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A structural and molecular dynamics approach to understanding the peptide-receptive transition state of MHC-I molecules*

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Abstract

The mature conformation of major histocompatibility complex class I (MHC-I) proteins depends on the presence of bound peptides, permitting recognition at the cell surface by CD8⁺ T lymphocytes. Newly synthesized MHC-I molecules in the endoplasmic reticulum are maintained in a peptide-receptive (PR) transition state by several chaperones until they are released concomitant with the loading of peptides. By determining the crystallographic structure of a region of an MHC-I molecule that is recognized by a unique monoclonal antibody and comparing this with docking and molecular dynamics simulations with the whole molecule, we demonstrate the movement of a hinged unit supporting the part of the binding groove that interacts with the amino terminal residues of the bound peptide. This unit contains a conserved 3₁₀ helix that flips from an exposed “open” position in the PR form to a “closed” position in the peptide-loaded (PL) mature molecule. These analyses indicate how this segment of the MHC-I molecule moves to help establish the A and B pockets critical for tight peptide binding and the stable structure required for antigen presentation and T cell recognition at the cell surface.

Keywords

Antigen presentation; MHC-I structure and function; Peptide loading; X-ray crystallography; Molecular dynamics

1. Introduction

Fundamental understanding of problems posed by the immune system often leads to far-ranging insight into rules that govern complex molecular, cellular, or organismic processes. The molecular and cellular events involved in antigen processing and presentation have

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taught us about protein chaperones, protein degradation, peptide generation and transport, and also protein assembly and folding. In particular, MHC-I molecules mature through a variety of steps: from their biosynthesis as separate heavy (HC) and light chains (β 2-microglobulin – β 2m), to their stabilization in the endoplasmic reticulum by the chaperones calnexin, calreticulin and tapasin, to their loading with peptides and the concomitant release of the trimolecular HC/ β 2m/peptide complex allowing final glycosylation and transport to the cell surface (Wearsch and Cresswell, 2008). Of particular interest is the conformational transition of the MHC-I heavy chain that accompanies peptide loading in the endoplasmic reticulum Golgi intermediate compartment (ERGIC).

Conformational changes that accompany peptide loading of MHC-I have been identified with a number of specific monoclonal antibodies (mAbs) (Yu et al., 1999). Some of these recognize peptide-dependent but not peptide specific epitopes of the MHC-I molecule, indicating the assumption of a mature PL conformation. Other mAbs of particular value are those that bind ER-resident PR MHC-I heavy chains, contrasting with those that recognize mature, cell surface expressed, PL forms of the molecule. The best understood of such antibodies is mAb 64-3-7, which binds H2-L^d and has been extensively characterized by biosynthetic labeling experiments and epitope mapping studies using synthetic peptides (Myers et al., 2000). Early pulse-chase biosynthetic labeling experiments clearly demonstrated the transition of immature 64-3-7⁺ molecules to 64-3-7⁻ ones, a transition that accompanied peptide acquisition. This loss of 64-3-7 reactivity accompanied the release of the MHC-I/ β 2m/peptide complex from its association with components of the peptide loading complex (PLC) which includes tapasin, calreticulin, ERp57, and the TAP1/2 peptide transporter. In parallel with the loss of 64-3-7 reactivity and the acquisition of bound peptide, H2-L^d gains reactivity with the peptide-dependent, but not peptide specific mAb, 30-5-7. To understand these changes, we have undertaken a more detailed analysis of the nature of the interaction of 64-3-7 with H2-L^d when it is in a PR conformation (Mage et al., 2012). First, we precisely mapped the part of H2-L^d that serves as the epitope for 64-3-7 by surface plasmon resonance (SPR), then we determined the X-ray crystal structure of the complex of 64-3-7 with the epitopic peptide to define the conformation of the region of H2-L^d bound by the mAb. Finally, we employed molecular docking and dynamics simulations to gather a visual understanding of the conformational changes that accompany the transition from PR to PL form.

2. Current status

Using synthetic peptides representing various amino and carboxyl terminal truncations, we confirmed and refined earlier mapping studies of the H2-L^d epitope bound by 64-3-7. This mAb binds a sequence of seven amino acids, representing residues 46–52 of the H2-L^d molecule, EPQAPWM. SPR binding studies determined the affinity of this interaction to be 127 nM. Analysis of the binding of the Fab to substituted synthetic peptides showed that polymorphic residues, Q48 and P50, as well as the conserved residue W51, were crucial to recognition. Most MHC-I molecules have R at position 48, and some have R at 50, leading to a lack of reactivity with 64-3-7. Only H2-L^d and H2-L^q have both Q48 and P50, and substitution of these two into other MHC-I molecules has been shown to confer 64-3-7 reactivity (Myers et al., 2000).

We co-crystallized three different peptides that included this core sequence with the Fab fragment prepared from mAb 64-3-7, collected high resolution X-ray diffraction data, and determined the three-dimensional structure of these peptide/Fab complexes. Structures determined from four different crystals were essentially identical, so we focused our attention on the one of the highest resolution which diffracted to 1.64 Å. ° This crystal was

obtained with a decamer peptide, YEPQAPWMEQ (residues 45–54), revealing good electron density for the Fab throughout, and for residues 46–53 of the bound peptide.

