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Conformational variation in structures of classical and nonclassical MHCII proteins and functional implications

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Summary

Recent structural characterizations of classical and nonclassical major histocompatibility complex class II (MHCII) proteins have provided a view into the dynamic nature of the MHCII peptide binding groove and the role that structural changes play in peptide loading processes. Although there have been numerous reports of crystal structures for MHCII-peptide complexes, a detailed analysis comparing all the structures has not been reported, and subtle conformational variations present in these structures may not have been fully appreciated. We compared the 91 MHCII crystal structures reported in the PDB to date, including a HLA-DR mutant particularly susceptible to DM-mediated peptide exchange, and reviewed experimental and computational studies of the effect of peptide binding on MHCII structure. These studies provide evidence for conformational lability in and around the α subunit 3_{10} helix at residues $\alpha 48-51$, a region known to be critical for HLA-DM-mediated peptide exchange. A biophysical study of MHC-peptide hydrogen bond strengths and a recent structure of the nonclassical MHCII protein HLA-DO reveal changes in the same region. Conformational variability was observed also in the vicinity of a kink in the β subunit helical region near residue $\beta 66$ and in the orientation and loop conformation in the $\beta 2$ Ig domain. Here, we provide an overview of the regions within classical and nonclassical MHCII proteins that display conformational changes and the potential role that these changes may have in the peptide loading/exchange process.

Keywords

major histocompatibility complex; human lymphocyte antigen; antigen presentation; peptide binding; protein structure; conformational change

Introduction

Major histocompatibility complex class II (MHCII) proteins are ~50 kDa heterodimeric transmembrane glycoproteins that comprise a critical part of the adaptive immune response to foreign pathogens. MHCII proteins are expressed by so-called ‘professional antigen-presenting cells’ such as dendritic cells, macrophages, and B cells, where they bind peptides derived from self and foreign proteins and display them at the cell surface for interaction with CD4⁺ T cells (Fig. 1). Newly synthesized MHCII α and β subunits are translocated to the lumen of the endoplasmic reticulum where they associate with a trimeric chaperone protein known as the class II MHC-associated invariant chain (1)(Unlike the MHCII α and β subunit, the invariant chain is not polymorphic). The resultant nonameric complex (2) is

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sorted to the endosomal compartment via AP-1 (3). Except for the regions involved in trimer formation and MHCII association, the invariant chain is unstructured and very sensitive to proteolysis (4) and quickly succumbs to degradation by endosomal-resident proteases known as cathepsins (5, 6). The majority of the invariant chain is removed, leaving a small peptide fragment known as CLIP (class II invariant chain peptide) bound in the MHCII peptide binding groove (4, 7).

Peptides destined for endosomal loading onto MHCII proteins can derive from self or foreign proteins brought into the endosomal pathway by fluid-phase endocytosis, receptor-mediated endocytosis, or autophagy (Fig. 1A). The relative efficiency of these pathways depends on the type and maturation state of the antigen-presenting cell. Regardless of the source of peptide, CLIP peptide must be removed from MHCII to allow antigenic peptide binding. This process is facilitated by the nonclassical MHCII protein HLA-DM (H2-M in mice). Nonclassical in this context means non-peptide binding and non-polymorphic, in comparison to the classical human MHCII proteins HLA-DR, HLA-DP, HLA-DQ and classical murine MHCII proteins I-E and I-A, which are highly polymorphic and bind peptides. *In vitro*, DM serves as a catalyst to promote both peptide binding and release (8, 9) and so can be considered as an enzyme (10, 11). *In vivo*, DM is required for efficient peptide loading, and in its absence, many cell-surface MHCII still carry CLIP (12). Despite numerous studies (discussed below), the mechanism by which DM mediates peptide exchange still has not been resolved. After loading, MHCII-peptide complexes traffic to the cell surface, where they can interact with T-cell receptors on CD4⁺ T cells. Recycling MHCII proteins can be reloaded by a similar process (13).

In some types of antigen-presenting cells, the regulation of peptide exchange by DM is modulated by another nonclassical MHCII protein HLA-DO (DO). The role played by DO in antigen presentation currently is poorly understood, although the unique pattern of expression in thymocytes, trophoblasts, certain immature dendritic cells, and B cells until germinal center entry, has led to suggestions of a role in induction or maintenance of T-cell tolerance (14). The observation of decreased autoimmunity upon DO overexpression in a mouse diabetes model is consistent with this idea (15). Most *in vitro* and *in vivo* studies of DO action have revealed an inhibitory function, with DO inhibiting DM-mediated catalysis of peptide binding or peptide release (16–19). However, some exceptions suggesting a more complicated role for DO have appeared (20, 21). Recent work from our laboratory has shown that DO inhibits DM-mediated peptide exchange through a competitive inhibition mechanism, with DO acting as substrate analog (data not shown).

