The Crystal Structure of H-2Db Complexed with a Partial Peptide Epitope Suggests a Major Histocompatibility Complex I Assembly Intermediate*

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In the absence of bound peptide ligands, major histocompatibility complex (MHC) class I molecules are unstable. In an attempt to determine the minimum requirement for peptide-dependent MHC class I stabilization, we have used short synthetic peptides derived from the Sendai virus nucleoprotein epitope (residues 324–332, \( ^1 \text{FAPGNYPAL} \)) to promote its folding in vitro of H-2D\(^b\). We found that H-2D\(^b\) can be stabilized by the pentapeptide \( ^5 \text{NYPAL} \), which is equivalent to the C-terminal portion of the optimal nonapeptide (the \( \alpha_3 \) and \( \beta_2 \)-m domains) that support a membrane distal \( \alpha_1 \)-\( \alpha_2 \) "superdomain." The peptide-binding site is formed by a deep cleft between two \( \alpha \)-helices in this superdomain. Antigenic peptides are always bound in the same orientation, with their N and C termini lying buried deep in pockets that define the ends of the peptide-binding groove (the A and F pockets, respectively). In addition, so-called "anchor residues" make allele specific interactions with polymorphic class I residues located deep inside the binding groove, in "specificity-determining pockets."

The peptide-MHC class I complex is formed in the endoplasmic reticulum (ER) and marks the end point of antigen processing (2). During antigen processing, proteins are unfolded and partially hydrolyzed in the cytoplasm, and the resulting polypeptides (of between 8 and 40 amino acids) are translocated across the ER membrane by the transporter associated with antigen processing. Once in the ER, some long peptide epitope precursors can undergo further trimming by the aminopeptidase ERAAP (3, 4) and are selected for assembly with newly synthesized MHC class I molecules that is dependent on their interaction with cofactor molecules such as calreticulin, tapasin, and ERP57.

The process results in the preferential release from the ER of class I molecules presenting peptides that bind stably. Recent evidence suggests that this selection of high affinity peptides in vivo may occur by a mechanism that is more complex and controlled than simple competition between potential ligands for binding to class I in the ER (2) and may involve editing of the MHC-bound peptide repertoire in the early secretory pathway of antigen-presenting cells.

It is not known whether the loading or editing of class I MHC peptide cargo is associated with a conformational change in the class I molecule. All MHC class I structures solved so far show that the antigenic peptides are deeply buried in the peptide-binding groove, with typically 80% of their surface area rendered solvent-inaccessible. It therefore seems likely that some form of conformational change would have to occur in the class I HC for this to happen, because peptides appear, from these structures, to be "trapped" in the peptide-binding site. Evidence for such a conformational change comes from kinetic analysis of peptide binding to class I (5), changes in fluorescence resonance energy transfer on peptide binding (6), and differential reactivity with monoclonal antibodies (7). Stable peptide binding, in turn, results in a more stable interaction between HC and \( \beta_2 \)-m, giving complexes that will persist at the cell surface long enough to induce a T cell response. In an attempt to visualize the structure of an MHC class I molecule bound to a suboptimal peptide (which is loaded in vivo was expected to be "edited out" of the repertoire of peptides prior to the presentation of class I MHC to the cell surface), we have cocrystallized H2-D\(^b\) with a (penta)peptide that has a low affinity for binding to H2-D\(^b\) and stabilizes it poorly. However, the structure of H2-D\(^b\) in this quasi-stable complex is indistinguishable from its structure when bound to a high affinity peptide ligand, despite the fact that its peptide-binding groove makes contact only with the C-terminal three amino acids, leaving most of the groove unoccupied. Thus, it appears that if a peptide-induced conformational change does occur in the peptide-binding domains of MHC class I, it is
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likely that it can be triggered by minimal occupancy of the peptide-binding groove.

