octameric peptide, Tax8 (LFGYPVYV), from human T cell lymphotropic virus-1 (HTLV-1) has been determined. This structure is compared with a newly refined, higher resolution (1.8 Å) structure of HLA-A2 complexed with the nonameric Tax9 peptide (LLFGYPVYV) with one more N-terminal residue. Despite the absence of a peptide residue (P1) bound in the conserved N-terminal peptide-binding pocket of the Tax8/HLA-A2 complex, the structures of the two complexes are essentially identical. Water molecules in the Tax8 complex replace the terminal amino group of the Tax9 peptide and mediate a network of hydrogen bonds among the secondary structural elements at that end of the peptide-binding groove. Thermal denaturation measurements indicate that the Tax8 complex is much less stable, \( \Delta T_{m} = 16^\circ C \), than the Tax9 complex, but both can sensitize target cells for lysis by some Tax-specific CTL from HTLV-1 infected individuals. The absence of a P1 peptide residue is thus not enough to prevent formation of a “closed conformation” of the peptide-binding site. TCR affinity measurements and cytotoxic T cell assays indicate that the Tax8/HLA-A2 complex does not functionally cross-react with the A6-TCR-bearing T cell clone specific for Tax9/HLA-A2 complexes.

The Journal of Immunology, 2000, 164: 6398–6405.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1. A.R.K. is supported by a Human Frontiers Long Term Fellowship. B.M.B. is supported by a fellowship from the Cancer Research Institute. D.C.W. is an investigator of the Howard Hughes Medical Institute.

2 Address correspondence and reprint requests to Dr. Don C. Wiley, Howard Hughes Medical Institute, Department of Molecular and Cellular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138. E-mail address: wiley@sta10.harvard.edu

Copyright © 2000 by The American Association of Immunologists

0022-1767/00/$02.00

3 Abbreviations used in this paper: Hc, heavy chain; \( \beta_{2m} \), \( \beta_{2}-\)microglobulin; \( T_{m} \), melting temperature; HTLV-1, human T cell lymphotropic virus-1; HAM/TSP, HTLV-I-associated myelopathy/tropical spastic paraparesis; CD, circular dichroism.
Table I. Crystalllographic data

<table>
<thead>
<tr>
<th></th>
<th>Tax8-A2</th>
<th>Tax9-A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>P1</td>
<td>P1</td>
</tr>
<tr>
<td>Molecules in AU</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cell parameter</td>
<td>(a = 50.34 , \text{Å}, b = 62.86 , \text{Å}, c = 74.84 , \text{Å}, \gamma = 77.96^\circ)</td>
<td>(a = 50.56 , \text{Å}, b = 63.79 , \text{Å}, c = 75.08 , \text{Å}, \gamma = 77.38^\circ)</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>2.15</td>
<td>1.8</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>38,048</td>
<td>72,184</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>78.0</td>
<td>85.3</td>
</tr>
<tr>
<td>(F_{o}R_f &gt; 3.0)</td>
<td>(2.26-2.15 Å) 38.3%</td>
<td>(1.94-1.80 Å) 59.9%</td>
</tr>
</tbody>
</table>

\(\text{R}_{merge} = \frac{\Sigma |I_{hkij}| - <I_{hkij}>^2}{\Sigma |I_{hkij}|^2} \times 100\%\).

The stability of the HLA-A2/Tax8 complex is markedly reduced, as expected for loss of the conserved interactions at the N-terminal amino acid of an antigenic peptide (Tax9) on the stability termini of bound peptides may dominate in the formation and stabilization of peptide/MHC complexes.

