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THE PEPTIDE-RECEPTIVE TRANSITION STATE OF MHC-I MOLECULES: INSIGHT FROM STRUCTURE AND MOLECULAR DYNAMICS

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Abstract

MHC-I proteins of the adaptive immune system require antigenic peptides for maintenance of mature conformation and immune function via specific recognition by MHC-I-restricted CD8⁺ T lymphocytes. New MHC-I molecules in the endoplasmic reticulum are held by chaperones in a peptide-receptive (PR) transition state pending release by tightly binding peptides. We show, by crystallographic, docking, and molecular dynamics methods, dramatic movement of a hinged unit containing a conserved 3₁₀ helix, that flips from an exposed “open” position in the PR transition state, to a “closed” position with buried hydrophobic side chains in the peptide-loaded (PL) mature molecule. Crystallography of hinged unit residues 46-53 of murine H-2L^d MHC-I heavy chain, complexed with mAb 64-3-7 demonstrates solvent exposure of these residues in the PR conformation. Docking and molecular dynamics predict how this segment moves to help form the A and B pockets crucial for the tight peptide binding needed for stability of the mature PL conformation, chaperone dissociation, and antigen presentation.

INTRODUCTION

In the adaptive immune system, CD8⁺ T lymphocytes, crucial effector cells, are activated by encounter of their TCR with peptide-bearing MHC-I molecules expressed on the plasma membrane of tumor, target, or infected antigen presenting cells (1). The loading of tightly binding self and pathogen peptides, crucial to the stability, cell-surface expression, peptide repertoire, and immune function of such MHC-I molecules (2, 3), takes place while the newly synthesized MHC-I molecules in the endoplasmic reticulum are held by chaperones in a peptide-receptive (PR) transition state. Similarly, MHC-II molecules, that generally acquire their peptide ligands in an endocytic compartment, are recognized by TCR on CD4⁺ T cells. Several classes of receptors on natural killer (NK) cells interact with specific MHC-I molecules complexed with peptide, though the influence of particular peptides in NK

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recognition is less specific (4). MHC-I polymorphisms and their accompanying peptide repertoires are associated with a variety of infections (e.g. HIV, HTLV, hepatitis C, malaria), autoimmune diseases (e.g. ankylosing spondylitis, asthma, birdshot retinopathy, Behcet's disease), drug hypersensitivities, and cancers (5). Lesions of the MHC-I pathway peptide transporters (TAP1 and TAP2), and the chaperone tapasin are associated with a group of immunodeficiency diseases called "bare lymphocyte syndrome (BLS) type 1" (6).

The usual formation of the MHC-I/ β 2-microglobulin(β 2m)/peptide complex exploits fundamental mechanisms of protein folding and assembly complemented by dedicated and generic components of the antigen presentation pathway. Thus, MHC-I molecules bind peptides derived from the natural degradative turnover of self proteins, from proteins over-expressed as a result of malignant transformation, from defective products of protein translation, and from peptides derived from infection by intracellular parasites such as viruses, in a complex but coordinated series of reactions (7–9). Newly synthesized MHC-I molecules enter the endoplasmic reticulum (ER) where they co-translationally assemble with the MHC-I light chain β 2m (10, 11), are stabilized in a peptide loading complex (PLC) that includes the chaperone lectins calnexin and calreticulin (12, 13), and tapasin (14–17). A complex of tapasin and endoplasmic reticulum protein 57, by stabilizing the PR form of MHC-I, permits access to peptides in the ER (15, 18, 19), which bind with a range of affinities (20). These peptides, generated in the cytoplasm by the proteasome, are transported to the ER via the transporter associated with antigen processing (TAP), and can be trimmed by ER resident proteases (21–25). Peptide editing, which occurs when new MHC-I molecules are bound to tapasin of the PLC in the ER (26), permits exchange of available peptides, and assures that MHC-I molecules that leave the ER and proceed to the cell surface have a mature conformation that is stabilized by high affinity peptides. When editing is hindered by mutations that interfere with proper delivery of peptides to the ER (3, 27), the resulting thermally labile MHC-I molecules reach the cell surface complexed with low affinity peptides. There, some peptides dissociate from the MHC-I molecules, hindering anti-viral immunity (28). Among the general features of peptide-binding MHC molecules that are crucial to peptide interaction and specificity are the presence of accommodating pockets of the peptide binding groove (29), designated A through F for MHC-I (30). Pocket A anchors the amine group of the amino terminal residue of the bound peptide, pocket B binds the side chain of peptide residue two, and pocket F accommodates the side chain of the carboxyl terminal residue.