**EXPERIMENTAL PROCEDURES**

**Peptides**—FAPGNYPAL corresponds to residues 324–332 of the Sendai virus nucleoprotein, binds to both H-2D^b and H-2K^b, and was synthesized by M. Pitkeathley and S. Shah at the Oxford Centre for Molecular Sciences (University of Oxford, Oxford, UK). All other peptides were synthesized by Research Genetics (Paisley, UK). Peptide purity was determined by reverse phase HPLC (C_8 microsorb column; Rainin, Woburn, MA) prior to use.

**Refolding of Recombinant H-2D^b**—Soluble H-2D^b HC corresponding to amino acids 1–270 was expressed as inclusion bodies in BL21 (DE 3) exactly as previously described (8). 50-μg aliquots of HC solubilized in 8 M urea were refolded in 2 ml of 100 mM Tris, pH 8.0, containing 2 mM EDTA, 0.5 M arginine, 0.5 mM oxidized, and 5 mM reduced glutathione, 36.5 μg of refolded purified human β2-m, and 30 μg of peptide dissolved in 100 mM Tris, pH 8.0. After 24 h at 4 °C, the yield of refolded H-2D^b was assessed using an ELISA-based inhibition assay as previously described (8). Briefly, 100 μl of clarified, refolding mix was preincubated with the conformation-sensitive monoclonal antibody B22.249 at 2.45 μg/ml before transferring the whole mixture in triplicate to wells of a 96-well flat bottomed plate coated with purified refolded H-2D^b-ASNENMDAM complexes (1 μM) and blocked (2% bovine serum albumin). After removal of the mixture, the wells were washed, and residual B22.249 binding was detected with alkaline-phosphatase-conjugated goat anti-mouse IgG antibody. Unless otherwise stated, the graphs show the averages of three titrations with each peptide performed on different days. The results are expressed as percentages of maximum inhibition, which is proportional to the yield of B22.249-reactive material in solution. The ELISA was standardized with purified, refolded H-2D^b-ASNENMDAM complexes of known concentration. Standard curves were very reproducible and characteristically gave half-maximum inhibition values (I_50) of 2.0 μg/ml.

For thermal denaturation measurements and crystallography, partially purified H-2D^b complexes produced in CHO cells (9) were dialyzed against phosphate-buffered saline and then denatured in 6 M guanidine HCl overnight at 4 °C. Heavy chain and β2-m were separated by gel filtration and recombined by adding heavy chain (final concentration, 0.8 μM) and β2-m (final concentration, 1.2 μM) to sufficient refolding buffer to dilute the urea to a final concentration of less than 100 μM. Refolded material was concentrated, purified by gel filtration, and confirmed to be correctly folded H-2D^b by immunoprecipitation with mAb B22.249. These purified class I molecules have been defined as "empty" according to several criteria (5) and do not crystallize. These molecules were then mixed with the desired concentration of peptide (HPLC purified to 99%) prior to use in experiments.

**Thermal Denaturation Measurements**—Purified, peptide-receptive H-2D^b molecules expressed in CHO cells were diluted to 270 nM in 2 ml of phosphate-buffered saline containing peptide at the specified concentration and were incubated at 4 °C overnight to allow binding. The mixture was then placed in a stirred 1-cm fluorescence cuvette and equilibrated to 10 °C for 10 min before being heated to 80 °C at a rate of 0.5°C/min. Fluorescence emission intensity was measured (λ_em = 280 nm, λ_exc = 345 nm) on a Perkin-Elmer LS50B spectrometer. The rate of change of emission intensity with temperature was calculated from the smoothed temperature curves.