In this paper we have studied the effect of removing the N-terminal amino acid of an antigenic peptide (Tax9) on the stability and structure of its interaction with HLA-A2, and on its recognition by T cells as an HLA-A2/Tax8 complex. Tax9 is the dominant antigenic peptide inducing cytotoxic T cells in human T lymphotrophic virus-1 (HTLV-1)-infected individuals (38), we find that it does not cross-react functionally with HLA-A2/Tax9-specific T cells in cell lysis assays and has a low affinity for an HLA-A2/Tax9-specific TCR. The stability of the HLA-A2/Tax8 complex is markedly reduced, as expected for loss of the conserved interactions at the N-terminal peptide-binding site, but the structure of the complex is remarkably similar with water molecules substituting for some of the peptide interactions in the binding site.

Materials and Methods

Protein purification and crystallization

The extracellular region of the HLA-A2 Hc and \(\beta_m\) were expressed separately in Escherichia coli as inclusion bodies (39). The inclusion bodies were refolded together in the presence of excess Tax8 (LLFGYPVYV) or Tax9 (LLFGYPVVYV) peptide. Briefly, milligram amounts of \(\beta_m\), Hc, and peptide were injected into 500 ml of a refolding buffer (100 mM Tris, 400 mM arginine-HCl, 2 mM NaEDTA, 0.5 mM oxidized glutathione, and 5 mM reduced glutathione, pH 8.3). The final concentrations of the complexes of HLA-A2/Tax9 were 2 mM reduced glutathione, pH 8.3). Although HLA-A2/Tax8 activates cytotoxic T cells in HTLV-1-infected individuals (38), we find that it does not cross-react functionally with HLA-A2/Tax9-specific T cells in cell lysis assays and has a low affinity for an HLA-A2/Tax9-specific TCR. The stability of the HLA-A2/Tax8 complex is markedly reduced, as expected for loss of the conserved interactions at the N-terminal peptide-binding site, but the structure of the complex is remarkably similar with water molecules substituting for some of the peptide interactions in the binding site.

Crystals of Tax8-A2 were obtained by vapor diffusion from hanging drops containing equal volumes of protein (5 mg/ml: 25 mM MES, pH 6.5) and 13–20% polyethylene glycol (PEG) 6000 (25 mM MES, pH 6.5), Tax8-A2 crystals were obtained by seeding with Tax8-A2 crystals. A 3-µl solution of seed crystals was incubated with a 3-µl solution containing 3 mg/ml Tax8-A2 and 400 µM Tax8 peptide in 25 mM MES (pH 6.4) and 0.1% NaN\(_3\). The drop was equilibrated against 13% PEG 6000, 25 mM MES, and 0.1% NaN\(_3\) at 18°C.

Data collection and structural refinement

Crystals of Tax8-A2 were transferred to a 20% glycerol solution in steps of 4–10% and flash-cooled in a stream of cryo-cooled nitrogen gas. Data were collected on a Mar345 detector (Mar Research, Hamburg, Germany) mounted on an Elliot GX-13 x-ray generator (GEC Avionics, London, U.K.). The structure of Tax8-A2 was refined by using the previously de-termed structure of Tax9-A2 as the starting model (Protein Data Bank code 1hhk; Ref. 34). A subset (10%) of the reflection data were flagged (R-free) and excluded from refinement protocols. As the crystals of Tax8-A2 and Tax9-A2 were isomorphous, the same set of flagged reflections previously used for monitoring Tax9-A2 refinement was used in the R-free data set for Tax8-A2. Because the structure of Tax9-A2 was previously refined to only 2.5 Å resolution (without waters), the data were extended to 1.8 Å and the model refined to that resolution with the inclusion of water molecules.

The starting model of Tax8-A2, stripped of water molecules and the peptide was initially subjected to rigid-body fitting using the program CNS (40). Electron density maps clearly indicated the position of Tax8 within the peptide-binding groove of HLA-A2. The structure was further refined by multiple cycles of energy minimization and model building using the programs CNS and O (40, 41). All reflections between 50 Å and the resolution limits of the data sets (2.14 Å for Tax8-A2, 1.8 Å for Tax9-A2) were used during refinement and electron density map calculations (Table I). Water molecules were gradually introduced during the course of model building and were selected from >3σ peaks in difference (\(F_c - F_o\)) electron density maps within 2.5–3.6 Å of hydrogen bond donors or acceptors.