The peptide binding, folding, maturation, and cell surface expression of MHC-I molecules have been examined extensively using a variety of monoclonal antibodies (27, 31–38), and by biochemical and biophysical methods (2, 39). The transition from unfolded to mature MHC-I can be monitored with the mAb 64-3-7 that binds the partly folded PR form of H-2L^d, but not the mature PL conformation that is detected with mAb 30-5-7 (31). Binding studies indicate that mAb 64-3-7 interacts with a segment of the α 1 domain of H-2L^d that includes residues 46-52 (40), and reactivity with 64-3-7 is lost when H-2L^d binds peptides, an event concomitant with dissociation from tapasin (41). Two residues, Q48 and P50, found only in H-2L^d and H-2L^q, allow binding to 64-3-7, and when substituted into other MHC-I molecules such as H-2K^d, H-2K^b (40), HLA-B27 (27) or HLA-A2 (42) confer reactivity with 64-3-7, indicating the general applicability of findings with this antibody.

Understanding the mechanism of peptide loading of MHC-I during its biosynthetic maturation is crucial for an appreciation of the details of a central step leading to T cell recognition and contributes to our broader knowledge of the general rules that govern the interaction of proteins with peptide ligands or peptide cofactors. By clarifying the molecular movements that accompany the binding of MHC-I to peptides, we may also learn about cooperative effects that influence protein folding and assembly. Ideally, structure

determination of the multimolecular PLC would provide crucial information addressing the mechanism of peptide loading, but the inherent lability of peptide-free MHC-I in solution (2) and its tendency to aggregate and precipitate have confounded efforts to isolate sufficient quantities of peptide-free complexes for crystallographic analysis. Since the mAb 64-3-7 recognizes a PR receptive conformation of PLC-bound H-2L^d, we reasoned that determining the structure of the segment of this MHC-I molecule in complex with this mAb would provide a snapshot of a part of the molecule in the PLC, and that molecular dynamics simulations would permit visualization of this conformation in the context of the whole MHC-I. To this end, we have determined the structure (to 1.64 Å resolution) of complexes of three H-2L^dα1 domain peptides (that include residues 46-53) with the Fab fragment of mAb 64-3-7. These structures reveal a conserved conformational feature, the 3₁₀ helix, which, because it is bound to 64-3-7, may be present and solvent-exposed in a proportion of the ensemble of molecules in the PR form.. We have examined the structure of residues 46-53 in molecular docking and molecular dynamics simulations in the context of H-2L^d that reveal conformational changes in the peptide binding groove that accompany the transition of H-2L^d from the PR to PL form. Interestingly, a segment of the α2 domain, pseudosymmetrical to the 64-3-7 binding site, determines tapasin interaction, known to stabilize the PR conformation prior to peptide binding. Based on this observation, we speculate that 64-3-7 and tapasin monitor the PR to PL transition using analogous structures located at opposite ends of the peptide binding groove.

MATERIALS AND METHODS

Monoclonal antibody preparation, sequence determination, and Fab preparation

MAb 64-3-7 (31) was purified from hybridoma cell culture supernatant by binding to protein A-sepharose. The amino-terminal protein sequences of the heavy and light chains of the mAb, determined by automated sequencing, were compared to the Kabat/Wu database (43) to design primers for DNA sequencing. cDNA prepared from 64-3-7 hybridoma cells was used to obtain the sequence of the V_H, C_{H1}, V_κ and C_κ domains of the antibody. The sequence has been deposited along with the structural model and structure factors in the protein data bank <http://www.rcsb.org/pdb/home/home.do> (44) under the PDB accession numbers 3UO1, 3UYR, 3V4U, and 3V52. The Fab fragment of 64-3-7, homogenous by SDS-gel electrophoresis and size exclusion HPLC, was prepared from the whole molecule by papain digestion followed by re-passage through protein-A sepharose (45, 46).

Surface Plasmon Resonance (SPR) binding studies

The sequences of peptides used in these experiments were: pL^d 35-55, RFDSDAENPRYEPQAPWMEQE (α1 domain segment); pK^b35-55, RFDSDAENPRYEPRARWMEQE; a control peptide was SNVREIKNRWRSTVQKLK. These peptides were synthesized with an additional amino terminal cysteine residue to permit directed coupling. Various smaller peptides are indicated in the figure legends. All peptides were analyzed by reverse phase HPLC or mass spectrometry and were of greater than 90% purity. For SPR experiments, 64-3-7 Fab was flowed past pL^d35-55 coupled via its amino terminal cysteine to a CM5 chip (47) in a BIAcore™ 2000. Three independent experiments using graded concentrations of the Fab were performed. Kinetics data were analyzed with BIAeval 3.2. Competition experiments were performed with a BIAcore™ T100. Control flow cells were either mock coupled, coupled to pK^b35-55, or to the control peptide. Details of the coupling have been described previously (46). Other details are in the relevant figure legends.