**Crystal Structure Determination**—Crystals of H-2D^b complexed with the pentapeptide NYPAL were grown by sitting drop vapor diffusion at 4 °C using microbricodes (10). The drops contained 1 μl of protein solution (typically 10 mg/ml H-2D^b NYPAL complex in 20 mM Tris, 150 mM NaCl, pH 7.5) plus 1 μl of reservoir solution (15–23% polyethylene glycol 6000, 100 mM ammonium sulfate, and 100 mM MES, pH 5.0). The crystals were harvested to a modified reservoir solution containing 25% polyethylene glycol 6000, 100 mM ammonium sulfate, 100 mM sodium chloride, and 50 mM MES, pH 5.0. For cryo-crystallographic data collection, the crystals were transferred stepwise to harvest buffer supplemented with progressively higher concentrations of glycerol up to a final concentration of 20%, flash cooled in liquid propane, and stored at −170 °C in liquid nitrogen. X-ray diffraction data were collected at station 7.2 of the UK Synchrotron Radiation Source (Daresbury) with a MarResearch 30-cm image plate detector. The data were autoindexed, integrated, scaled, and merged using the programs DENZO and SCALEPACK (11). Crystallographic statistics are reported in Table 1. The structure of H-2D^b complexed with nonamer peptides has previously been determined in an essentially isomorphous unit cell (9). These coordinates (minus peptide) were therefore used as an initial model for refinement of the H-2D^b NYPAL crystal structure using standard protocols in the programs XPLOR (12) and, latterly, CNS (13). Electron density of residues YPAL of the peptide was unambiguous in 2F_o – F_c and F_o – F_c ϕ_r_e_l maps from the earliest stages of the refinement. These residues were added to the model manually using the program O (14). Subsequent refinement cycles and manual rebuilding allowed two glycerol molecules and several water molecules to be positioned within the peptide-binding groove. The statistics for the final refined structure are presented in Table 1. Protein

**TABLE 1**

Statistics for crystallographic data collection and refinement

| Parameter | Value |
|-----------|-------|
| Space group | P2_1 |
| Unit cell a (Å) | 61.3 |
| Unit cell b (Å) | 58.6 |
| Unit cell c (Å) | 74.0 |
| Resolution range (Å) | 30-2.55 |
| Completeness (outer) (%) | 95.8 |
| Total observations | 39,041 |
| Uniqueness | 15.880 |
| Average I/σ(I) (outer) | 19.8 |
| Rmerge (outer) (%) | 4.9 |

**Model refinement**

| Parameter | Value |
|-----------|-------|
| Resolution range (Å) | 20-2.55 |
| Reflections (working set/test set) | 14,465/1,232 |
| Rmerge/R_factor (%) | 23.9/29.7 |
| Root mean square deviation from standard stereochemistry | 0.007 |
| Bonds (Å) | 1.33 |
| Angles (°) | 3.116 |
| Number of atoms | 3.116 |
| Protein | 12 |
| Glycerol | 101 |
| Water | 89.3 |
| Ramachandran plot | 10.1 |
| Most favored (%) | 0.3 |
| Additional (%) | 0.3 |
| Generous (%) | 0.3 |
| Disallowed (%) | 0.3 |

**B factors (Å)**

| Component | Value |
|-----------|-------|
| MHC heavy chain + β2-m | 33.8 |
| Main chain | 35.3 |
| Side chain | 34.2 |
| Peptide | 35.3 |
| Main chain | 35.3 |
| Side chain | 59.6 |
| Glycerol (2 molecules) | 34.2 |
| Waters in groove (17 molecules) | 30.0 |
| Waters outside groove (84 molecules) | 30.0 |

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6 J. Tormo, unpublished data.
stereochemistry was monitored using the program PROCHECK (15).

