Three-dimensional structure of a peptide extending from one end of a class I MHC binding site

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Class I major histocompatibility complex (MHC) molecules present peptides to CD8+ T cells for immunological surveillance (reviewed in ref. 1). The structures of complexes of class I MHC molecules with octamer, nonamer and decamer peptides determined until now2,4 show a common binding mode, with both peptide termini bound in conserved pockets at the ends of the peptide binding site. Length variations were accommodated by the peptide bulging5,6 or zig-zagging7 in the middle. Here we describe the structure of a decamer peptide which binds with the carboxy-terminal residue positioned outside the peptide binding site. Several protein side chains have rearranged to allow the peptide to exit. The structure suggests that even longer peptides could bind. The energetic effect of the altered mode of binding has been assessed by measuring the stability of the complex to thermal denaturation. Peptides bound in this novel manner are stable at physiological temperature, raising questions about their role in T-cell recognition and their production by proteolytic processing.
Each HLA-A2.1/peptide complex was purified and melting temperature assay carried out as described previously. Briefly, the complexes (4-12 μM) were subjected to increasing temperature while the change in circular dichroic signal at 218 nm was monitored every 1.0 °C in an Aviv 60DS spectropolarimeter. The Tm was derived from the least-squares fit of the plot of molar ellipticity versus temperature using a two-state unfolding algorithm. Data shown above are the average of two independent experiments with 2-3 melting curves per experiment. The error associated with the fitted parameter Tm is less than 0.5 °C. ΔTm is the change in the melting temperature from that of the nonamer MLLSVPLLP.

Following the observation that long peptides without appropriately positioned anchor residues bind class I molecules in vivo and the suggestion that a decamer from the signal sequence of calreticulin might not bind in the carboxyterminal end of the binding site of HLA-A2, we determined the structure of that decamer (MLLSPVPLLP) complexed with HLA-A2 by X-ray diffraction (Fig. 1, methods). Initial electron density maps were interpretable for the entire length of the peptide. The C-terminal glycine projects out of the peptide binding cleft (Figs. 1 and 2a). The first nine residues of the decamer bind to HLA-A2.1 almost identically to conventional nonamers; the locations of the aminoterm inus and the P2 anchor residue overlap those from a collection of viral peptides bound to HLA-A2 (Fig. 2b) and the P9 side chain and carbonyl oxygen overlap the P9 anchor and one oxygen of the carbohydrate groups of conventionally bound nonamers (Fig. 2c). The pattern of hydrogen bonds made at the C terminus is different, however. In the conventional mode of binding (Fig. 2d), four hydrogen bonds are made to the peptide carboxyterminal oxygens, three from conserved MHC residues (Thr 143, Lys 146, Tyr 84) and one from a nonconserved residue (Thr 80 through H2O). Only one of those bonds (Thr 143) is observed in the calreticulin decamer complex, although one new hydrogen bond is also formed between Lys 146 and the exiting P10 carboxyterminal group of the calreticulin decamer (Fig. 2c).

Two of the four class I residues that participate in hydrogen bonding to conventionally bound peptides have relocated in the calreticulin peptide/HLA-A2 complex. Tyr 84 has rotated away from the peptide P9 carboxyl removing the potential for a hydroxyl bond (Fig. 2e). Lys 146 has rotated out of the site to form a salt bridge with the P10 carboxylate. These movements create an opening through which the peptide C terminus can extend out into solvent (compare Fig. 3a and b).

We have assessed the energetic consequences of this novel binding mode by comparing the thermal stabilities of the calreticulin decamer complex to that of a complex with a peptide with the same first 9 amino acids (MLLSPVPLLP), containing anchor residues at conventional positions (P2 Leu, P9 Leu) (Table 1). The complex with the nonamer peptide thermally denatures at 65.8 °C, a temperature comparable to the naturally occurring antigenic peptide of the influenza virus M1 protein (residues 58-66) complexed to HLA-A2.1. The decamer, MLLSPVPLLP, denatures at 14 °C lower, 51.7 °C. This 14 °C difference is about half that observed (~2 °C) when all four C-terminal hydrophobic bonds (Fig. 2d) are eliminated by replacing the calreticulin complex with a methyl group on the influenza virus M1 peptide. Because the crystal structure of the calreticulin decamer suggests that long peptides might extend even further out of the binding site, we tested a synthetic 11-residue oligonucleotide with arginine (a non-anchor residue for HLA-A2) added to the C terminus of the decamer. That complex denatured at 48.0 °C, an additional loss of only 3.7 °C compared to the decamer, arguing that it also extends out of the site.

