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The H-2K\textsuperscript{k} MHC Peptide-Binding Groove Anchors the Backbone of an Octameric Antigenic Peptide in an Unprecedented Mode\textsuperscript{1,2}

Christine Kellenberger,\textsuperscript{3*} Alain Roussel,\textsuperscript{+} and Bernard Malissen\textsuperscript{*}

A wealth of data has accumulated on the structure of mouse MHC class I (MHCI) molecules encoded by the H-2\textsuperscript{b} and H-2\textsuperscript{d} haplotypes. In contrast, there is a dearth of structural data regarding H-2\textsuperscript{k}-encoded molecules. Therefore, the structures of H-2K\textsuperscript{k} complexed to an octameric peptide from influenza A virus (HA\textsubscript{259–266}) and to a nonameric peptide from SV40 (SV40\textsubscript{560–568}) have been determined by x-ray crystallography at 2.5 and 3.0 Å resolutions, respectively. The structure of the H-2K\textsuperscript{k}-HA\textsubscript{259–266} complex reveals that residues located on the floor of the peptide-binding groove contact directly the backbone of the octameric peptide and force it to lie deep within the H-2K\textsuperscript{k} groove. This unprecedented mode of peptide binding occurs despite the presence of bulky residues in the middle of the floor of the H-2K\textsuperscript{k} peptide-binding groove. As a result, the Cα atoms of peptide residues P5 and P6 are more buried than the corresponding residues of H-2K\textsuperscript{k}-bound octapeptides, making them even less accessible to TCR contact. When bound to H-2K\textsuperscript{k}, the backbone of the SV40\textsubscript{560–568} nonapeptide bulges out of the peptide-binding groove and adopts a conformation reminiscent of that observed for peptides bound to H-2L\textsuperscript{d}. This structural convergence occurs despite the totally different architectures of the H-2L\textsuperscript{d} and H-2K\textsuperscript{k} peptide-binding grooves. Therefore, these two H-2K\textsuperscript{k}-peptide complexes provide insights into the mechanisms through which MHC polymorphism outside primary peptide pockets influences the conformation of the bound peptides and have implications for TCR recognition and vaccine design. The Journal of Immunology, 2005, 175: 3819–3825.

Major histocompatibility complex-encoded molecules govern adaptive immune responses by presenting antigenic peptides to TCRs. MHCI\textsuperscript{4} molecules are specifically devoted to present small peptide fragments (typically eight or nine amino acids in length) mostly generated as byproducts of aberrant protein synthesis (1). Approximately 35 of them are polymorphic. Hydrogen bonds between conserved side chains of the peptide-binding groove and main chain polar atoms from the peptide provide a general, sequence-independent mechanism for binding many different peptides. These conserved hydrogen bonds are confined to both ends of the peptide-binding groove and involve peptide residues found at position 1 (P1), and at both the penultimate P(\textOmega-1) and C-terminal P(\textOmega) positions (numbering according to Madden (2)). This conserved bonding fixes the positions of the peptide N and C termini and forces the central part of peptides longer than eight amino acids to bulge out of the groove, to zigzag within the groove, or, more rarely, to extend from the termini of the groove (3).

Peptides capable of binding with high affinity to a given MHCI allele share two or three primary anchor residues, the side chains of which are specifically accommodated into MHC cavities or pockets. The polymorphic residues that line the pockets determine groove specificity and restrict the repertoire of peptides capable of binding to a given MHCI allele. Six pockets, denoted A through F, have been defined in MHCI peptide-binding grooves (2). In every MHCI allele, the side chain of the peptide residue found at the P\textOmega position constitutes a primary anchor and is deeply buried in the F pocket. The position of the second primary anchor is more variable and usually occurs at P2, P3, or P5.

Most studies on mouse MHCI molecules have focused on the H-2K\textsuperscript{b} allele and, to a lesser extent, on H-2D\textsuperscript{d}, resulting in the elucidation of their structures in complex with various peptides (4–10) and with several TCRs (11–15). More limited structural data have been obtained for H-2D\textsuperscript{d} (16, 17) and H-2L\textsuperscript{d} (18, 19). Considering that no structural information is available yet for MHCI molecules encoded by the H-2\textsuperscript{k} haplotype, we crystallized and solved the structure of the H-2K\textsuperscript{k} molecule in complex with an octapeptide derived from influenza A virus (HA\textsubscript{259–266}; FEANGNLI) (20) and with a nonapeptide derived from SV40 (SV40\textsubscript{560–568}; SEFLLEKRI) (21). These two H-2K\textsuperscript{k}-peptide complexes provide insights on the mechanisms by which MHC polymorphism outside primary peptide pockets influences the course of the bound peptides and have important implications for TCR recognition.
Materials and Methods

Protein production, crystalization, and x-ray data collection

These procedures have been previously described for H-2K\(^{\alpha}\) complexes (22).