RESULTS

Refolding H-2D\(^b\) with the Pentapeptide \(\text{NYPAL}\)^5—Previous data, using a cell lysate-based MHC class I assembly assay, have suggested that the first contact between a potential peptide ligand and the MHC class I peptide-binding groove might be between the C terminus of the peptide and the so-called F pocket of the binding groove (16, 17). However, using this assay, no binding to H-2D\(^b\) could be detected for peptides that are shorter than the optimal nonamer by one amino acid residue or more at the N terminus, at concentrations up to 1 mM (8).\(^7\) Accordingly, we assessed the ability of the C-terminal portion of the Sendai virus nucleoprotein peptide (\(\text{NYPAL}\)^5) containing both H-2D\(^b\) anchor residues (underlined) to support the assembly of soluble H-2D\(^b\) HC and \(\beta2\)-m in \textit{vitro} refolding reactions. Small scale experimental refolds were set up, each containing 50 \(\mu\)M of denatured H-2D\(^b\) HC and 36.5 \(\mu\)M of \(\beta2\)-m (expressed in \textit{Escherichia coli}) plus peptide concentrations ranging from 1 mM (equivalent to a 1,000-fold molar excess of peptide over HC) to 100 \(\mu\)M. The relative yield of correctly folded and assembled H-2D\(^b\) was then assayed by inhibition ELISA.

Fig. 1 shows that, in contrast to the data obtained in cell lysates, \(\text{NYPAL}\)^5 is capable of supporting refolding, albeit less effectively than the full-length peptide. Binding was dependent on the inclusion of residues Asn\(^5\) and Tyr\(^6\), because the C-terminal tripeptide \(\text{PAL}\)^3 was even less efficient at supporting refolding than \(\text{NYPAL}\)^5. The N-terminal pentapeptide \(\text{FAPGN}\)^5 did not support refolding above background at any concentration tested. In separate experiments with the irrelevant peptide \(\text{TYQRTRALV}\), no refolding could be detected in this assay at any concentration tested.

\(\text{H-2D}^b\text{NPAL}^5\text{ Complexes Are of Intermediate Stability—}\)We next assessed the thermal stability of the complex formed between H-2D\(^b\) and \(\text{NYPAL}^5\) using intrinsic tryptophan fluorescence. For these experiments, class I peptide complexes were made by refolding peptides with denatured, soluble H-2D\(^b\) HC and \(\beta2\)-m produced in CHO cells. These complexes were chosen in preference to the molecules produced in \textit{E. coli} to allow maximum compatibility with structural analyses, because prior experience indicated that CHO derived H-2D\(^b\) complexes showed a greater propensity to yield diffracting crystals.\(^5\) Fig. 2 shows that at 50 \(\mu\)M, when \(\text{NYPAL}^5\) binding was saturated, a single transition point at 40 °C was seen. H-2D\(^b\) had a melting temperature \(T_m\) of −54 °C when bound to \(\text{FAPGNYPAL}^5\) and a \(T_m\) of 32 °C in the absence of any bound ligand. The H-2D\(^b\) NPAL\(^5\) complex was therefore of intermediate stability between empty MHC class I molecules and the complex made with wild type peptide FAPGNYPAL and had a comparable thermal transition temperature to complexes formed with the peptide \(\text{FAPGAYPA}^\alpha\) in which the two anchor residues (Asn\(^5\) and Leu\(^6\)) were changed to alanine \(T_m = 40 °C\).\(^9\) The addition of 22 \(\mu\)M \(\text{FAPGN}\)^9 had no stabilizing effect but increased the melting temperature to 38 °C when added to 225 \(\mu\)M. At this concentration, even the irrelevant peptide TYQRTRALV had a slight stabilizing effect, increasing the melting temperature by 3–4 °C (data not shown).

Crystal Structure of the H-2D\(^b\) NPAL\(^5\) Complex—The crystal structure of H-2D\(^b\) with bound \(\text{NYPAL}^5\) at 2.55 Å resolution (Fig. 3a) reveals no significant differences in the main chain conformation of the class I heavy chain residues (root mean square deviation for 177 Ca

\(^{7\ a}\) A. Glithero, unpublished observation.

\(^{8\ a}\) J. Tormo and A. Glithero, unpublished observations.