Crystallization, X-ray Data Collection, Structure Solution and Refinement

Crystals of the 64-3-7 Fab complexed with peptide were obtained in 0.1M LiSO₄, 0.1M Tris pH 8.5, 30% (w/v) PEG 3000, following incubation at 4° C. Diffraction data were collected at NSLS Beamline X29A with an ADSC CCD detector at wavelength 1.07500 Å for crystals designated M8, M3, M4, and M1 which were produced with the 64-3-7 Fab and peptides representing H-2L^d residues 45-54, 46-53, 46-53, and pL^d35-55 (with the added Cys residue alkylated) respectively. For each Fab/peptide complex crystal, data were collected, then scaled with HKL2000 (48). An initial solution for dataset M1 was obtained by molecular replacement with PHASER (49), as implemented in the CCP4 suite (50). The heavy chain of the blue fluorescent antibody EP2-19G2 (PDB code 3CFC (51)), and the light chain of Fab M75 (PDB code 2HKF (52)) were used as models for molecular replacement. The initial solution was rigid-body refined and rebuilt manually with Coot (53), replacing the necessary residues with the amino acids determined by DNA sequencing. Structures of the three other Fab/peptide complexes were determined by molecular replacement using the partially refined M1 structure. For all four structures, rounds of refinement with Refmac5 in CCP4 were interspersed with manual rebuilding in Coot. All structures were refined further in PHENIX (54) with model building in Coot. In general, cycles of manual model building followed by refinement included real-space refinement in Coot, and real-space, individual B-factor, TLS, and simulated annealing refinement in PHENIX, with final placement of waters and correction of N/Q/H flips with MOLPROBITY ((55)). Final analysis was performed with MOLPROBITY (54). Data collection and refinement statistics are provided in Supplemental Table I. Graphics were generated with PyMOL (The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC) and VMD (56). Amino acid sequence alignments and display were accomplished with ClustalW (57) and WebLogo (58), respectively.

Molecular Docking and Molecular Dynamics

To perform all-atom, perturbation docking of the Fv of 64-3-7 to a crystallographic model of H-2L^d/β2m (1LDP (59)), we used Rosetta Dock (v.2.3)(60). The H-2L^d molecule was prepared for docking by the *in silico* removal of bound antigenic peptide and carbohydrate, an energy minimization, and a short (~10 nsec) molecular dynamics run under isobaric-isothermal conditions. Parameters for molecular dynamics simulations are detailed below. The lowest energy conformation of this peptide-free 1LDP complex was taken from the production run and used for docking. Prior to docking, the Fv (lacking pL^d 46-53) was placed 25 Å from the side of H-2L^d that contained residues 46-53. During the run, the Fv was permitted to change its orientation before docking by translating, rotating, and rocking relative to H-2L^d. Backbones were held rigid with the exception of H-2L^d residues 44-55, and the Fv CDR3 loops, and all sidechains were allowed to flex. Ten thousand docking solutions were generated and the top 10% scoring solutions (in terms of lowest energy) were clustered based on an all-atom RMSD of 5Å. Representative solutions from the top ten scoring clusters were retained. (Nine of the ten selected models contained an α-helix conformation for these residues.) Of these, one docking solution contained a conformation of residues 46-53 of H-2L^d that was similar to that of the peptide in the X-ray determined Fab/peptide complex. The conformation of H-2L^d from this solution was used as input for the molecular dynamics simulations with the Fv. Calculations were performed using the high performance computational resources of the Biowulf/Linux cluster at the NIH, Bethesda, MD (<http://biowulf.nih.gov>).

For both the energy minimization and molecular dynamics experiments, molecules were explicitly solvated with TIP3P water molecules and Na⁺ and Cl⁻ counterions using VMD (56), and simulations were performed using NAMD (v.2.7) (61). For the 1LDP/Fv dynamics, φ/ψ torsion angles for residues 46-53 as well as the χ¹ and χ² values for Trp51

were taken from the crystal structure and were used as constraints during the dynamics simulation. Electrostatic interactions were handled using a Particle-Mesh Ewald summation and included periodic boundary conditions. The CHARMM27 (62) forcefield was used with CHARMM atom types and charges. The system was warmed slowly to 310K in 10K increments with each increment running for 5psec providing enough time for system equilibration at a given temperature. Once the 310K target temperature was reached, a system was further equilibrated for 50psec. Data were gathered from a production run that lasted 10 nsec for 1LDP alone, and were extended to 100 nsec for the Fv/H-2L^d complex. A1fsec integration timestep was used along with a 12 Å cutoff. Langevin dynamics were used to maintain temperature and a modified Nosé-Hoover Langevin piston was used to control pressure.