Structure determination and refinement

The structure of the H-2K\(^{\alpha}\)-HA\(_{259-266}\) complex was determined by molecular replacement with the Amore program (23). When used as a search model, a peptide-stripped H-2K\(^{\alpha}\) molecule (PDB ID code 1LEG) failed to provide a solution when considered as a whole unit. To reach a solution, we had to separate it into the \(\alpha/\beta2\) and \(\alpha/\beta3\) domain sets. One single complex was identified in the asymmetric unit, and the HA\(_{259-266}\) peptide was then built in the resulting Fo-Fc electron density map (Fig. 1D) using the Turbo Frodo program (24). Refinement was conducted using the Refmac 5.0 program (ccp4 package; (www.ccp4.ac.uk)) with Translation Ligation refinement. Crystallographic statistics for each complex have been reported previously and are detailed in Table I (22). The \(R_{\text{free}}\) and \(R_{\text{free}}\) values for the refined model are 21.2 and 24.5, respectively. Strong 2Fo-Fc densities were observed, in particular in a cavity between the \(\alpha/\beta2\) and \(\alpha/\beta3\) domains. These densities remained, however, non-interpretable and were attributed to water molecules for the sake of refinement. Furthermore, the region that links the \(\alpha2\) and \(\alpha3\) domains (aa 176–181) could not be built due to the lack of electron density.

The structure of the H-2K\(^{\alpha}\)-SV40\(_{560-568}\) complex was solved by molecular replacement using the structure of H-2K\(^{\alpha}\)-HA\(_{259-266}\) complex being treated as \(\alpha/\beta2\) and \(\alpha/\beta3\) independent parts. The nonapeptide was built unambiguously in the Fo-Fc density map (Fig. 2B). The refinement was achieved using the CNS (25) and Refmac 5.0 programs. The final electron density map was of good quality for the \(\alpha1\), \(\alpha2\), and \(\alpha3\) domains and for the peptide, whereas \(\beta2\) was poorly defined, probably due to internal mobility. The final \(R_{\text{free}}\) values are 23.1 and 30.9, respectively (Table I). In contrast to that observed for the H-2K\(^{\alpha}\)-HA\(_{259-266}\) complex (see above), no electron density was present in the cavity between the \(\alpha/\beta2\) and \(\alpha/\beta3\) domains. The relative positions of the \(\alpha1/\alpha2\) and \(\alpha3/\alpha2\) domains differed in the two H-2K\(^{\alpha}\)-peptide complexes and diverged from those observed in MHCI structures reported to date. The differences in the relative positions of the \(\alpha/\beta2\) and \(\alpha/\beta3\) domains are common and are probably due to packing differences in the crystals. The two H-2K\(^{\alpha}\) complexes also showed small, but significant, deviations (range, 0.65–1.15 \(\AA\)) in two strands of the \(\beta\) sheet (residues 95–96 of strand S4 and residues 116–118 of strand S5). The present analysis is limited to the structure of the \(\alpha/\beta2\) domains of both H-2K\(^{\alpha}\)-peptide complexes.

Comparison with human and mouse MHCI crystal structures

The structures of the two H-2K\(^{\alpha}\)-peptide complexes were compared with human (HLA-A2, HLA-B8, HLA-B27, HLA-B35, HLA-B44, HLA-B51, and HLA-B53) and mouse (H-2Db, H-2Dd, H-2K\(^{\alpha}\), and H-2L\(^{\beta}\)) MHCI structures. The amino acid sequences of these MHCI molecules were aligned (data not shown). A first round of superimposition of each molecule on the H-2K\(^{\alpha}\)-\(\alpha/\alpha2\) domains (residues 1–175) was performed using the Cu atoms of amino acids found at positions 50, 100, and 150. The superimposition was then refined using all the Ca atoms using less than 0.5 \(\AA\) for the same cutoff distance. In our strategy, the initial 1-Å cutoff distance was decreased to 0.5 \(\AA\) in a second step. After this last calculation step, the quality of the superimposition can be estimated by the number of Ca atoms pairs distant of <0.5 \(\AA\). For instance, when H-2K\(^{\alpha}\) was compared with H-2K\(^{\beta}\), of the 175 Ca pairs that were considered, 90 of them were distant by <0.5 \(\AA\).