\(^{9\ a}\) M. Kojima, unpublished observation.
positions of the α1α2 peptide-binding domain is 0.4 Å) compared with H-2Dd bound to 1FAPGNYPAL5 (Fig. 3b and Ref. 9). Indeed, a structural superposition limited to the portions of the α1 and α2-helices flanking the N-terminal half of the binding groove (residues 56–70 and 156–172) yields an even lower root mean square deviation (0.3 Å). Similarly, the variation of crystallographic B factors within the structure does not indicate any significant increase in flexibility for this half of the H-2Dd-binding groove arising from the absence of bound peptide (the mean B factor for the main chain atoms of the peptide-binding groove is 35 Å² and for residues 56–70 and 156–172 is 33 Å², a ratio of 1:0.94); the variation in flexibility matches that of a H-2Dd-binding groove containing full-length peptide (the corresponding values are 33 and 31 Å², which yield the same ratio). Residues 57–61 are involved in a lattice contact within the crystal. No other parts of the binding groove α1- and α2-helices make any such interactions; thus the potential influence of the crystalline environment on the structural stability appears to be very limited.

As expected by analogy with the equivalent residues in the structure of the full-length wild type (WT) peptide 1FAPGNYPAL5-H-2Dd complex 3NYPAL5, occupies the C-terminal half of the H-2Dd-binding groove. The electron density occupying the N-terminal half of the binding groove is discontinuous and is consistent with a number of tightly bound water molecules supplemented with two glycerol molecules (Fig. 3b), presumably recruited from the cryo-protectant used for flash cooling the crystals (see “Experimental Procedures”). Khan et al. (18) have reported a stable binding groove structure for a HLA-A2 peptide complex in which one pocket of the binding groove (the A pocket that normally houses the N terminus of the peptide) is not filled by peptide. For the current structure less than half the binding groove is occupied by peptide. However, structural superposition with the WT peptide-H-2Dd structure and related complexes reveals no differences in the main chain conformation of the class I heavy chain residues. Similarly, the variation of crystallographic B factors within the structure does not indicate any significant increase in flexibility in the N-terminal half of the H-2Dd-binding groove arising from the absence of bound peptide.

Peptide residues 5YPAL5 are well ordered within the H-2Dd3NYPAL5 crystal structure, but unexpectedly there is no well ordered electron density corresponding to the N-terminal asparagine (Asn5). The predominance of asparagines at position 5 in the H-2Dd peptide-binding motif corresponds to the use of Asn5 as an anchor residue (9, 19), but in 3NYPAL5 the absence of well ordered electron density indicates that this residue is highly flexible. The conformation of the peptide at Tyr6 is consistent with Asn5 being oriented away from the peptide-binding groove. In Fig. 4 the conformation of the half-peptide is compared with those of related full-length peptides from previously determined H-2Dd complexes (9). The half-peptide structure shows strikingly good agreement with that of the O-β-linked N-acetylgalactosamine (O-GlcNAc) substituted peptide FAPGS(O-GlcNAc)YPAL (K2G) rather than with that of the standard Asn5 anchored WT peptide. In the K2G structure the bulky O-GlcNAc ring linked to a serine (Ser5) in the peptide is too large to be accommodated in the peptide-binding groove like an Asn5 anchor residue. As a result the peptide undergoes a major local rearrangement to orientate the glycan bearing Ser5 side chain away from the peptide-binding groove, whereas the aromatic ring of the tyrosine (Tyr6) reoriented downwards to stack parallel with that of MHC residue Tyr156 at the side of the groove. Clearly in the 3NYPAL5 complex there is no such steric imperative forcing the exposure of the Asn5 side chain, but rather the reorientation appears to arise from a subtle change in the energetics of the peptide binding. The standard full-length peptide conformation, using Asn5 as an anchor residue, allows MHC residue His155 to hydrogen bond to the main chain carbonyl of peptide residue 4, but this arrangement precludes any stacking interaction between Tyr6 and Tyr156. In the half-peptide complex, the option of a peptide main chain hydrogen bond to the His155 side chain is removed, apparently tipping the energetic balance in favor of the use of Tyr6 as an anchor rather than Asn5. In H-2Dd3NYPAL5, as in the K2G complex, two water molecules substitute for the polar groups of a Asn5 anchor side chain to maintain hydrogen bonds to MHC residue Gln97.