RESULTS

Direct binding of mAb 64-3-7 to H-2L^d peptides and affinity measurement

Our strategy for elucidating the nature of the PR form of MHC-I molecules has been to exploit the unique properties of the mAb 64-3-7, first mapping the 64-3-7 binding site, then defining it crystallographically, extending the crystallographic findings with additional binding studies, and finally exploring the structure of the PR form with molecular docking and dynamics simulations. Initial characterization of the 64-3-7 binding site on H-2L^d, based on amino acid sequence comparisons, site directed mutagenesis of related MHC-I molecules, and competition for mAb binding to H-2L^d-positive cells by synthetic peptides, revealed the importance of Q48, P50, and W51 for recognition, and established a segment of the H-2L^dα1 domain, consisting of residues 46 to 52, EPQAPWM, as the minimal epitope of this mAb (27, 34, 40). To rigorously define the epitope recognized by 64-3-7 and to obtain quantitative data on this interaction, we examined the direct binding of the mAb with immobilized synthetic peptides using SPR (Figure 1A). A global fit of the data from three experiments for binding of the Fab to the coupled peptide pL^d35-55 indicated a K_D of 127±1.4nM (Figure 1A). Similar experiments using a biosensor surface coupled with a variant peptide containing Q48R and P50R, pK^b35-55, or with an irrelevant but similarly coupled control peptide, revealed no detectable binding (data not shown).

Using the pL^d35-55-coupled surface as the ligand, competition experiments with synthetic peptides and the purified mAb confirmed the minimal core size of the epitope (Figure 1B). Clearly, this core sequence EPQAPWM is sufficient to compete effectively for antibody binding, while the two overlapping 6-mers (EPQAPW and PQAPWM) show only intermediate activity, and the pentamer (QAPWM) fails to block even at high concentration.

Crystal Structure of the 64-3-7 Fab/peptide complex

To identify directly those atoms that are solvent-exposed in 64-3-7-reactive H-2L^d molecules, to distinguish residues whose side chains directly interact from those that might be involved in the maintenance of the 64-3-7 epitope conformation, and to delineate the extent and details of the antibody-bound surface of the pL^d46-53 peptide, and by inference of PR H-2L^d, we obtained X-ray quality crystals of the 64-3-7 Fab complexed with peptides of several different lengths all containing the core sequence of H-2L^d residues 46-53. Crystals analyzed included one consisting of the alkylated version of the peptide pL^d35-55 used as the ligand in the binding study, two obtained with the octamer EPQAPWME, residues 46-53, and one obtained with the decamer peptide representing residues 45-54, YEPQAPWMEQ. The best of these four crystals (M8) diffracted to 1.64 Å (Figure 2, Supplementary Table I).

Although there are minor differences among the four refined structures, they are remarkably similar, and all four reveal reliable electron density for peptide residues E46 to E53, and density for backbone atoms of Q54 was observed in M1 and M8. These structures are virtually identical (RMSD for the six possible binary comparisons of the four structures ranges from 0.105 to 0.314 Å, and of the four bound peptides against each other from 0.148 to 0.489 Å). We will confine our discussion to the crystal designated M8, which contained peptide 45-54 (YEPQAPWMEQ).

Organization of the complex, structure of the bound peptide, and extent of solvent-exposure in the peptide-receptive (PR) molecule

The overall structure of the 64-3-7 Fab is, as expected, that of a typical murine IgG2b antibody (63, 64), that makes contacts to the peptide antigen through characteristic residues of the CDR loops of both the IgG2b heavy chain and the κ light chain. (See Supplementary Table II for an enumeration of the atomic contacts and hydrogen bonds). The peptide residues most important for contacting the antibody are Q48 (24 total atomic contacts), P50 (17 contacts), and W51 (25 contacts), with only four involving M52 (Figure 2C).

Figure 3 shows the buried surface portions of H-2L^d peptide segment residues 48-52. Because they are bound to antibody in this complex, these H-2L^d side chains must of necessity be solvent-exposed in some fraction of the molecules representing the PR form of H-2L^d. These differences were quantified by calculating the solvent-accessible surface area per residue for the pL^d segment residues 46-54 in the Fab/peptide complex, compared with the solvent accessibility of the same residues in the H-2L^d structure, 1LDP (59) (Figure 3E). As illustrated in Figure 3, residues 48-52 are largely buried in the complete PL H-2L^d crystal structures, and residues 47-51 are buried in the complexes with the Fab, indicating solvent-exposure in the PR form. Thus, in the transition from the PR form to the PL form, residues 47-52 are likely to move from a solvent-exposed conformation in which they are accessible to mAb 64-3-7, to the mature structure in which these residues are buried and inaccessible to solvent and incapable of binding to mAb 64-3-7. This structural view of the residues of H-2L^d that interact strongly with 64-3-7 is consistent with published binding studies (27, 40) and our additional direct and competition studies presented above. More important, however, is the generally applicable finding that this antibody identifies a secondary structure element, a 3_{10} helix, that is present in at least a significant proportion of PR molecules, and that is preserved in the transition from the PR to PL form of H-2L^d, strongly implying that the same transition occurs for other MHC-I molecules as they bind peptide and are released from tapasin and the PLC.

