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Crystal structure of a TAPBPR-MHC-I complex reveals the mechanism of peptide editing in antigen presentation

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Central to CD8⁺ T-cell mediated immunity is the recognition of peptide-major histocompatibility complex class I (pMHC-I) proteins displayed by antigen-presenting cells. Chaperone-mediated loading of high-affinity peptides onto MHC-I is a key step in the MHC-I antigen presentation pathway. However, the structure of MHC-I with a chaperone that facilitates peptide loading has not been determined. We report the crystal structure of MHC-I in complex with the peptide editor TAPBPR (TAP binding protein, related), a tapasin homolog. TAPBPR remodels the peptide-binding groove of MHC-I, resulting in the release of low-affinity peptide. Changes include groove relaxation, modifications of key binding pockets, and domain adjustments. This structure captures a peptide-receptive state of MHC-I and provides insights into the mechanism of peptide editing by TAPBPR and, by analogy, tapasin.

Cell surface peptide-major histocompatibility complex class I (pMHC-I) complexes play a crucial role in adaptive and innate immunity by functioning as ligands for immunosurveillance by CD8⁺ T cells and natural killer cells (1, 2). Key to the intracellular assembly of pMHC-I complexes is the selective loading of high-affinity peptides, a process termed peptide editing. A dynamic series of steps involving peptide binding and exchange is orchestrated in the endoplasmic reticulum (ER) by tapasin or the closely related TAPBPR (3) to ensure that peptides of optimal affinity and length are assembled with MHC-I for display on the cell surface. Although the X-ray structure of tapasin in complex with the peptide-loading complex component Erp57 has been determined (4), structural data addressing the mechanism of peptide editing has been limited, likely due to the difficulty in generating crystallizable peptide-free (PF) or peptide-receptive (PR) MHC-I.

TAPBPR interacts directly with PF or PR MHC-I molecules, such as HLA-A*02:01 (HLA-A2) and H2-D^d (D^d), generated by photolysis of co-assembled photosensitive peptides (5, 6). Attempts to crystallize purified TAPBPR-MHC-I complexes generated in this manner (fig. S1) were unsuccessful. Thus, we developed an alternative strategy to prepare partially peptide-filled yet stable pMHC-I molecules. Previous studies of MHC-I interactions with the related molecule, tapasin (7–11), as well as molecular dynamics (MD) simulations (12–17) suggest that the flexibility of the MHC-I F pocket, which accommodates the C-terminal amino acid of the peptide (18), is modulated by tapasin. Thus, we reasoned

that pMHC-I molecules assembled with peptides truncated from the C terminus, leaving the F pocket unoccupied, would serve as effective ligands for TAPBPR. However, C-terminal truncated peptides failed to efficiently refold many MHC-I molecules, so we adapted an approach developed for the HLA-DM-HLA-DR1 complex (19, 20) and examined both human and mouse pMHC-I structures to identify those that would be amenable to disulfide linkage at central peptide positions (see materials and methods). Exploiting the stabilizing effect of a Gly-Leu dipeptide (see materials and methods), we successfully generated D^d73C molecules disulfide-linked to each of three peptides (table S1): full-length pI10C5 (RGPGCAFVTI) and two truncated versions (pA6C5 (RGPGCA) and pR5C5 (RGPGC)).

We next examined the binding of peptide-D^d73C complexes to human TAPBPR by surface plasmon resonance (SPR) (Fig. 1). D^d73C-10mer bound to TAPBPR with an equilibrium constant, K_D , of 0.19 μ M and a dissociation rate constant, k_d , of 0.024 s⁻¹ (Fig. 1A). By contrast, the D^d73C-6mer (Fig. 1B) or D^d73C-5mer (Fig. 1C) bound with approximately ten-fold greater affinity (K_D of 0.0094 μ M and 0.0091 μ M respectively) due primarily to smaller k_d values of 0.0013 s⁻¹ and 0.0011 s⁻¹, respectively. Statistical evaluation of the binding parameters is shown in Fig. 1, D and E. As shown previously for TAPBPR-MHC-I complexes prepared with HLA-A2, H2-D^b, H2-L^d, as well as H2-D^d (6), high-affinity peptide led to the dissociation of TAPBPR from D^d73C-5mer (fig. S2). The rate of dissociation by peptide was related to the affinity of the peptide for the MHC-I molecule (fig. S2) (5).