The conformation of the C-terminal three peptide residues (YPAL5) is common to the half-peptide and full-length peptide complexes (Fig. 4). Thus, the standard C-terminal hydrogen bond network and burial of anchor residue Leu9 in the hydrophobic F pocket of the H-2Dd peptide-binding groove is conserved.

Mechanisms Conserving the Structural Integrity of the N-terminal Half of the Peptide-Binding Groove—During refinement of the H-2Dd3NYPAL5 structural model, a number of discrete peaks of electron density were observed in the empty N-terminal half of the peptide-binding groove. These patches of electron density have been satisfactorily modeled by tightly bound water molecules and two glycerol molecules (see “Experimental Procedures”; Fig. 5). The polar groups presented by these small molecules fulfill the hydrogen bonding requirements of MHC residues in the peptide-binding groove. The N-terminal four residues of the WT peptide 1FAPGNYPAL5 make polar interactions to the H-2Dd only through their main chain groups, contributing direct hydrogen bonds to four H-2Dd residues (Tyr171, Tyr7, Tyr159, and Gln63). Of these residues, only Tyr159 lacks a hydrogen bond to the most tightly bound water/
glycerol positions selected for refinement in the 2.55 Å resolution H-2D\(^{b}\) pNYPAL\(^{9}\) structure.

The peptide binding characteristics of the N-terminal half of the MHC class I groove are classically described in terms of three pockets, designated A, B, and D (20). The key feature of the A pocket is normally the incorporation of the N-terminal amino group of a peptide into a network of conserved hydrogen bonds; the bulk of this network remains intact in the H-2D\(^{b}\) pNYPAL\(^{9}\) structure (Fig. 5). A similar substitution by water molecules has been seen in the crystal structure for a peptide lacking the N-terminal residue bound to the human MHC class I molecule HLA-A2 (18). There are also some minor reorientations of the side chains of MHC residues Glu\(^{163}\) and Trp\(^{167}\) to partially occupy space occluded by the phenylalanine side chain (F1) in the WT and K2G complexes. Again these changes match those observed for an empty A pocket in HLA A2 (18). As a result of the shift by Glu\(^{163}\), a salt bridge is formed with Lys\(^{66}\). This salt bridge, covering the top of the A pocket, is also present in the H-2D\(^{b}\) ASNENMETM complex (19) and is likely to be a general feature of the H-2D\(^{b}\) when not obstructed by the presence of a large N-terminal peptide residue (for example phenylalanine in the WT peptide).

The B and the D pockets include the only hydrophobic residues (Tyr\(^{7}\) and Tyr\(^{19}\), respectively) that would be potentially exposed to solvent by the absence of full-length peptide in the H-2D\(^{b}\) pNYPAL\(^{9}\) complex. The two glycerol molecules are situated near these tyrosine residues and substitute, at least partially, for the absence of peptide residues 2 and 3. The position of peptide residue 2 in particular is closely duplicated, with the glycerol molecule mimicking its shielding of the Tyr\(^{a}\) aromatic ring (Fig. 5). The fact that glycerol was introduced into the system only after crystal growth (see “Experimental Procedures”) suggests that the contribution of these two molecules cannot be critical to the formation of the peptide-binding groove structure, although the possibility remains that trace amounts of glycerol present in the polyethylene glycol solution used for crystallization might have interacted with heavy chain at an earlier stage. Nevertheless, the subsequent siting of the glycerol molecules highlights features favoring the incorporation of peptide, rather than purely polar water molecules, into the groove. As in the A pocket, the remaining vacant space in the B and D pockets is filled with a network of water molecules forming hydrogen bonds to the predominantly polar surface of this portion of the H-2D\(^{b}\) groove.