Quantitative measurement of peptide contact residues with SPR binding studies using substituted synthetic peptides

Based on comparison of the reactivity of 64-3-7 with different MHC-I molecules and on the results of staining experiments using cells treated with mutant peptides, we previously concluded that H-2L^d polymorphic residues Q48 and P50, and also the conserved residue W51 were all crucial to recognition (40). These results are consistent with the structure of the Fab/peptide complex described here. We performed additional binding inhibition studies by SPR that extend these findings. Figure 1C summarizes these data, indicating that the heptamer EPQAPWM inhibits binding effectively, that hydrophobic substitutions at position 52, Ile or Val for Met, have little influence on binding, and that even an Ala substitution at that position has only a small effect. Alanine substitutions throughout the minimal heptamer reveal that P50 and W51 are the most important for the interaction. Substitution of Q48 by Ala has little or no effect, while substitution by Arg, the residue found in H-2K^d and H-2K^b, and of P50 by Arg obliterates the interaction. This indicates that the ability of Q48 and P50 to confer 64-3-7 reactivity on other MHC-I molecules is primarily due to removal of

interfering residues (typically Arg at these positions), consistent with the large steric influence of the Arg substitutions (see Supplemental Figure 1).

Preservation of a 3_{10} helix

A 3_{10} helix (65) is found in a sampling of MHC-I molecules in the region of residues 49-53 (66). Such a 3_{10} helix is also present in the structure of the peptide segment bound to 64-3-7 (Figure 4A-D) and thus is likely to be present in a proportion of the molecules that are in the PR transition state. The same region of the two published structures of full length H-2L^d in complex with peptides, 1LDP (59) and 1LD9 (15), also reveals a 3_{10} helix, i.e. H-bonding from the backbone carbonyl oxygen atom of residue *i* to the backbone amino H atom of residue *i* + 3 (Figure 4D). (Also, all other structures of H-2L^d or its platform domain in complex with different TCR (67–69) (70) show the same 3_{10} helix.) Thus, we infer that this segment moves as a unit from the PR to the PL form of the molecule, accompanied by some changes in side chain rotamer configurations, the most striking of which is rotation of the W51 χ^2 dihedral angle by about 180° between the two forms (Figure 4A and 4B).

Molecular Docking and Molecular Dynamics

We exploited molecular docking and dynamics simulations to visualize the conformation of the unloaded PR form of the H-2L^d molecule in complex with mAb 64-3-7. Molecular docking (protein-protein docking) allows the prediction of contacts between two molecules based on the known structures of the individual uncomplexed components. We used Rosetta (60) as described in the Materials and Methods to visualize the docked complex of the Fv of 64-3-7 with H-2L^d from which the bound antigenic peptide had been removed *in silico*. Contacts between 64-3-7 and H-2L^d residues 46-53 were similar in both the M8 peptide crystal structure and the docked model (Figure 5A). We observed the preserved 3_{10} helix in residues 48-53 in both the docked and the crystal structures. The backbone configuration of residues 48-53 in the crystal structure was the same as in the docked model (RMSD for the superposition was 1.23 Å) (Figure 5A). Additional views of the docking and the dynamics simulation of the PR conformation are available in Supplemental Movie 1. The most important feature revealed by the dynamics simulation is that when the region 46-53 is exposed to solvent, as it must be to allow 64-3-7 binding, residues contiguous with this epitopic segment are necessarily drawn away from their native configuration (Figure 5B). Thus, as W51 moves towards the Fv, it no longer is capable of providing the support of the native position of Y171 of H-2L^d, a residue intrinsic to the formation and structure of the A pocket (Figure 5C). In addition, Y45, towards the amino terminal end of the 3_{10} helical segment, is also pulled away and is incapable of contributing its essential role in the formation of the B pocket (Figure 5B and D).

DISCUSSION

The general problem of understanding the dynamic changes that accompany the binding of a small molecule ligand by a receptor glycoprotein has been addressed using structural (NMR and X-ray crystallographic), thermodynamic, spectroscopic, and molecular modeling techniques (71–73). When high resolution structures of the liganded and free forms of the receptor are known, visualization of the transition from free to bound conformations may be made (74). However, in the absence of knowledge of the experimentally determined structure of either one of these two forms, computational methods for elucidating such transitions may be employed. Understanding the structural transition that accompanies the peptide loading of MHC-I molecules in the endoplasmic reticulum is fundamental not only to the specific rules that govern a key step in antigen presentation and the immune response; it also provides an opportunity to explore the value of a complementary approach in which

X-ray crystallography and computational docking/dynamics methods are employed in concert.