The most important finding in the X-ray structures was that residues 47–51 are buried in the complexes with the Fab. The peptide residues with the most atomic contacts to the Fab are Q48, P50, and W51. Since 64-3-7 binds H2-L^d free of peptide in the PR form, this means that these residues must be exposed to solvent when H2-L^d is in this configuration. Since these same residues are not solvent-exposed in structures of mature H2-L^d complexed with peptide (the PL form), this indicates that the transition from the PR form (stabilized in the PLC) to the PL form involves movement of this residue 46 to 53 region of H2-L^d from a solvent-exposed to a solvent-inaccessible position. The most striking feature in the structure of the Fab/peptide complex is that the backbone of the bound peptide describes a 3₁₀ helix, a backbone configuration also found in the PL form of the molecule, elucidated in the crystal structures of mature H2-L^d. Such a 3₁₀ helix is a common feature observed in crystal structures of almost all MHC-I molecules that bind a peptide or other antigenic ligand, and suggests that this conserved structural feature plays a key role in the transition of PR to PL MHC-I molecules.

To visualize the transition of the whole MHC-I molecule from its PR to PL form, we extended our studies with molecular docking and molecular dynamics simulations. Molecular docking (protein–protein docking) was employed to explore the nature of the interaction of the Fab with the whole H2-L^d molecule. Using Rosetta Dock (Gray et al., 2003), we computationally docked the Fv portion of the Fab to the H2-L^d structure from which the bound peptide was removed. In this computational exercise we constrained the structure of the Fv but allowed flexibility of amino acid side chains as well as of the backbone of the CDR3 regions. We also permitted backbone and side chain flexibility of H2-L^d residues 44–55, the region of interaction mapped by our mutational and crystallographic studies. Remarkably, among the lowest energy docked structures was a conformation of H2-L^d residues 46–53 very similar to that of the X-ray determined structure. This conformation was then used for an extended molecular dynamics simulation, which revealed the mobility of this region, and the consistent preservation of the 3₁₀ helix in the backbone structure.

To further visualize the transition of residues 46–53 from the PR to the PL form, we superposed the structures of the docked with the native molecules. This graphically illustrates the excursion of the 3₁₀ containing region, anchored by residues G39 and D56. The superposed structures illustrate the remarkable movement of the region of the MHC-I molecule containing the 3₁₀ helix as a hinged segment, transitioning from an “open” solvent accessible configuration to a “closed”, tightly packed solvent sequestered configuration. Fig. 1 shows the details of the two structures and compares them to a swinging door.

How do these structures and simulations assist us in understanding the molecular events that take place in the structurally more intricate PLC? What is functionally different about the PR and PL forms of the MHC-I molecule? A crucial feature of MHC-I molecules is that they employ structural pockets of the binding groove to determine the site for the tight interaction of bound antigenic peptide. These pockets, named A through F, vary among different forms of MHC-I molecules, and define the peptide binding specificity of the particular MHC-I molecule. The A pocket, relatively invariant, stabilizes the amino terminal amine of the bound peptide through conserved interactions and hydrogen bonds involving Y59 and Y171. (Residues Y59 and Y171 are structurally supported by W51 and M52.) The B pocket, somewhat polymorphic, exploits residue 45 (Y in H2-L^d) as a focus for interacting with the side chain of the bound peptide position 2. The F pocket accommodates the side chain of the carboxyl-terminal amino acid of the bound peptide, and its variation defines the

motif of this ultimate peptide side chain. Of particular interest in understanding the transition from a PR to PL form, is that the conformation of the 3_{10} helix-containing segments 46–52 in the PR form removes residue Y45 as well as residues W51 and M52 from positions that are crucial to the support of the B and A pockets respectively. This implies that the PR form of the molecule lacks well-formed B and A pockets, and is positioned somewhat like an open baseball glove, waiting for the appropriate peptide to enter. We envision the binding of peptide by the MHC-I molecule to be a cooperative event, in which the encounter of the peptide with the PR binding groove, then coordinates the stabilization of the 46–52, 3_{10} helix-containing segment, as it swings in hinge-like fashion from the open to closed configuration. As the 46–52 segment swings in, pockets A and B are formed, allowing the tight binding of the peptide via its amino terminus and second residue side chain. It is indeed remarkable that a similar 3_{10} -helix containing region in MHC-II molecules appears to be involved in peptide loading of MHC-II molecules as catalyzed by HLA-DM in the endosome (Painter et al., 2011).

Our data only directly influence our understanding of the structural transition in the region that binds the amino terminal part of the peptide, and do not address the other crucial aspect of the transition from a PR to PL conformation—the release of MHC-I/ β 2m/peptide from the components of the peptide loading complex. The major interaction of the PR HC/ β 2m peptide-free heterodimer is with tapasin which engages a region of the MHC-I α 2 domain around residues 128–136, and seems to involve indirectly residues that line the F pocket. The cooperative stabilization of the bound peptide at both its amino and carboxyl termini results in the reorganization not only of the 46–52 segment of the α 1 domain, but also of the pseudo-symmetrical 128–136 segment of the α 2 domain. Needless to say, further experiments are needed to clarify the details of these molecular transitions.

3. Future perspectives

Our current studies explore an important, but small segment of the MHC-I molecule in its PR conformation. It may yet be possible to assemble all the interacting components of the PLC, MHC-I HC, β 2m, calreticulin, tapasin, and ERp57 into a crystallizable unit. Alternatively, careful exploitation of computational approaches including molecular docking and molecular dynamics simulations, along with quantitative measurements of the binding interactions among the components of the PLC should lead to a more refined understanding of this molecular machine that plays such an important role in antigen presentation.

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Fig. 1. Structural transition of an $\alpha 1$ domain region behaves like a door on a hinge. (A) The 3_{10} helix of amino acid residues 49–53 of H2-L^d is indicated by the cylinder, and the transition from the PR form to the PL conformation of the mature H2-L^d molecule (PDB code, 1LDP) is illustrated as an axial rotation of the cylinder. (B) Schematically, the solvent exposed (solid fill) residues of the PR form swing to a solvent inaccessible configuration (striped fill) PL form as though positioned on a swinging door. The arrow indicates the direction of the closing door transition. No specific steps in the transition are implied.