Results

Overall structure of the H-2K\(^{\alpha}\) complexes

The structures of the H-2K\(^{\alpha}\)-HA\(_{259-266}\) and H-2K\(^{\alpha}\)-SV40\(_{560-568}\) complexes were determined by molecular replacement at 2.5 and 3.0 \(\AA\) resolution, respectively. In both complexes, H-2K\(^{\alpha}\) showed the typical MHCI canonical fold. Superimposition of the \(\alpha/\alpha2\) domains of H-2K\(^{\alpha}\)-HA\(_{259-266}\) and of H-2K\(^{\alpha}\)-SV40\(_{560-568}\) gives average root mean square deviations of 0.57 and 0.40 \(\AA\) for 162 and 108 Ca atom positions, respectively. The \(\alpha1\) helices were well superimposed, and the \(\alpha2\) helices were shifted by 0.5–1 \(\AA\). Structural divergences between the two H-2K\(^{\alpha}\) complexes were noted at residues 39–41 (corresponding to a solvent-exposed loop connecting strands S3 and S4), residues 53–58 (corresponding to the end of a 3\(\alpha\) helix before the \(\alpha1\) helix), and residues 103–106 (corresponding to a loop connecting strands S4 and S5), with maximal deviations of 2.71, 2.64, and 2.96 \(\AA\) at residues 41, 54, and 105, respectively.

Octapeptide backbone conformation

The P1 residue at the N-terminal end of HA\(_{259-266}\) forms hydrogen bonds with residues Tyr77, Tyr159, and Tyr171 of H-2K\(^{\alpha}\). At the C-terminal end, the P(1\(\alpha\)) residue binds to H-2K\(^{\alpha}\) Thr147, and the P1\(\alpha\) residue binds to H-2K\(^{\beta}\) Asn77, Tyr84, and Thr143 (Table II). This network of interactions is highly conserved among human and mouse MHCI molecules. The overall conformation adopted by...
The deep course adopted by the central region of H-2Kb-octamer complexes is reminiscent of that of H-2Kb-bound octapeptides (Fig. 1, A and B). The Cα trace of H-2Kb-octapeptides differs slightly from H-2Kb-bound peptides at P2, P5, and P6. For instance, the Cα atom of P2 is higher up by ~1 Å, whereas the P5 and P6 Cα atoms are more buried than those of H-2Kb-bound octapeptides. The protrusion of the Cα atom at P2 of H-2Kb might be explained by the presence of bulky residues (His9, Tyr45, and Tyr99) that decreases the volume of the B pocket (see below), and by the need to accommodate the long side chain of the Glu residue found at P2 of H-2Kb-octamers.

<table>
<thead>
<tr>
<th>Data collection</th>
<th>H-2Kb/HAA(259–266)</th>
<th>H-2Kb/SV40(560–568)</th>
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</thead>
<tbody>
<tr>
<td>Resolution (Å)</td>
<td>15.0–2.5</td>
<td>15–3.0</td>
</tr>
<tr>
<td>Space group</td>
<td>P321</td>
<td>P2</td>
</tr>
<tr>
<td>Cell dimension</td>
<td>111.76; 109.42</td>
<td>85.13; 72.63</td>
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<tr>
<td>Number molecules/asymmetric unit</td>
<td>26,053</td>
<td>18,302</td>
</tr>
<tr>
<td>Number of reflections</td>
<td>8.9</td>
<td>4.6</td>
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<tr>
<td>Completeness (%)</td>
<td>99.3</td>
<td>90.2</td>
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<tr>
<td>Rsym (%)</td>
<td>6.9 (33.8)</td>
<td>15.8 (39.7)</td>
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</table>