During the course of refinement, it was observed that the N-terminal leucine residue (Leu2) of Tax8 has a backbone conformation that is distinct from that residue in Tax9, resulting in a net shift of 1.0 Å in the position of the α-amino group of Tax8. Simulated annealing omit maps, in which the peptide and surrounding water molecules were removed, confirmed that the N terminus was both well positioned and ordered in both molecules of the asymmetric unit. The conformation of Leu2 precludes formation of a salt bridge with Glu\(^6\) of the B pocket; in the Tax9 structure, the identical amide nitrogen hydrogen bonds to Glu\(^6\). To further test the validity of the Tax8 model, one further cycle of positional refinement was performed in

Table II. Refinement statistics

<table>
<thead>
<tr>
<th></th>
<th>Tax8</th>
<th>Tax9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution (Å)</td>
<td>2.15</td>
<td>1.8</td>
</tr>
<tr>
<td>(R_{merge}) (%)</td>
<td>25.41 (2.26–2.2 Å)</td>
<td>25.02 (1.86–1.8 Å)</td>
</tr>
<tr>
<td>(R_{cryst}) (%)</td>
<td>19.18 (2.26–2.2 Å)</td>
<td>19.77 (1.86–1.8 Å)</td>
</tr>
<tr>
<td>rms deviations from ideality</td>
<td>21.6%</td>
<td>24.9%</td>
</tr>
<tr>
<td>Bonds (Å)</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Angles (°)</td>
<td>1.6</td>
<td>2.4</td>
</tr>
<tr>
<td>Dihedrals (°)</td>
<td>25.3</td>
<td>26.2</td>
</tr>
<tr>
<td>Improper (°)</td>
<td>0.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Luzzati e.s.d. (Å)</td>
<td>0.23</td>
<td>0.21</td>
</tr>
<tr>
<td>Protein atoms</td>
<td>6306</td>
<td>6322</td>
</tr>
<tr>
<td>Water molecules</td>
<td>496</td>
<td>607</td>
</tr>
<tr>
<td>Ramachandran map</td>
<td>Most favored (%)</td>
<td>89.3</td>
</tr>
<tr>
<td>Disallowed (%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(R_{merge} = \frac{\Sigma |F_o| - |F_c|}{\Sigma |F_o|}\). \(R_{merge}\) calculated using 10% of the reflections, randomly generated and excluded from refinement protocols.

a Estimated coordinate accuracy of the structures from the method of Luzzati (51).

b Analyzed using the program PROCHECK (52).
which the α-amino group was manually re-fitted by adjustment of torsion angles to within 3.2 Å of the side-chain of Glu83(Oe1). Following crystallographically restrained energy minimization in the CNS program, it was again observed that the N terminus shifted away, to a position 3.6 Å from the side-chain of Glu 63(O). Again, observed that the N terminus shifted away, to a position 3.6 Å from the side-chain of Glu 63(O).

Characterization of Tax8-A2

The 2G4 T cell clone has a TCR (A6-TCR) specific for Tax9 complexed with HLA-A2, and the interaction of this receptor with a number of altered peptides complexed with HLA-A2 has been previously investigated (37, 42). The binding of Tax8-A2 to the A6-TCR was investigated here using an equilibrium BIACore assay as described previously (37). Briefly, recombinant A6-TCR with a single free thiol at the C terminus of the β-chain was coupled to a CM5 sensor chip using standard thiol coupling. Multiple concentrations of Tax8-A2 were injected at a flow rate of 10 μl/min, and the responses at equilibrium (300 s after injection) were determined. The temperature of the sample was maintained at 4°C, and Tax8-A2 dilutions were made from a highly concentrated stock immediately before injection to minimize dissociation of the Tax8 peptide from HLA-A2. The responses from injections over a mock surface were subtracted from the data, and all injections were repeated twice. Equilibrium responses were fit against the concentration of injected Tax8-A2, assuming a single-site binding model. Solution conditions were 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, and 0.005% polysorbate-20 (pH 7.4) at 25°C.