We crystallized the chromatographically purified TAPBPR-D^d73C-5mer complex, merged the four best X-ray data sets, and solved a structure consisting of the TAPBPR luminal domains, D^d73C, and h β_2 m. Crystals were of the P3₁ space group with four complexes in the asymmetric unit (table S2). The refined structure, at 3.4-Å resolution, had an $R_{\text{work}}/R_{\text{free}}$ (%) of 24.2/26.9, and satisfied standard validation criteria for proteins solved at this resolution. We focus our discussion on the first of four complexes in the asymmetric unit and note differences where relevant. In addition, crystal structures of unliganded D^d73C-10mer, D^d73C-6mer, and D^d73C-5mer were determined as summarized in table S2 and fig. S3.

The overall structure (Fig. 2) of the TAPBPR-D^d73C-5mer complex revealed a heterotrimer of TAPBPR-D^d73C heavy chain- β_2 m consistent with previous analytical ultracentrifugation and SPR data (6). The D^d73C- β_2 m heterodimer was nestled in a glove-like basket consisting of the N- and IgV-like domains of TAPBPR that clasp the $\alpha 2$ -1 helix of D^d73C and extend a loop (residues 210 to 213, connecting strands β_{13} and β_{14} (fig. S4)) beneath the peptide-binding platform. This loop interacted with β_2 m and supported D^d73C β -strands 6-8 on the floor of the binding groove (Fig. 2, A to C). The composite TAPBPR N/IgV domain, which extends from the N terminus to residue 281, consisted of 19 β -strands with one short 3_{10} -helix at residues 254-256. These β -strands were structurally similar to those of tapasin in the tapasin/ERp57 complex and reflect the sequence conservation among different TAPBPR species (fig. S4). The C-terminal domain (residues 282-377) revealed a typical IgC structure that notably forms part of an Ig-domain trimer coordinating with the Ig-like β_2 m and D^d73C $\alpha 3$ domains (Fig. 2D). The TAPBPR-D^d73C interface (Fig. 2, E and F) buried an area of 1,884 Å² with 1,310 Å² contributed by D^d73C and 574 Å² by β_2 m. The interface was dominated by 17 hydrogen bonds between TAPBPR and the D^d73C heavy chain, three salt bridges, with a total of 22 TAPBPR residue contacts (see table S3). Interactions with β_2 m were less extensive, with five hydrogen bonds and a total of nine TAPBPR residue contacts. This TAPBPR-D^d73C structure was consistent with extensive mutagenesis data reported previously (21) (tables S4 and S5).

A comparison of the free and TAPBPR-bound forms of D^d73C-5mer revealed a distortion of the platform domain in the TAPBPR-complexed state (Fig. 3, A and B). In the region of direct contact between D^d73C-5mer and TAPBPR, the $\alpha 2$ -1-helix was pulled away by as much as 3 Å (by interaction between residues 138 to 150, particularly R144, R145, and E148 of D^d73C, and residues of the palm of the glove of the TAPBPR N/IgV domain (Fig. 3, A and C, and table S3)). In addition, β -strand 8 of D^d73C was drawn down approximately 1.8 Å (Fig. 3B and movie S1), and β -strand 5 was pulled by an interdigitating loop at the heel of the TAPBPR glove by as

much as 1.4 Å (Fig. 3D). Other intermolecular interactions are listed in table S3. Interaction with TAPBPR distorted the peptide-binding groove. Notably, the side chain of Y84, which canonically coordinates both the C terminus of bound peptide and K146 of $\alpha 2$ -1, flipped away from the groove to interact with E102 of TAPBPR (Fig. 3, E and F). The changes in this region deformed the F pocket, which normally accommodates the side chain of the C-terminal amino acid of the bound peptide. TAPBPR not only distorted the localized region of contact, but also exerted distal effects on the organization of A and B pocket residues that coordinate the N terminus of the bound peptide. In two of the four molecules in the asymmetric unit, the D^d73C R66 side chain extended across the binding groove to hydrogen-bond with Y159 effectively closing off this section (Fig. 3, G and H). As a consequence of these dynamic structural changes in the binding groove, electron density for the covalently bound 5mer peptide and the GL dipeptide (used in refolding), and the accompanying peptide-stabilizing hydrogen-bonding network, clearly seen in the unliganded D^d73C-5mer (fig. S3, C, F, and G), was no longer visualized in the TAPBPR complex. This lack of electron density of the covalently bound peptide, taken together with the widening of the groove at the F pocket region and rearrangements of key peptide-binding residues of D^d73C, is consistent with the view that the covalently bound 5mer is mobile in the complex and is no longer tethered to the binding groove.