The class II region of the major histocompatibility locus (MHC) comprises genes for both classical and nonclassical MHC proteins (Fig. 1B,C). With our recent determination of a crystal structure for DO (in complex with DM) (data not shown), structural information now is available for an example of each of the MHC locus proteins. Crystal structures of the nonclassical MHCII proteins, DM and DO, have revealed these accessory proteins to be structural homologues of classical MHCII proteins, with the largest deviations in structural similarities along the MHCII peptide binding groove. There have been 91 classical MHCII-peptide crystal structures solved to date (Table 1), and although there is remarkable similarity among them all, there are subtle differences in key regions of the MHCII known to be important for peptide binding and DM-mediated peptide exchange.

MHC structural overview

The first crystal structure of an MHCII protein, determined almost two decades ago, was for HLA-DR1 [DRA*01:01, B1*01:01; see Marsh *et al.* (22) or <http://hla.alleles.org> for a description of the HLA allele nomenclature], bound to a mixture of B-cell-derived naturally

processed peptides (23). The structure revealed an architecture composed of a membrane distal peptide-binding groove formed by two α helices flanking an 8-stranded β pleated sheet floor, with both α and β subunits contributing approximately equal halves (Fig. 2A). The $\alpha 1$ and $\beta 1$ domains together comprise the peptide binding domain (Fig. 2B). The membrane proximal regions consist of two immunoglobulin (Ig)-like domains, $\alpha 2$ and $\beta 2$, one from each subunit. Membrane-spanning and short cytosolic domains were not included in that structure and have not been characterized since for any MHCII protein. Although this structure was generated from an MHCII protein in complex with a mixture of peptides in the binding groove, the extended polyproline type II conformation of the peptide backbone was evident (24). This conformation can be thought of as a twisted β strand with an approximate three-residue repeat, which directs some of the bound peptide side chains toward pockets in the MHCII peptide binding site, with the intervening residues exposed for interaction with TCR (Fig. 2C). The pockets conventionally are numbered relative to the largest HLA-DR1 pocket termed P1, which accommodates a hydrophobic residue near the peptide N-terminus. Pockets were also observed to accommodate the P4, P6, and P9 residues (25). The same basic peptide conformation has been observed in all subsequent structures, including one in which the peptide has been reported to bind in the opposite orientation (26). The MHCII peptide-binding groove was shown to be open-ended, thereby allowing peptides of variable length to bind. Subsequently, it has been shown that MHCII proteins can bind peptides with as little as two residues (27) as well as full length unfolded proteins (28), although studies of the spectrum of naturally processed peptides shown that most fall into the range of 13–25 residues, with an average length of ~15 (29).

Since the first crystal structure was solved, there have been an additional 91 MHCII–peptide complexes deposited in the PDB (Table 1). In addition to many peptide complexes of HLA-DR1, structures for other mouse and human MHCII proteins have allowed for a comparative structural approach. Subsequent studies of MHCII structures have confirmed the role of four main side chain specificity pockets, P1, P4, P6, and P9, with additional contributions from P3, P7, and P10 (30, 31). Due to the polymorphic nature of MHCII proteins, there are differences in the architecture, charge, and shape of the binding pockets, with consequent differences in peptide binding specificity. In many cases, the contributions of the pockets to the overall peptide-binding affinity can be considered independent, leading the use of simple position-specific scoring matrices (i.e. motifs) in predicting relative peptide binding affinities for many MHCII alleles (32). However, the predictive ability of these algorithms is somewhat limited as compared to similar approaches applied to MHCI proteins (22, 33), and cooperative effects have been shown to modulate binding affinity for at least some peptides (34).

The canonical conformation of peptides bound to MHCII proteins appears to be maintained by a hydrogen-bonding network that extends from the main chain of the peptide to both main chain and conserved side chain residues of the MHCII (24, 25) (Fig. 3). The hydrogen bond network is highly conserved despite differences in peptide sequences and MHCII proteins and therefore confers a stereotyped mode of binding across the spectrum of peptide-MHCII interactions. The disruption of the hydrogen bond network has been posited as a key determinant for DM-mediated peptide exchange. Because DM must work on highly polymorphic proteins, disruption of the hydrogen bonds that are invariant would provide a unifying mechanism that could extend across the repertoire of peptide-MHCII complexes (8). However, numerous studies have suggested a more complicated mechanism of action for DM (discussed below).