Newly synthesized MHC-I molecules complexed with $\beta 2m$ are poised in the ER in a PR form, ready to bind and be stabilized in the mature PL form by peptides destined for display at the cell surface. To study this transition, we used a unique antibody, 64-3-7, that binds the PR form but not the PL form of the mouse MHC-I molecule, H-2L^d, as well as other MHC-I molecules with appropriate substitutions. We report the X-ray crystallographic structure of this antibody bound to a peptide segment of the PR form of H-2L^d. The structure of the peptide segment, defined by binding studies to be a core heptamer (Figure 1 and (40)) is well visualized in the complex. Because this segment of the H-2L^d α 1 chain is antibody-accessible, it is solvent-exposed in some proportion of PR molecules (Figure 3C). The published crystal structures of H-2L^d (1LDP(59), and 1LD9 (15)), reveal that residues P50, W51, M52 are sequestered from solvent and interact with other residues in mature PL H-2L^d molecules (Figure 3D). However in the PR form, some of these residues, particularly the side chains of W51 and M52, interact with 64-3-7 and thus are in a different configuration (Figure 4A and B).

Residues 49-53 form a 3_{10} helix in the peptide segment bound to 64-3-7, and hence this conformation may be found in the PR form (Figure 4B). They also form a 3_{10} helix in the PL form of the molecule (Figure 4B and Figure 5A and B). This suggests that the secondary structure of this segment, although changing its position, undergoes minimal change in conformation on peptide binding. A smaller peptide, PQAPWM, that only binds weakly, failed to crystallize with 64-3-7 Fab. Perhaps the longer peptides are better able to maintain the helical conformation needed for binding to 64-3-7. (However, our data do not distinguish between a preexisting helix and one selected from an ensemble of molecules by the antibody. That 64-3-7 is an effective reagent in Western blotting is consistent with both possibilities but also suggests that an antibody that sees a “linear epitope” may recognize a secondary structure element.) Nevertheless, extended molecular dynamics simulations of the docked H-2L^d, with release of constraints on the 3_{10} helix, and removal of both the Fv and the antigenic peptide, revealed preservation of at least one i to $i + 3$ H-bond between the five residue pairs (49 to 52, 50 to 53, 51 to 54, 52 to 55, and 53 to 56) a majority of the time (data not shown). This would support the view that the 3_{10} helix is not “induced” by the antibody. We previously suggested that this 46-53 segment may form part of a conserved hinge (66), which on changing from the PR to the PL conformation moves to a position that supports binding of the antigenic peptide. Figure 6 illustrates how residues 46-53 may participate as a hinged unit. Measurement of the deviation of backbone atoms between 1LDP and the corresponding atoms of 1LDP as docked to the Fv, suggests that the backbone bonds that “define” the borders of the hinges are in the vicinity of A40 and E53 (Figure 6).

We observe that classical MHC-I molecules of a number of species including human and mouse, that bind peptide ligands, contain the 3_{10} helix, and share striking sequence similarity (Supplemental Figure 1). Our observations on the dynamic mobility of the 3_{10} helix-containing segment provide a rationale for the conservation of this structural feature. This suggests that this helix-containing hinge plays a critical role in supporting and stabilizing peptide binding, and thus antigen presentation.

Molecular dynamics simulations have been applied to MHC-I (75-78) and MHC-II (79) in efforts to explore both peptide bound and peptide free states and to elucidate the differences between variant HLA-B*44 molecules that differ in their tapasin dependence (77, 78). In simulations of peptide-free MHC-I the binding region at the N-terminus of the peptide remained close to that of the PL state, whereas the region known to bind the C-terminus showed greater dynamic fluctuations. This suggested that F pocket deviations were

dependent on tapasin for the tapasin-dependent protein HLA-B*44:02. Greater deviations were observed for the tapasin-independent HLA-B*44:05 and were proposed to account for the differences. Here, we have exploited the unique mAb 64-3-7 which captures at least a subset of the PR form of MHC-I molecules, to first identify the conformation of its MHC-I epitope, and then extended this observation by docking and dynamics studies. These reveal that the 64-3-7⁺ molecules have significant distortions in residues crucial to the formation of the A and B pockets that accommodate the amino terminal regions of the antigenic peptide. Because 64-3-7 is a unique antibody that binds PR MHC-I with sufficient affinity to immunoprecipitate PR H-2L^d molecules in the PLC, we can only address the conformation of the residues recognized. It would, of course, be advantageous to have a library of antibodies that would decorate different regions of PR H-2L^d to probe other parts of the peptide free, PR molecule, but we know of no other mAbs suitable for such a study. Our results complement those obtained by dynamics simulations alone, and together suggest that the PR form of the MHC-I molecule has unformed A and B pockets and is thus unable to anchor the peptide amino terminus and the residue at P2, respectively. They also suggest that under the influence of tapasin (or in the absence of tapasin for tapasin-independent molecules) such molecules may have an unformed F pocket as well. It is remarkable that the two regions involved, residues 46-53 (for the A and B pockets) and the region surrounding residue 116 (critical to the F pocket) are located at symmetrical parts of the α 1 and α 2 domains, respectively.