A comparison of the free and TAPBPR-bound forms of D^d73C-5mer also revealed dramatic movements of the MHC-I $\alpha 3$ and β_2 m domains, which shifted 6.4 Å and 4.4 Å, respectively, to engage TAPBPR (fig. S5 and movie S2). The repositioning of the D^d73C $\alpha 3$ and β_2 m domains in the TAPBPR complex illustrates the coordinated and dynamic structural changes that occur when D^d73C interchanges between PR and peptide-loaded conformations.

An unanticipated result was the identification of important contacts of TAPBPR with β_2 m. In the complex, several regions of TAPBPR- β_2 m contact were noted, including residues of the $\beta 1$ strand at the N terminus of β_2 m, the loop at 59-60, which abuts loop 211-212 of TAPBPR, and C-terminal residues 92-94 of strand $\beta 7$ that bind residues 330 to 332 of the IgC domain of TAPBPR (Fig. 4, A to C). The significance of the β_2 m 58-60 loop is evidenced by reduced TAPBPR binding to D^d73C-5mer assembled with a β_2 m D59A mutant (Fig. 4, E and G to I). Ala substitutions of β_2 m residues I7, or I92/K94 do not affect TAPBPR binding as severely as D59A (Fig. 4, D and F to I). Functional and binding studies have suggested the mutual contribution of β_2 m and bound peptide to MHC-I stability (22-25). Additionally, nuclear magnetic resonance studies of MHC-I reveal peptide-influenced changes in chemical shifts of the β_2 m 58-60 loop which abuts the floor of the peptide-binding platform (26). Thus, β_2 m 58-

60 loop interactions with TAPBPR may not only stabilize PR MHC-I but also communicate the occupancy of the peptide binding groove—whether empty, partially occupied, or occupied by a low- or high-affinity peptide.

The crystal structure of the TAPBPR–Dd73C–5mer complex provides an X-ray structure of TAPBPR and permits direct comparison with the related tapasin. As implied by previous low-resolution SAXS structures (6) and anticipated by mutational and molecular dynamics studies (21, 27), TAPBPR and tapasin, despite only 22% protein sequence identity, reveal remarkable structural similarity (see Fig. 2G). Mutational analyses (4, 6, 8, 9, 21, 28, 29) and molecular dynamics simulations (12, 15, 17), mapped the general region of the tapasin–MHC-I interaction to the MHC-I α 2-1-helix and the α 3 domain, sites that overlap with the TAPBPR-binding site (21). Notably, residues that interact with MHC-I are broadly conserved between TAPBPR and tapasin (fig. S4). Finally, the TAPBPR–D^d73C complex structure provides a basis for building a homology model of tapasin–HLA-B*44:02 (fig. S6) to guide further tests of the mechanism underlying the catalytic role of tapasin.

A comparison of the role of TAPBPR with that of HLA-DM, which similarly stabilizes a peptide-receptive conformation of the MHC-II molecule, revealed that these two classes of chaperone function by destabilizing diametrically opposite regions of the binding groove—TAPBPR affecting the F pocket (C-terminal portion) of the MHC-I groove directly, and HLA-DM distorting the P1 pocket (N-terminal portion) of the MHC-II. Although large structural changes in HLA-DR on HLA-DM binding in the P9 region have not been observed, a clear-cut effect of P9-substituted peptides on the stability of HLA-DM–HLA-DR complexes has been noted (19).

TAPBPR and tapasin function as both chaperone and peptide editor. As viewed through the X-ray structure of a peptide-dissociable TAPBPR–D^d73C–5mer complex described here, in which the 5mer represents a low affinity peptide, chaperone and editor functions are the result of the same structural effects: stabilization of an MHC-I conformation showing global differences in the α 2-1 helix, the accessibility of the full peptide binding groove, including the A and F pockets, and the relative disposition of the α 3 and β 2m domains. We propose a model of TAPBPR-mediated peptide editing in which TAPBPR catalyzes dissociation of low affinity peptides, stabilizes PR (empty) MHC-I, and is released by high affinity peptide (Fig. 4J). By analogy, tapasin follows the same general structural principles.