T cell assays

T cell-mediated cytoxicity was quantitated by a time-resolved fluorometric assay using HLA-A2-transfected cells as targets as previously described (36). Effector cells were the A6-TCR-expressing CD8+ T cell clone 2G4 that recognizes the Tax11–19 peptide presented by HLA-A2 and was isolated from a patient with HAM/TSP (43).

For the antagonism assay, HLA-A2-transfected Hmy2.C1R cells were treated with 100 μg/ml mitomycin C (Sigma, St. Louis, MO) for 2 h at 37°C, washed three times with PBS, and then pulsed with 1000 nM of candidate antagonist peptides for 2 h at 37°C. The cells were washed, and 1 × 10^6 cells were incubated with 1 × 10^6 2G4 T cells for 1 h at 37°C. Tax peptide was added at concentrations of 0.1–10 nM, and the cells were incubated for 48 h. Supernatants were collected and assayed for IFN-γ content as previously described (36).

Results

Peptide residues P2-P9 of the Tax8-A2 and Tax9-A2 complexes bind very similarly

The structures of Tax8-A2 and Tax9-A2 were refined to reasonable agreement between observed and calculated structure factors, as well as good stereochemistry and geometry (Table II). Although data extending to 2.15 Å were used in the refinement of Tax8-A2, the data were incomplete in the highest resolution shell (Table I). The number of observed reflections corresponds to a 100% complete data set to 2.30-Å resolution. The overall structure of the Tax8-A2 complex is identical to Tax9-A2 and is shown in Fig. 1. The quality of the structures permits a detailed discussion of the peptide-binding groove and its bound solvent.

The conformation and position of Tax8 within the peptide-binding groove of HLA-A2 is identical to the structure observed in Tax9-A2 (Fig. 2). Hydrogen bonds and hydrophobic contacts are

For the antagonism assay, HLA-A2-transfected Hmy2.C1R cells were treated with 100 μg/ml mitomycin C (Sigma, St. Louis, MO) for 2 h at 37°C, washed three times with PBS, and then pulsed with 1000 nM of candidate antagonist peptides for 2 h at 37°C. The cells were washed, and 1 × 10^6 cells were incubated with 1 × 10^6 2G4 T cells for 1 h at 37°C. Tax peptide was added at concentrations of 0.1–10 nM, and the cells were incubated for 48 h. Supernatants were collected and assayed for IFN-γ content as previously described (36).

Results

Peptide residues P2-P9 of the Tax8-A2 and Tax9-A2 complexes bind very similarly

The structures of Tax8-A2 and Tax9-A2 were refined to reasonable agreement between observed and calculated structure factors, as well as good stereochemistry and geometry (Table II). Although data extending to 2.15 Å were used in the refinement of Tax8-A2, the data were incomplete in the highest resolution shell (Table I). The number of observed reflections corresponds to a 100% complete data set to 2.30-Å resolution. The overall structure of the Tax8-A2 complex is identical to Tax9-A2 and is shown in Fig. 1. The quality of the structures permits a detailed discussion of the peptide-binding groove and its bound solvent.

The conformation and position of Tax8 within the peptide-binding groove of HLA-A2 is identical to the structure observed in Tax9-A2 (Fig. 2). Hydrogen bonds and hydrophobic contacts are
preserved from Phe$^3$ to Val$^9$ of Tax8 and the corresponding MHC binding pocket, indicating that loss of the position 1 Leu (P1) does not induce global shifts or conformational changes in the peptide or MHC. Following alignment of the HLA Hc, the root-mean-square difference in the coordinates of the common atoms of Tax8 and Tax9 was 0.36 Å. However, the $\alpha$-amino group of Leu$^2$ (Tax8) is positioned about 1 Å from the equivalent P2 amide nitrogen of Tax9 (see Materials and Methods), making a hydrogen bond with the hydroxyl of Tyr$^{159}$ rather than Glu$^{63}$ as in Tax9 (compare Fig. 3, a and b). The distance between the side chain of Glu$^{63}$ and the P2 amino group of Tax8 is 3.9 Å, which may still permit a favorable electrostatic interaction.