Our docking simulation, followed by molecular dynamics of 64-3-7 with the peptide-free H-2L^d (Supplemental Movie 1), shows the 46-53 peptide segment bound to the Fv domain of the mAb. The great similarity in binding and conformation between the crystal structure and the docking cross-validates both structures (Figure 5A). The mobility of the 46-53 segment seen in the PR structure as visualized in the docked model has major effects on critical parts of the peptide binding groove. Antigenic peptides bind tightly to their respective MHC-I molecules by virtue of side chain interactions with several pockets found in the MHC groove (29, 30). Although crucial pockets may differ for various MHC-I gene products, the “A” and “F” pockets perform similar functions for most MHC-I molecules. The A pocket coordinates the amino-terminal amino group with conserved H bonds, and the F pocket accommodates the side chain of the carboxy-terminal amino acid. Other pockets, particularly the “B” pocket, originally called the “45” pocket, play important roles in most, but not all MHC-I molecules. For H-2L^d, whose peptide binding motif commonly includes a proline at position 2 of the peptide (24), a small B pocket is particularly important. The molecular dynamics simulation of the docked antibody with the PR form of H-2L^d indicates that residues Gln49, Pro50, Trp51, and Met52 (which directly bind 64-3-7) are distant from their positions in the mature structure, as are residues adjacent to the 46-53 region. For example, Trp51 is distant from its contacts in the PL form, and thus does not buttress Tyr171, which is a critical component of the A pocket. Tyr45, adjacent to the 46-53 peptide segment, is freely accessible to solvent in the dynamics generated PR form, thus incapable of participating in the formation of the B pocket (Figure 5D and Supplemental Movie 1).

The results reported here establishing a role for the 3₁₀ helix-containing hinge located in the α 1 domain of peptide-binding MHC-I molecules must also be interpreted in terms of the relationship of peptide binding to PR MHC-I and the release of the PL molecules from the PLC. The release of the newly loaded MHC-I molecule from the PLC indicates that significant conformational change takes place in the tapasin-binding region concomitant with peptide binding, which requires (or induces) the formation of well-formed pockets, in particular A, B, and F. Thus, a largely cooperative effect on peptide binding is accompanied by the 3₁₀ helix hinge flip of segment 46-53 which occurs coordinate with a significant conformational change in the tapasin binding site. Site-directed mutagenesis and coprecipitation experiments have mapped the tapasin binding site to a region surrounding

MHC-I residue T134 that lies in a position in the $\alpha 2$ domain pseudo-symmetrical to the location of the 3_{10} helix hinge described here (27, 28, 41, 80). Supporting the importance of a structural contribution of the F pocket and residues surrounding it in tapasin dependence, recent studies comparing tapasin interactions and MHC-I conformational flexibility as assessed by molecular dynamics simulations exploiting the single amino acid variants HLA-B*44:02 (tapasin dependent) and HLA-B*44:05 (tapasin independent) suggest that conformational changes reminiscent of those that take place at residues 46-52 may also take place around residues 114 and 116 which form the foundation of the F pocket (5).

Peptide editing for MHC-II molecules, which takes place in an endosomal compartment, is dependent on the catalytic chaperone DM, whose binding site has been mapped to a region homologous to the 64-3-7 binding site on MHC-I. Although the precise mechanism by which DM effects peptide exchange on MHC-II remains controversial (81–87), recent evidence suggests that the ability of MHC-II to interact with DM is driven by a conformational change in the region of the MHC-II α chain 3_{10} helix (88). In this regard, tapasin and DM may both function as chaperones that give MHC-I and MHC-II molecules, respectively, a chance to fold around tightly binding antigenic peptides needed for molecular stability and effective immune function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