This structural description of the interaction between MHC-I and TAPBPR provides insights into the molecular mechanism of peptide editing, an evolutionarily conserved and critical step in generating stable cell surface pMHC-I complexes for immunological surveillance. Additionally, this

work provides a basis for further structural and computational approaches modeling additional components of the TAPBPR- and tapasin-mediated peptide presentation pathways.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/cgi/content/full/science.aao5154/DC1
Materials and Methods
Figs. S1 to S6
Tables S1 to S5
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Movies S1 and S2

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Fig. 1. Kinetics and affinity of the interaction between TAPBPR and D^d73C containing disulfide-linked peptide. (A to E) SPR analysis of the interaction between TAPBPR and D^d73C-10mer (A), D^d73C-6mer (B), and D^d73C-5mer (C). Data are shown in red and fits of the data to a 1:1 binding model are overlaid in black. The results of three independent experiments ($M \pm SD$) are shown as scatter plots in (D) and (E). ***, $p < 0.05$ (one-way ANOVA).

Fig. 2. Overall structure of the TAPBPR–D^d73C–hβ₂m–5mer complex. (A) Ribbon representation of TAPBPR (pink), D^d73C heavy chain (blue), and β₂m (green), with individual domains of TAPBPR and structural elements of D^d73C labeled. (B) Similar to (A) but with D^d73C and β₂m in surface representation. (C) Similar to (A) but with TAPBPR in surface representation. (D) Ribbon and surface representation of the Ig-domain trimer composed of the TAPBPR IgC domain, D^d73C α3, and β₂m. (E) Surface representation of D^d73C and β₂m with interface residues highlighted in blue and green, respectively, D^d73C–β₂m was rotated to the left by approximately 90°. (F) Surface representation of TAPBPR with interface residues highlighted in pink (rightward rotation of 90°).

Fig. 3. TAPBPR remodels D^d73C binding groove. (A to C) Superposition of the platform domain of D^d73C in the unliganded (gray) and TAPBPR-bound (blue) forms. TAPBPR is pink. Groove widening (B, C) at α2-1 helix is indicated by arrows. (D) Comparison of floor of the peptide-binding groove of D^d73C in the unliganded (gray) and TAPBPR-bound (blue) forms. Movement of the β strands is depicted by arrow. (E) Contact between Y84 in the α1 helix of D^d73C (blue) with E102 of TAPBPR (pink). (F) Y84 of D^d73C-5mer (gray) contacts GL dipeptide (cyan) in F pocket in absence of TAPBPR. (G) Steric blocking of the A/B pocket of D^d73C in the TAPBPR-bound form by side-chain interactions between R66 and Y159. Electron density for these residues is depicted in dark blue. (H) Disposition of R66 and Y159 in unliganded D^d73C-5mer when the groove is occupied by peptide.

Fig. 4. Importance of β_2m -TAPBPR contacts and mechanism of peptide editing as viewed through the structure of TAPBPR-MHC-I complex. (A to C) close-up view of contact residues between $h\beta_2m$ and TAPBPR: (A) β_2m I7 to TAPBPR L334; (B) β_2m D59 to TAPBPR K211; (C) β_2m I92/K94 to TAPBPR S330/F331. (D to F) Summary of binding of D^d73C-5mer complexes assembled with the indicated mutant $h\beta_2m$ chains. (G to I) Summary of binding parameters as compared with WT ($M \pm SD$). Asterisks indicate degree of statistical significance of means of multiple determinations: ****, $p < 0.001$; ***, $p \leq 0.006$; **, $p < 0.03$ (one-way ANOVA). (J) Model for TAPBPR function in peptide presentation: 1. Various peptides, ranging from low-affinity (LA) to high-affinity (HA) are provided to the ER following cytoplasmic proteolysis and TAP-mediated transport; 2. TAPBPR binds to MHC-I bearing a low-affinity peptide (MHC-I/LA) or a peptide of suboptimal length, and catalyzes peptide dissociation; 3. TAPBPR binding remodels the peptide groove causing release of the low-affinity peptide, and stabilization of PF MHC-I (TAPBPR=MHC-I=PR); 4. upon binding a high-affinity peptide, MHC-I-PR conformation changes to MHC-I-HA (high affinity), dissociates from TAPBPR (see fig. S2) and is transported to the cell surface. This model addresses only the effects of interactions of TAPBPR with MHC-I. Further experiments are needed to structurally clarify the recently described recycling pathway that employs UDP-glucose:glycoprotein glucosyltransferase (UGT1) (30).