Structural alignments of all MHCII show heterogeneity in the α subunit 3_{10} helix and the β subunit helical kink

We have examined the 91 MHCII-peptide complex structures deposited in the PDB in order to determine regions of conformational heterogeneity. Although overall the structures are highly similar, alignments of the peptide binding domains of these MHCII proteins revealed structural heterogeneity in three regions: the α subunit 3–10 helical region $\alpha 45$ –54 (Fig. 4A,B), the $\beta 2$ Ig-like domain (Fig. 4C), and the pronounced kink in the β subunit helical region $\beta 62$ –71 (Fig. 5A).

The largest degree of structural heterogeneity is found in the $\beta 2$ Ig-like domain, whose orientation relative to the peptide binding domain varies by ~ 10 degrees (Fig. 4A, curved arrow). The motion is best described as a pivot about a point near the attachment to the peptide-binding domain and thus represents a rigid body motion rather than a conformational change within the Ig domain. After alignment of the Ig domains (Fig. 4C), less variation is observed, but there is still substantial conformational heterogeneity in the lower loops connecting the beta strands. The largest variation is seen for the A–B loop between the first two strands of the Ig domain, residues 105–112 (Fig. 4A,C). Residues in this region also tend to exhibit higher atomic displacement factors (B-factors) as compared to the rest of structure. Whether or not this sort of flexibility will be present for the full-length membrane-embedded protein is not known.

Outside the end of the $\beta 2$ -domain, the greatest structural heterogeneity observed within the set of MHCII crystal structures is in the α subunit 3_{10} helical region (Fig. 4A,B). In this region, residues $\alpha 45$ –51 form a short 3_{10} helix, at the N-terminal end of the peptide binding groove and roughly perpendicular to it, which connects the β sheet platform with the long α helix that defines the binding groove (Figs 2A and 3A). The 3_{10} helical conformation is similar to a conventional alpha helical conformation but wound more tightly, with 3 rather than 3.4 residues per turn and NH-CO hydrogen bonds between i and $i+3$ residues forming 10-atom rings. The 3_{10} helix is thought to be less stable than the alpha helix and extended 3_{10} helices are relatively rare in protein structures (35). In this region, the majority of the MHCII structures group together with C α deviation of about 2 Å. There are however large deviations for the HLA-DQ proteins and one of the mouse I-Ag7 structures (Fig. 4B, arrow). The greatest variance in distance is seen for the HLA-DQ protein structure 3PL6 at residue $\alpha 50$, which deviates from the HLA-DR1 structure 1DLH by 6.7 Å at this position. Interestingly, another HLA-DQ protein structure 1UVQ deviates greatly in this region as well, with multiple residues deviating from 1DLH by 4 Å. Even though both of these HLA-DQ alleles share the same α chain, HLA-DQA*01:02, they show as much variation between each other as they do when compared to 1DLH. Unlike the other HLA-DQ alleles, this α chain contains 2 glycines at the $\alpha 52$ and $\alpha 53$ positions that may confer structural lability in this region allowing multiple conformers to be adopted.

Aside from the HLA-DQ alleles carrying the Gly-Gly sequence at $\alpha 52$ – $\alpha 53$, the other MHCII protein structures exhibit smaller but still substantial deviations in the 3_{10} helix $\alpha 45$ –51 region. These deviations are not due to insertions or deletions. Although there is sequence variation in this region, only one protein, HLA-DQ (1SV9), has a deletion (at the $\alpha 52$ residue). Interestingly, insertion of a residue to repair the deletion restores DM susceptibility to this otherwise resistant allele (36). In addition to 1SV9, the largest variations in this region are observed for the HLA-DQ proteins 1UVQ, 4DP8, 2NNA, 3PL6, the mouse IAg-7 protein 1F3J, and the HLA-DR1 α F54C mutant 3QXD. The variation is best described by a partial unwinding of the 3_{10} helical structure, which is more pronounced in the C-terminal region of the helix. The largest outliers are 3PL6 and 1F3J, with $\alpha 48$ –51 NH-CO $i+3$ hydrogen bonds lengths greater than 4.5 Å, and a conformation intermediate

between 3_{10} and conventional α helix (Fig. 4B, grey arrow). Both of these structures do however conform to a 3_{10} helix for one turn between residues $\alpha 45$ -48. The other HLA-DQ structures that vary from the normal distribution in this region have 3_{10} helices that extend fully from $\alpha 45$ -51, although they all have an increased NH-CO $i+3$ hydrogen bond lengths of ~ 3.8 Å, as compared to 1DLH and the other structures for which this distance is ~ 3.3 Å.