Two water molecules occupy the N-terminal peptide residue binding pocket in the Tax8 complex

In the Tax8 complex two water molecules, Wat-1 and Wat-2, partially fill the space occupied by the P1 peptide residue, Leu$^1$, in the Tax9 complex (Fig. 3). Wat-1 provides a bridge from the N terminus of Tax8, via hydrogen bonds, to the carboxylate group of Glu$^{63}$ (Fig. 3). Wat-2 is in the position that is occupied by the $\alpha$-amino group of Leu$^1$ in Tax9 (Fig. 3, a and b). This water provides a bridge from the hydroxyl group of Tyr$^{59}$, in the $\beta$-sheet forming the floor of the peptide-binding groove, via hydrogen bonds to Tyr$^{171}$ in $\alpha$-helix forming one side of the binding site (Fig. 3). In the Tax9 complex, the same hydrogen bonded bridge between elements of secondary structure is made through the amino group at Leu$^1$ of the bound peptide (Fig. 3b). Wat-2 is also within hydrogen bonding distance of Wat-1 (distance of 2.82 Å; Fig. 3a) and a third water (Wat-3) found in both the Tax8 and Tax9 complexes (Fig. 3).

The absence of the P1 peptide residue apparently causes some small conformational changes in HLA-A2. The indole ring of Trp$^{167}$ and the side chain of Glu$^{163}$ are about 1 Å closer to the space that would be occupied by Leu$^1$ of Tax9 (data not shown). Despite these observed structural differences at the binding site for the peptide N terminus, the side chain of the P2 peptide residue,
Table III. Hydrogen bonds in the peptide N-terminal region of the binding groove

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Water</th>
<th>Heavy Chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tax9-A2 structure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu(^1) (N)</td>
<td>Tyr(^7) (OH)</td>
<td>(2.82 Å)</td>
</tr>
<tr>
<td>Leu(^1) (N)</td>
<td>Tyr(^7) (OH)</td>
<td>(2.84 Å)</td>
</tr>
<tr>
<td>Leu(^1) (O)</td>
<td>Tyr(^7) (OH)</td>
<td>(2.71 Å)</td>
</tr>
<tr>
<td>Leu(^2) (N)</td>
<td>Glu(^6) (OE1)</td>
<td>(2.91 Å)</td>
</tr>
<tr>
<td>Leu(^2) (O)</td>
<td>Lys(^6) (NZ)</td>
<td>(3.02 Å)</td>
</tr>
<tr>
<td>Tax8-A2 structure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu(^2) (N)</td>
<td>Wat-1 (3.04 Å)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wat-1</td>
<td></td>
</tr>
<tr>
<td>Leu(^2) (N)</td>
<td>Glue(^6)</td>
<td>(3.02 Å)</td>
</tr>
<tr>
<td>Leu(^2) (O)</td>
<td>Tyr(^7) (OH)</td>
<td>(3.29 Å)</td>
</tr>
<tr>
<td></td>
<td>Lys(^6) (NZ)</td>
<td>(3.32 Å)</td>
</tr>
<tr>
<td>Leu(^2) (N)</td>
<td>Wat-2</td>
<td></td>
</tr>
<tr>
<td>Leu(^2) (O)</td>
<td>Wat-2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tyr(^7) (OH)</td>
<td>(2.53 Å)</td>
</tr>
<tr>
<td></td>
<td>Lys(^6) (NZ)</td>
<td>(2.72 Å)</td>
</tr>
</tbody>
</table>

*Only hydrogen bonds differing between the Tax8-A2 and Tax9-A2 structures are listed.