Refinement statistics

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<th>23.1 (26.7)*</th>
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<td>Rsym</td>
<td>24.5 (33.1)*</td>
<td>30.9 (37.4)*</td>
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<tr>
<td>Total number of atoms</td>
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<td>6,480</td>
</tr>
<tr>
<td>from protein</td>
<td>3,132</td>
<td>6,334</td>
</tr>
<tr>
<td>from water</td>
<td>182</td>
<td>146</td>
</tr>
<tr>
<td>Rmsd from ideal geometry</td>
<td>0.009</td>
<td>0.010</td>
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<tr>
<td>Bond length (Å)</td>
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<td>1.21</td>
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<tr>
<td>Ramachandran plot</td>
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<td></td>
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<tr>
<td>Most-favored regions (%)</td>
<td>91.4</td>
<td>82.5</td>
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<tr>
<td>Allowed regions (%)</td>
<td>8.0</td>
<td>14.8</td>
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<tr>
<td>Generously allowed regions (%)</td>
<td>0.6</td>
<td>2.7</td>
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<tr>
<td>B factors</td>
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<td></td>
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<tr>
<td>α-chain</td>
<td>29.8</td>
<td>22.6</td>
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<tr>
<td>β-chain</td>
<td>27.7</td>
<td>43.8</td>
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<td>Peptide</td>
<td>27.2</td>
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<tr>
<td>Average water molecules</td>
<td>47.8</td>
<td>11.4</td>
</tr>
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</table>

* R factor and R free in the last shell (2.5–2.56).

The specificity of the B pocket

The Cα atom found at P6 of H-2Kb-octamer complexes. An absence of contact with the MHC is also found for the P6 main chain of peptides bound to H-2Dβ, thereby favoring some conformational malleability, and in HLA-A2-peptide complexes, where Cα displacements can even occur across residues P4 to P6 (26). Therefore, the H-2Kb-HA259–266 complex, the bidentate anchoring of the P6 main chain through both Asp77 and Tyr116 probably prevents any wobbling of the P6 Cα atom and constitutes a distinctive attribute among the MHCI molecules studied to date.
Therefore, the structural environment of the B pocket is largely conserved between mouse H-2Kk and human HLA-B44 and is consistent with their preference for a long, negatively charged side chain as found in Glu.

Nonapeptide backbone conformation

Although both H-2Kk and H-2Kb bind predominantly octapeptides, they are also capable of accommodating nonapeptides (see http://syfpeithi.bmi-heidelberg.com/). Studies of the binding of a series of nonapeptides to H-2Kb revealed that no canonical binding mode exists, except for the conformational constraint imposed by the presence of a central primary anchor at P5 or P6, that is accommodated by the deep pocket that characterizes the groove of H-2Kk (4, 6, 8). Considering that H-2Kk lacks such a deep recess, we analyzed next how it accommodates the SV40 560–568 nonapeptide. Compared with the HA259–266 octamer, the extra residue present in the SV40 560–568 nonapeptide is accommodated through a prominent bulge in its central part. Comparison of SV40 560–568 with nonapeptides bound to human and mouse MHCI molecules shows that it adopts a Cα trace reminiscent of that of peptides bound to H-2Lk (Fig. 3C), which all use Tyr116 to make an indirect hydrogen bond with the P7 carbonyl oxygen of nonapeptides. Therefore, the binding of the SV40 560–568 nonamer to H-2Kk involves fewer hydrogen bonds than that of the HA259–266 octamer.

Solvent exposure of H-2Kk-bound peptides

The quality of the electron density map enabled us to build unambiguously the HA259–266 octapeptide except at P6 where the asparagine shows a double conformation (Fig. 1D and Table II). In conformer A, the side chain of AsnP6 is clamped by two hydrogen bonds involving Asp156 and by two indirect bonds with Asp114 and Tyr116. As a consequence, AsnP6 is buried within the cleft and oriented toward the α2 helix. In conformer B, the side chain of AsnP6 is hydroxylated and points outside the groove. Superimposition of HA259–266 with H-2Kk-bound octapeptides shows that conformer B of AsnP6 lies deeper in the groove by 1–2 Å (for the Cα atom) and 2–5 Å (for the apex of the side chain; Fig. 1B).

The degree of solvent exposure of HA259–266 bound to H-2Kk and of several octapeptides bound to H-2Kb was calculated for each position using the Turbo-Fredo program (Fig. 4). Peptide side chains at positions 2, 3, 5, and 8 are deeply buried in both H-2Kk and H-2Kb, whereas side chains at P4, P6, and P7 are pointing up and show variable degrees of solvent exposure (P4, 53–104 Å²; P6, 16–148 Å²; P7, 22–92 Å²). Among the P6 residues examined, AsnP6 of HA259–266 is the least accessible. Thus in the H-2Kk–HA259–266 complex, there are only two residues, AsnP4 and LeuP7, that remain available for TCR contact.