DISCUSSION

The C-terminal pentapeptide pNYPAL\(^{9}\) binds to H-2D\(^{b}\) suboptimally, resulting in a complex that is more stable than empty MHC class I molecules but less stable than the complex made with wild type peptide FAPGNYPAL. In this respect, the H-2D\(^{b}\) pNYPAL complex shares characteristics with MHC class I molecules loaded in vivo in the absence of the ER resident cofactor tapasin (2). The H-2D\(^{b}\) pentapeptide-binding groove exhibits a pronounced division in character between the N- and C-terminal ends, being relatively hydrophobic in the portion of the groove that interacts with the C-terminal part of a peptide ligand and relatively polar in the portion that interacts with the N-terminal part of a ligand. It is interesting to note that there appears to be a strong (although not exclusive) trend toward a more hydrophobic character to the right-hand end of the peptide-binding groove when all of the MHC class I molecules are considered. It is unclear whether alleles with strong P2 anchors will behave the same as H2-D\(^{b}\), which does not have a primary anchor position at P2. Nevertheless, in common with all MHC class I molecules, it does have a relatively standard binding groove architecture, including a B pocket, which normally accommodates the P2 side chain (alanine in the case of the Sendai virus nucleoprotein epitope FAPGNYPAL) and an A pocket, which binds the N terminus of standard length peptides.

The crystal structure of the pNYPAL\(^{9}\) complex indicates that the hydrogen bonding requirements of side chains in the N-terminal half of the peptide-binding groove can be largely satisfied by solvent. This suggests that although the C-terminal end of the H-2D\(^{b}\) peptide-binding groove may require interactions with peptide ligand for stability above the T\(_{m}\), the N-terminal end of the groove might be largely “self-stabilizing.” This notion is supported by recent molecular dynamic modeling indicating that compared with the N-terminal region, the part of the peptide-binding groove at the peptide C terminus is far more flexible in the absence of bound peptide (21) and consistent with our observation that very little stabilizing effect could be achieved with the N-terminal pentapeptide FAPGN. This could be because the conserved hydrogen bonds that are normally made to the C terminus and penultimate carbonyl of bound peptide are less easily satisfied by solvent or that there are a number of hydrophobic side chains toward the right hand end of the groove that would need to be covered to ensure molecular stability (Fig. 5). Relevant to this point is the fact that the immunoproteasome tends to generate peptides with C termini that require no further processing before binding to class I MHC molecules once they enter the ER (22). One model for peptide binding in vivo would therefore be the capture of the C terminus and stabilization of the F pocket followed by binding of the N-terminal half of the peptide. The peptide dependence for class I stabilization around the C terminus of bound peptide is consistent with there being a conformational change in the peptide-binding domains of class I MHC upon peptide binding, an idea that is supported by extensive serological data (23, 24).