Leu\(^2\), occupies the identical hydrophobic pocket in both Tax8 and Tax9 complexes, forming contacts with Met\(^{45}\) and Phe\(^{6}\) in the B pocket (16).

Solvent molecules in the peptide-binding sites of both Tax8 and Tax9 complexes

In the middle portion of the peptide, three buried water molecules form part of the interface between Tax8 and Hc (Wat-4, Wat-5, Wat-6; Fig. 4). These waters are also present in the structure of Tax9-A2 refined here, but were not visible in the previously published, lower resolution (2.5 Å) structure (34). The carbonyl oxygen of Tyr\(^{6}\) is linked via Wat-4 to the e-amino group of Arg\(^{97}\). The carbonyl oxygen of Val\(^{7}\) is linked via Wat-6 to the carbamate oxygen of Asp\(^{72}\). These water-mediated, hydrogen bond bridges are formed with the part of the peptide that is central to recognition by TCRs (35, 37). Upon binding of both the A6- and B7-TCRs, a conformational change takes place in the Tax9 peptide such that the side chain of Pro\(^{6}\) becomes buried in the pocket occupied by two of these water molecules (35), and the Val\(^{7}\) side chain projects outward toward the TCR. Consequently, two water molecules, Wat-4 and Wat-5, must be displaced upon TCR binding. Another water molecule, Wat-7, bridges the C-terminal carboxylate of the peptide via hydrogen bonds to the side chain of Thr\(^{80}\) (Oy1) in both the Tax8-A2 and Tax9-A2 structures.

Thermal stability of the HLA-A2/Tax8 complex

The thermal denaturation curves of Tax8-A2 and Tax9-A2 were determined by monitoring the loss of secondary structure using CD. The mid-point of the transitions were 49°C for Tax8-A2 and 65°C for Tax9-A2 (Fig. 5), indicating a significant destabilization of the peptide-A2 complex following loss of the N-terminal residue of the peptide. A second transition observed in the denaturation curves beginning near 80°C corresponds to the unfolding of β2m (27, 32). Consequently, the early loss of secondary structure observed for Tax8-A2 is attributed to loss of the peptide and unfolding of the Hc, as previously shown (27, 32). Due to linkage between peptide binding and stable folding of the Hc/β2m heterodimer, it follows that Tax8 binds the A2 molecule with a much lower affinity than Tax9 (28, 44). These results are consistent with peptide-binding studies of the murine class I MHC H2-K\(^{d}\), in which deletion of the N-terminal residue of a nonomer peptide significantly reduced the stability of the MHC/peptide complex (45).

TCR binding and T cell activation

Binding affinity of Tax8-A2 to the A6-TCR, specific for HLA-A2/Tax9, was measured using an equilibrium plasmon resonance experiment. The A6-TCR clone, isolated from a patient with the HTLV-1-associated autoimmune disorder HAM/TSP, is a class I-restricted TCR specific for HLA-A2 (43). Equilibrium binding experiments (Fig. 6a) revealed that the association of the A6-TCR with Tax8-A2 is about 16-fold weaker ($K_a = 15 \pm 2 \mu M$) relative to full-length Tax9 peptide–HMC complex ($K_a = 0.9 \pm 0.1 \mu M$; Ref. 37). Although we attempted to minimize such contributions during the experiment, it is possible that this result is influenced slightly by dissociation of Tax8 from the MHC (see Materials and Methods).