In the case of the SV40 560–568 nonamer bound to H-2Kk, the side chains found at P1, P2, P5, and P7 of are not available for TCR contact, as shown by their low solvent exposure (Table III). In contrast, residues P4, P6, and P8 of SV40 560–568 are exposed to solvent. Moreover, the side chain of GluP6 of SV40 560–568 shows a similar exposure as the TyrP6 of the H-2Kb-bound, Dev8 octapeptide and the IleP6 of the H-2Lk-bound, p29 nonapeptide (Table III and Fig. 3C). Therefore, the prominent accessibility of
GluP6 of SV40560–568 contrasts with the almost buried conformation of AsnP6 of HA259–266.

Discussion

The middle section of H-2Kb-bound peptides is deeply embedded in the peptide-binding groove as a consequence of the deep pocket that lies in the midportion of the H-2Kb peptide-binding groove and accommodates the primary anchor residue found at P5. In contrast, the corresponding pocket of H-2Kk is lined by residues bulkier than those found in H-2Kb. Despite this marked difference in midgroove architecture that should result in different peptide conformations, the central part of the HA259–266 octameric peptide also adopts a flat conformation that runs even deeper than that of H-2Kb-bound octapeptides. This unexpected finding is due to the binding of the main chain of the octamer by residues from the floor of the H-2Kk groove, in particular Arg97. The structure of H-2Kk-HA259–266 highlights the fact that bulky residues belonging to the β sheet of an MHC-I peptide-binding groove can be used to anchor the central region of an octapeptide main chain. This is an unprecedented observation that differs, for instance, from H-2Kb-octamer complexes, where the central region of the peptide backbone is contacted exclusively by the side chains of amino acids belonging to α-helices. The contributions of Arg97, Tyr99, and Tyr116 to peptide binding have already been evoked for HLA-B35 (28) and HLA-B27 (29). In both these instances, however, hydrogen bonds were made with the side chains and not the main chain of the bound peptides.

The crucial role played by residue Arg97 in the architecture of the H-2Kk peptide-binding groove can be emphasized by analyzing other MHC alleles with an Arg residue at position 97. This comparison shows that the side chain of Arg97 adopts four distinct conformations according to the nature of neighboring residues (Fig. 5). Two of these conformations are alternatively used by HLA-A2 depending on the sequence of the peptide to which it binds (30), one is used by the two closely related HLA-B35 and HLA-B53 alleles (31), and the last one is used by both HLA-B44 (27) and the two H-2Kk complexes reported in this paper. As pointed out by Madden et al. (30), alternate conformations of Arg97 and Tyr116 in HLA-A2 are used to bind to different peptide sequences. The flexibility of Arg97 is also involved in the structural reorganization that occurs within the Tax-HLA-A2 complex upon TCR binding (32, 33) (Fig. 5). Some conformational malleability has also been reported for Arg97 of HLA-B53 (31). Therefore, the coincident position noted for Arg97 in the two H-2Kk complexes reported in this study suggests that it is probably rigid and markedly contrasts with the malleability of Arg97 in HLA-B53 and HLA-A2. This unique feature has probably been selected to allow Arg97 of H-2Kk to directly bind octamers and nonamers.

Taking into account the likely rigidity of Arg97, simulation studies were undertaken to determine the repertoire of residue conformations that can be accommodated within the H-2Kk groove, especially at position P5. These studies demonstrate that in the absence of major structural rearrangement of the backbone, it is impossible
to fit an aromatic residue at P5 and also suggest that most H-2K\(^b\)-bound peptides will conserve a C\(^r\)/H9251 trace similar to that of HA259–266 as long as their P5 residue is not bulkier than Leu/Ile. Consistent with this prediction, the syfpeithi database (http://syfpeithi.bmi-heidelberg.com) shows that 19 octapeptides of the 22 reported to bind H-2K\(^b\) have small side chains at P5. A minimal epitope of Mycobacterium tuberculosis (MT) recognized by CD8 T cells in the context of H-2K\(^b\) molecules has been recently reported (34). Docking of this MT-derived peptide into the H-2K\(^b\) binding groove can be achieved without steric clash, and this suggests the possibility of accommodating an Ala at P5. Together, these data suggest that the C\(^r\) trace adopted by the HA259–266 octamer constitutes a generic feature of octapeptides bound in the H-2K\(^b\) groove.