The structure presented here is relevant to recent studies suggesting that in vivo, the acquisition of peptides by class I molecules occurs in two stages. In this model, class I molecules are first assembled with β2-m, but the resulting empty class I molecules are not competent to be exported from the ER and are degraded intracellularly. Binding of suboptimal peptides (of which, by definition, pNYPAL\(^{9}\) would be an example) is required to render MHC class I molecules competent for export. In the absence of an interaction with any of the ER cofactors, class I MHC molecules loaded with such suboptimal peptides appear at the cell surface in an unstable form (25). Some of these may even be recognized by T cells (26, 27). Normally, however, the class I suboptimal peptide complex would bind to cofactor molecules in the ER, including calreticulin, tapasin, and ERp57, and enter a process of “peptide optimization.” Here the peptide cargo of class I molecules is edited by tapasin with an important nonqualitative contribution from calreticulin and possibly ERp57 (28). Tapasin-mediated peptide editing is thought to occur through an analogous process to HLA-DM-assisted editing of the peptide cargo bound to class II MHC molecules, although the molecular mechanism of either process is not known. Interestingly, HLA-DM is thought to interact with the class II MHC peptide-binding site close to the region that binds to the N terminus of peptide cargo and where nucleation of peptide binding is thought to occur (29). The tapasin-binding site maps to the region around the F pocket of class I MHC, which, as our data suggest, could be the site of an encounter complex between peptide and class I. Thus, these peptide-editing cofactors may be strategically situated to aid nucleation events occurring between peptide and MHC, which are at opposite ends of the peptide-binding groove of class I versus class II MHC molecules (24). Although it is thought that peptide editing involves the exchange of low affinity peptides for those with a higher affinity, it could also involve proteolytic trimming of N-terminally extended suboptimal peptides tethered to class I molecules via their C termini (30).
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It has been suggested that a peptide-dependent conformational change in class I HC contributes to the release of class I molecules from ER, and there are several lines of evidence to suggest that such a conformational change occurs (5) and that it may be a property of optimal peptides (peptides with anchor residues that are able to interact with the A and F pockets simultaneously) but not suboptimal ligands (peptides missing one or more anchor residues or of a length or composition that does not allow simultaneous occupancy of the A and F pockets) (5). Our data, however, show that the overall backbone structure of H-2D\textsuperscript{b} is essentially identical whether it is bound to NY-PAL or FAPGNPAL. Thus, any major conformational change that occurs in the class I molecule upon peptide binding appears to be as readily induced by the suboptimal C-terminal half-peptide as by the optimal ligand. Although it is possible that a different structure would have been obtained with murine β2-m, no structural differences have so far been observed between H-2 D\textsuperscript{b} complexed to human (9) versus mouse (19) β2-m that indicate that this would be likely.

Prior to interacting with suboptimal peptides or following their dissociation, it is possible that truly empty class I molecules could exist in a different conformation and that stabilization of this structure inside the cell depends on its interaction with cofactors such as tapasin, calreticulin, and ERp57. The chemical nature of the F pocket plays a key role in determining the dependence of different class I alleles on the cofactor tapasin and consequently in determining the extent to which different class I MHC molecules can undergo “spontaneous” peptide editing (2, 25, 26). If this fact reflects the influence of tapasin on a peptide-induced conformational change in class I HC contributes to the release of class I molecules from ER and there are several lines of evidence to suggest that such a conformational change occurs (5) and that it may be a property of optimal peptides (peptides with anchor residues that are able to interact with the A and F pockets simultaneously) but not suboptimal ligands (peptides missing one or more anchor residues or of a length or composition that does not allow simultaneous occupancy of the A and F pockets) (5). Our data, however, show that the overall backbone structure of H-2D\textsuperscript{b} is essentially identical whether it is bound to NY-PAL or FAPGNPAL. Thus, any major conformational change that occurs in the class I molecule upon peptide binding appears to be as readily induced by the suboptimal C-terminal half-peptide as by the optimal ligand. Although it is possible that a different structure would have been obtained with murine β2-m, no structural differences have so far been observed between H-2 D\textsuperscript{b} complexed to human (9) versus mouse (19) β2-m that indicate that this would be likely.

Prior to interacting with suboptimal peptides or following their dissociation, it is possible that truly empty class I molecules could exist in a different conformation and that stabilization of this structure inside the cell depends on its interaction with cofactors such as tapasin, calreticulin, and ERp57. The chemical nature of the F pocket plays a key role in determining the dependence of different class I alleles on the cofactor tapasin and consequently in determining the extent to which different class I MHC molecules can undergo “spontaneous” peptide editing (2, 25, 26). If this fact reflects the influence of tapasin on a peptide-induced conformational change in the class I MHC molecule, then the structure presented here will help to elucidate the molecular mechanism of tapasin.

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