The biological activity of the Tax8 peptide was measured in a specific lysis (cytotoxicity) assay (Fig. 6b). The data show that, compared with the wild-type Tax9 peptide, Tax8 has a very limited ability to activate A6-TCR-bearing T cells (barely detectable lysis at a million-fold increase in peptide concentration). In addition, no TCR antagonism (vs Tax9) was detected with Tax8 peptide (Fig. 6c). However, Tax8 could sensitize targets cells for lysis by some Tax-specific CTL (38).
antagonize A6-bearing T-cells for IFN-γ activity with the A6-TCR is shown as a reference (2.5:1). A control peptide (influenza M1 57–68) that lacks biological and Tax8 (M5) to lysis by the A6-TCR bearing CTL clone 2G4 (E:T assay as described.rium binding of Tax8-A2 to the A6-TCR performed using a BIAcore residues in the P1 binding site making the conserved array of the peptides were 8-mer, 9-mer, or 10-mer, have had a peptide bound peptides determined by x-ray crystallography, whether Until now, all the structures of class I MHC molecules with Structure and stability of Tax8/HLA-A2 Discussion Structure and stability of Tax8/HLA-A2 Until now, all the structures of class I MHC molecules with bound peptides determined by x-ray crystallography, whether the peptides were 8-mer, 9-mer, or 10-mer, have had a peptide residue in the P1 binding site making the conserved array of hydrogen bonds to nonpolymorphic MHC residues (Fig. 3b). In the human class I molecule HLA-B35, a short peptide was accommodated by stretching out a kink usually found in longer peptides (46), and in murine class I molecules the shape of the bottom of the binding site is conducive to the binding of shorter peptides (21, 47). The Tax8/Tax9 pair of peptides were studied here, because it seemed likely that the 8-mer would bind just like the 9-mer, except with the P1 site empty. This offered the opportunity to see whether the loss of the conserved hydrogen bonds, which both held bound peptides in the site and tethered the secondary structures of the binding site together, would affect the conformation of the binding site. In particular, we hoped to see whether the binding site might adopt a partially “open state” as proposed to exist from the slow binding and dissociation kinetics of peptides.

In the Tax8 complex, as a result of no peptide residue occupying the P1 binding site, we observed a loss of three hydrogen bonds directly from the bound peptide to the MHC molecule, relative to the Tax9 complex. However, the shift in the primary amine position of Tax8, and the addition of two water molecules, created a new network of hydrogen bonds that satisfies all of the hydrogen bonding acceptors and donors of the conserved MHC residues (Fig. 3). Furthermore, the new network of hydrogen bonds in the Tax8 complex cross-links together the same secondary structure elements as in the Tax9 complex. Thus, although the structure indicates that the Tax8 peptide might be expected to bind more weakly than Tax9, due to the loss of the three conserved hydrogen bonds from peptide to MHC, the restoration of a hydrogen bonding network using water molecules apparently serves to stabilize the “closed conformation” of the MHC binding site.

In previous studies with a different peptide, the influenza virus matrix peptide (GILGFVFTL), the N-terminal amino group of the peptide was replaced, synthetically, by a methyl group (32). The modified N terminus prevented the formation of two of the hydrogen bonds that group normally makes with conserved residues of the MHC molecule (28). This loss of interactions resulted in a decrease of the thermal denaturation temperature of the peptide/HLA-A2 complex of 21°C, corresponding to a ΔΔG° value of about 4.6 kcal/mol (28, 32). A structure of the complex revealed that the substituted methyl group rotated away from the hydrogen bonding groups of the MHC molecule and was replaced by a water molecule occupying the position vacated by the peptide N terminus (28) and located identically to water Wat-2 observed in the Tax8 complex (Fig. 3a). The bound water molecule formed a similar set of hydrogen bonds to the conserved MHC residues as the full-length 9-residue matrix and Tax peptides, but did not provide any bridging hydrogen bonds to the modified peptide. Apparently the loss of these two hydrogen bonds to the N-terminally modified peptide resulted in the large (~4.6 kcal/mol) decrease in stability of the peptide/MHC complex.