After the generation of an allele-neutral TCR repertoire in early thymocytes, a phase of molecular matching arises through positive selection of TCRs capable of coping with a composite surface made of self-peptide side chains and of MHC determinants that are both conserved and allele specific. There are probably fewer constraints linked to matching the few allele-specific residues found on the top of the MHC helices and available for TCR contacts than adapting to the diversity of peptide side chains and to the generic features that are imposed on the bound peptides by the architecture of a given MHC peptide-binding groove. For instance, TCR read-out of peptides that follow a flat and deep course within the MHCI groove, as exemplified by octapeptides bound to H-2K\(^b\), generates structural constraints distinct from the readout of H-2D\(^b\)- and H-2L\(^b\)-bound peptides that protrude out of the C-terminal part of the peptide-binding groove. Such a challenge is in part met by the flexibility of the TCR Ag-binding site (35, 36). Conformational adjustments at the level of the bound peptides also maximize matching at the TCR-peptide-MHC interface (37, 38). We showed that in the case of H-2K\(^b\), the HA259–266 octapeptide displays a low degree of solvent exposure. Moreover, our modeling studies suggest that an MT-derived and H-2K\(^b\)-bound peptide is even less exposed to TCR scrutiny than peptide HA259–266; the solvent accessibilities of the P4, P6, and P7 residues of the MT-derived peptide are 91, 17, and 68 Å\(^2\), respectively. No TCR structure bound to H-2K\(^b\)-octamer complex is yet available, and it remains to be determined how the TCR binding site manages to accommodate these peptides that lack prominently exposed residues and appear rather featureless to the TCR. Along that line, the crystal structure of a human TCR directed to a peptide derived from influenza virus and presented by HLA-A2 showed that very few docking possibilities exist to specifically recognize peptide lacking surface-exposed side chains (39).

Table III. Solvent accessibility of peptide residues in the H-2K\(^b\)-HA(259–266) and H-2K\(^b\)-SV40(560–568) complexes\(^a\)

<table>
<thead>
<tr>
<th>Residue of HA(259–266)</th>
<th>Solvent Accessible Surface (Å(^2))</th>
<th>Residue of SV40(560–568)</th>
<th>Solvent Accessible Surface (Å(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe(^{p1})</td>
<td>32</td>
<td>Ser(^{p1})</td>
<td>7</td>
</tr>
<tr>
<td>Glu(^{p2})</td>
<td>7</td>
<td>Glu(^{p2})</td>
<td>8</td>
</tr>
<tr>
<td>Ala(^{p3})</td>
<td>10</td>
<td>Phe(^{p3})</td>
<td>10</td>
</tr>
<tr>
<td>Asn(^{p4})</td>
<td>104</td>
<td>Leu(^{p4})</td>
<td>78</td>
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<tr>
<td>Gly(^{p5})</td>
<td>7</td>
<td>Leu(^{p5})</td>
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</tr>
<tr>
<td>Phe(^{p6})</td>
<td>16/25</td>
<td>Lys(^{p7})</td>
<td>3</td>
</tr>
<tr>
<td>Asn(^{p8})</td>
<td>92/82</td>
<td>Arg(^{p9})</td>
<td>108</td>
</tr>
<tr>
<td>Leu(^{p9})</td>
<td>7</td>
<td>Ile(^{p10})</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^a\)The values were determined using the program Turbo-Frodo. The water molecules were omitted for the calculation. For positions P6 and P7 of HA(259–266), the two values correspond to conformers A and B.
In conclusion, the two H-2K\(^b\) crystal structures reported in this paper together with data resulting from the sequencing of naturally processed peptides eluted from H-2K\(^b\) show that the H-2K\(^b\) binding motif is composed of two primary anchors at P2 and P4. Pocket B accommodates residue P2 and shows a strong preference for negatively charged residues with long aliphatic chain (Glu), whereas pocket F prefers aliphatic residues (Ile and Val) at the peptide C terminus. The dominant solvent-accessible residues of the H\(_{2}A_{599–666}\) octapeptide are found at P4 and P7, whereas P6 is poorly solvent accessible. In the SV\(_{40}560–568\) nonapeptide bound to H-2K\(^b\), residues at position P4, P6, and P8 are fully solvent accessible. The structure of H-2K\(^b\) in complex with an octapeptide also shows that side chains from residues of the floor of the peptide-binding groove can be used to directly lock an octapeptide main chain. This constitutes a unique feature among the MHCI-peptide complexes studied to date and results in a central peptide region deeply embedded in the groove and poorly accessible to TCR contact. Given that the structures reported in this study represent a novel conformation of bound peptides not hitherto used for modeling TCR-peptide-MHCI interactions, they may help ab initio epitope identification.

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Disclosures
The authors have no financial conflict of interest.

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