SPR shows the affinity and specificity of mAb 64-3-7 for its binding site on the α chain of the H-2L^d MHC-I molecule. (A) Soluble Fab fragment of mAb 64-3-7 was offered to a biosensor surface coupled with pL^d35-55, at a flow rate of 30 μ l/min. Arrows indicate the initiation of the injection and the start of the buffer washout respectively. Fab was offered at graded concentrations of 70, 140, 280, 560, and 1120 nM (dotted lines), performed in triplicate, and data, fit globally (solid black lines) to a single site Langmuir model, indicated a K_D of 127 ± 1.4 nM. (Curve fitting in the dissociation phase was limited to the initial seventy seconds of the washout to minimize the contribution of rebinding.) (B) Competition with truncated peptides defines the core epitope as the EPQAPWM heptamer. Graded

concentrations (11, 33, and 100 μM) of the indicated peptides were used in competition for binding as described in Materials and Methods. (C) The indicated peptides (at 33 μM) were used in binding inhibition studies as detailed in Materials and Methods, and the % inhibition of steady state binding in the absence of competing peptide was determined for each of them.

Figure 2. Peptide segment residues 46-53 contact CDRs of 64-3-7 H and L chains. (A) Cartoon illustration of Fv region of 64-3-7 is shown with stick representation of peptide segment 46-54. (B) "Top" view of (A) showing CDR loops surrounding segment 46-54. (C) Residues contacting segment 46-53 are indicated and color-coded to (B). (For clarity, CDR3H contacts are shown here in black).

Figure 3.

Residues 48-52 are buried in mAb 64-3-7. (A) Top side-by-side stereo view of peptide segment E46 (bottom) to Q54 (top) bound to 64-3-7 H and L chains. Peptide segment, green; H chain, blue; L chain grey. (B) Side stereo view of (A). (C) Buried Residues Q48-W51 shown as sticks inside pink surface; exposed E46, P47, and M52-Q54 as sticks inside grey. (D) Stereo view of H-2L^d (1LDP) with buried W51 and M52. (E) Residues A49, W51, and M52 of segment 46-53 have low relative fractional accessible surface area (RSA) in PL H-2L^d (1LDP), but have high RSA in PR H-2L^d, as calculated as $(RSA_{PR} = 1 - RSA_{Bound})$, where $RSA_{Bound} = RSA$ in the Fab and docked Fv peptide complexed structures. Calculation of RSA was performed with VADAR (<http://redpoll.pharmacy.ualberta.ca/vadar>)(89).

Figure 4.

The 3₁₀ helix is present in residues 49-53 of the H-2L^d MHC-I α chain in PR and PL conformation. (A) Residues 49-53 in the PL mature molecule 1LDP. The region of the 49-53 3₁₀ helix is illustrated by the cylinder. α1 and α2 helices are indicated, as are residues Y59 and Y171 that line the A pocket. (B) Residues 49-53 as bound to mAb 64-3-7. Note the side chain rotamer shifts for W51 and M52 between (A) and (B). (C) Side-by-side stereo view of (B), the Fab-bound conformation. (D) Location of the 3₁₀ helix in H-2L^d, in an average of eight MHC-I molecules, and in the M8 peptide bound to the Fab, is shown by measurement of the distance from the carbonyl of the indicated residue *i* to the amine H of residue *i* + 3. The eight averaged MHC_I molecules, plotted as mean +/- S.E.M., are H-2L^d (Avg. of 1LD9 and 1LDP), HLA-Aw68(1HSB), HLA-A2.1(3HLA), HLA-B27(1HSA), HLA-Cw4(1QQD), H-2K^d(1KPU), H-2D^b(3CC5), N*0101(bovine, 3PWU).

Figure 5. Comparisons of Docked and Crystal Structures

(A) Alignment of 64-3-7 Fv cocrystallized with 46-53 segment, and docked 64-3-7Fv with H-2L^d (showing only 46-53 segment). Crystal H chain, slate; docked H chain, light blue; crystal L chain, gray; docked L chain, tan; crystal 46-53, green; docked 46-53, magenta. (B) Superposition of 64-3-7 docked with H-2L^d, and PL H-2L^d heavy chain (1LDP (only $\alpha 1$ and $\alpha 2$ domains shown)) reveals extended conformation of residue 44 to 56 hinge region in the docked structure. PL H-2L^d, cyan; docked H-2L^d, magenta. (C) W51 and M52 buttress the invariant Y59 and Y171 at the amino terminal end of the peptide binding site (3TF7). (D) Y45 provides a floor for the "B" pocket, shown adjacent to L2 of peptide QLSPFPFDL (3TF7).

Figure 6. Location of residues that identify the hinge segment. Corresponding backbone atoms of superposed structures of the H-2L^d heavy chain of the PL (1LDP) and the Fv-docked heavy chain (PR) were examined. (A) Distance between corresponding atoms (N, CA, CO) of PL and PR forms. (B) Location of the hinge termini at D39 and G56. H-2L^d (PL), cyan; Fv-docked molecule (PR), magenta. Distances in angstroms for several corresponding CA-CA comparisons are shown.