Peptides bound to class I molecules in vivo are derived from cellular proteins by one or two mechanisms. Most appear to arise from cytoplasmic proteolysis followed by transport into the endoplasmic reticulum (ER) (reviewed in ref. 1), but some derive from signal sequences that enter the ER lumen as part of a secreted protein. The decamer (MLLSPVPLLP) studied here is the N-terminal 10 amino acids of the 17 amino-acid signal sequence of calreticulin. It is found bound to HLA-A2 even in cells lacking peptide transporter functions. The nonamer, MLLSPVPLL, binds with substantially higher affinity than the decamer (Table 1), yet is not detected bound to HLA-A2 in vivo. These observations suggest that the nonamer is not produced in vivo.

It has been suggested that peptides may be trimmed, by for example a carboxy peptidase, while bound to class I molecules. The X-ray structure (Fig. 3b) shows that the peptide carboxyterminal oxygens, even in this peptide which extends out of the site, are bound too closely to the MHC protein to fit simultaneously into the active site of a protease. Instead, trimming of successive

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TABLE 1 Melting temperatures (Tm) of complexes containing peptides related to MLLSPVPLLP

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Source</th>
<th>Tm (°C)</th>
<th>ΔTm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLLSPVPLL</td>
<td>Calreticin</td>
<td>65.8</td>
<td>—</td>
</tr>
<tr>
<td>MLLSPVPLL</td>
<td>Calreticin</td>
<td>51.7</td>
<td>-14.1</td>
</tr>
<tr>
<td>MLLSPVPLL.G</td>
<td>Experimental</td>
<td>48.0</td>
<td>-17.8</td>
</tr>
</tbody>
</table>

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Fig. 1. "Omit" [I2FJ - Fc] electron density for the peptide MLLSPVPLLP. The electron density is calculated from phases derived from iterative cycles of non-crystallographic real-space averaging of the two molecules in the asymmetric unit using a model without peptide. The path of the peptide is clearly visible and the glycine at P10 is located out of the peptide binding site.

METHODS. Soluble HLA-A2 complexed with the peptide MLLSPVPLLP was produced as described previously and co-crystallized. X-ray data were collected to 1.9 Å resolution on single crystals (~180° C, 0.15° oscillation frames with a Xentronics detector on an Enraf-Nonius Kappa GX-13 rotating anode generator with a 100 μm focal cup and focusing mirrors). The space group was determined by examination of the difference in intensities. Peptide maps bound to conventional peptides were found in the asymmetric unit with a model peptide. The path of the peptide is clearly visible and the glycine at P10 is located out of the peptide binding site.

Fig. 2. Omit [I2FJ - Fc] electron density for the peptide MLLSPVPLLP. The electron density is calculated from phases derived from iterative cycles of non-crystallographic real-space averaging of the two molecules in the asymmetric unit using a model without peptide. The path of the peptide is clearly visible and the glycine at P10 is located out of the peptide binding site.

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C-terminal peptide residues might occur by a cycle of dissociation of a few C-terminal residues, proteolytic cleavage of the exposed terminal residue, and rebinding. By such a "nibbling" mechanism a nonamier might never be produced if the decamer bound tightly enough to be transported to the cell surface with the MHC molecule.

The extraction of long peptides from class I molecules in vivo and the thermal stability well above 37°C measured here, suggest that the unconventional mode of peptide binding observed crystallographically may be found on cell surfaces. It has been argued from both peptide pool sequences and thermodynamic data, that peptides could extend from either end of the binding site. The peptide extensions out of the binding site must be geometrically different from those found on class II MHC molecules (compare Fig. 3a here with Fig. 4 in ref. 18) because of differences in the shape of the binding sites. It will...
be interesting to see if the novel surfaces presented by peptides extending out of the ends of class I MHC binding sites are recognized by αβ or γδ T cells.

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