The thermal denaturation temperature of the Tax8 complex was observed in this paper to be 16°C lower than that of the Tax9 complex. This difference also presumably results partly from the loss of direct hydrogen bonds between the peptide and MHC molecule. Although the bridging water molecules Wat-1 and Wat-2 form a network of hydrogen bonds, the new hydrogen bonds do not directly link the peptide to the MHC molecule (Fig. 3a). Thus, as in the earlier study of the modified matrix peptide/MHC complexes, eliminating the conserved hydrogen bonds from the peptide N terminus to the MHC molecule in the Tax8 complex also very substantially decreased the stability of the MHC molecule. In both cases, despite drastic changes in the stability of the MHC molecules, when hydrogen bonding interactions with the peptide were removed, the conformation of the peptide-binding site of the destabilized molecules was not changed. Instead, in both cases, water molecules were observed to bind and created networks of hydrogen bonds that apparently maintained the binding site structure. This suggests the possibility that even an empty binding site, devoid of peptide, which is quite unstable relative to peptide/MHC complexes (27), might maintain this closed conformation as the result of binding water molecules at its ends. “Closed-empty” and “closed-full” binding sites may look similar and dominate at equilibrium, with “open-empty” and “open-full” states being more transient states separated from the closed states by the high free energy.
was too fast to measure by surface plasmon resonance, consistent
agonist. The kinetic off-rate of the A6-TCR with Tax8/HLA-A2
range that often either signals as a partial agonist or an antagonist
16-fold decrease in TCR affinity, combine somehow to decrease
atomic contact to the P1 sidechain (Leu1 ). If we model a TCR
peptide residues P4 to P8, but the TCR does cover and make one
both cases the major contacts between TCR and peptide are with
have been determined complexed with Tax9/HLA-A2 (35–37). In
empty, so that the TCR of Tax8-specific CTL must bind to a pep-
sequence or conformation.

In published studies, it is the kinetic off-rate of the TCR from the
MHC/ligand complex that most often correlates with the nature of the T cell response (48). The kinetic off-rate of A6-TCR with
Tax9/HLA-A2 is 0.093 s–1 (37, 42), in the range of a typical agonist. The kinetic off-rate of the A6-TCR with Tax8/HLA-A2 was too fast to measure by surface plasmon resonance, consistent with the extremely low T cell response observed (Fig. 6, B and C).
The affinity for a Tax9 specific αβTCR ectodomain for the
Tax8/HLA-A2 complex was measured as 16-fold lower than its affinity for Tax9/HLA-A2 (Fig. 6A). This affinity difference is at
least qualitatively consistent with the modeling that indicates a potential cavity in the interface. A 16-fold magnitude of affinity difference in other TCR/peptide/MHC complexes has been observed to be sufficient to alter T cell responses dramatically (37, 42, 49, 50). Qualitatively consistent with this expectation, we observed markedly reduced (>106 reduction) activity in Tax8-in-
duced cell lysis of a Tax9-specific T cell line (Fig 6B) and no antagonism of Tax9 activity at 1 µM Tax8 peptide concentration (Fig. 6C). Quantitatively, however, the absolute affinity, Kd = 15 ± 2 µM, of A6-TCR for the Tax8/HLA-A2 ligand, is in the range that often either signals as a partial agonist or an antagonist (37, 42), rather than as a null as observed here. This comparison suggests that the Tax8/HLA-A2 complex may have unusual properties as a cell surface ligand for a T cell. Apparently the decreased stability of the Tax8/MHC complex (T1/2 = 49°C), coupled with the 16-fold decrease in TCR affinity, combine somehow to decrease the effectiveness of the Tax8/MHC complex as a ligand in cellular assays (since neither alone prevents either partial agonist or antagonist responses).

Acknowledgments

We thank R. Crouse, A. Haykov, and C. Garnett for technical support.

References


25. Parker, K., M. DiBrino, L. Hull, and J. E. Coligan. 1992. The β2-microglobulin dissociation rate is an accurate measure of the stability of MHC class I hetero-


27. Fahnstock, M. L., I. Tamar, L. Nahri, and P. J. Bjorkman. 1992. Thermal stability comparison purified empty and peptide-filled forms of a class I MHC mol-