Aligned with the 3_{10} helix is another similarly oriented short helical region from the β subunit that correspondingly connects the $\beta 1$ domain α helix to the lower $\beta 2$ Ig domain (Fig. 4A,B). This region however is found in a conventional α helical conformation and is much less conformationally heterogeneous in MHCII structures determined to date. Some structural variation is observed in this region for the several I-A^d (1IAO) and I-A^b (1MUJ, 3C60, 3RDT, 1LNU, 3C5Z, 3C6L) structures, which all have proline at position $\beta 86$.

The third region that exhibits significant structural variation in MHCII protein structure lies within the $\beta 1$ helix at the kinked region from residues $\beta 62$ -70 (Fig. 5). This region has extensive polymorphic variation and is also a site that has been implicated as undergoing peptide-induced conformational changes (see below). We aligned the peptide binding domains ($\alpha 1$ -85 and $\beta 1$ -90) of all the structures to 1DLH in order to examine regions of conformational variability (Fig. 5A,B). The majority of structures cluster together with C α deviations throughout the peptide binding site of less than 2 Å. The major exception to this (in addition to the 3_{10} helix) is in the region around Asp $\beta 66$, which shows a 3.7 Å range even between structures that cluster tightly throughout the rest of the peptide binding domain (Fig. 5B). There are two groups of outliers in this region. One consists of several I-A proteins that have a two-residue deletion in the $\beta 63$ -67 region, which causes the kinked region to tilt inward toward the peptide binding groove by approximately 4 Å (Fig. 5B, open arrow). The other group is consists of the two HLA-DRB5*01:01 structures 1H15 and 1ZGL. This group is displaced in the opposite direction at Asp $\beta 66$, moving up an away from the peptide by ~ 3 Å. In both cases in the crystal lattice there are intermolecular contacts at Asp $\beta 66$, one by a crystal contact (1H15) and one by a TCR contact (1ZGL). The latter is described in detail by Mariuzza's group (37), who compare the MHCII structure with and without TCR and find that the peptide binding groove is opened by 1.5 Å. They note the extensive contact at the β kinked region and the change induced there by the TCR V β contact. The conformational changes described by Li *et al.* (37) in the TCR-bound HLA-DRB5*01:01 are very similar to those induced in the other HLA-DRB5*0101 structure (1H15) at the crystal contact. We aligned the two molecules in the asymmetric unit of 1H15 and found that the crystal contact induces a 3 Å displacement of the beta Asp $\beta 66$ away from the peptide binding domain (Fig. 5C,D). There are extensive crystal contacts at other regions in this structure that do not result in conformational changes, which suggests this region in particular is labile. This alternate conformation exists despite the fact that this structure was constrained with an r.m.s. of 0.5 Å for all C α atoms (38).

Dynamic nature of the HLA-DR1 α F54C mutant MHCII-peptide complex

The structural variation in published MHCII-peptide structures could be due to structural differences induced by peptide or MHC sequence changes. However, in some cases the structural variation is observed even for multiple copies of the same protein within the crystallographic asymmetric unit, and so is likely to represent actual structural lability rather than peptide-dependent changes or allelic variation. For example, the $\beta 2$ Ig domain hinge motions were noted even in the first MHCII structures, which contained a dimer in the asymmetric unit (23, 25).

We recently reported the 2.3 Å crystal structure of HLA-DR1 (α F54C) bound to CLIP, along with WT HLA-DR1 bound to CLIP that crystallized in the same unit cell (39). The α F54C

point mutation in the peptide binding site makes the protein exquisitely susceptible to DM-mediated peptide release, more than 100-fold compared to WT HLA-DR1, and also increases the affinity for DM. The crystal structure revealed that the mutation facilitated a conformational change in one molecule of the asymmetric unit. The conformational change involved reorientation of the α -subunit 3₁₀ helix, along with concerted rotamer changes in residues Leu α 45, Phe α 48, Phe α 51, and Phe β 89 (Fig. 5B). Similar rotamer changes for residues Leu α 45, Phe α 48, and Phe α 51 are seen for all HLA-DQ structures that have the HLA-DQA*0301 α chain, 2NNA, 1JK8, and 4D8P. The conformational change in the α F54C mutant appeared to be induced by a crystal contact at Phe α 51, which contacted the β 2 domain of another molecule in the crystal. The ability to adopt the altered conformation was induced by the mutation, as the WT HLA-DR1-CLIP crystal had similar crystal contacts, but no change was induced in the WT structure. The Phe α 51 side chain is known from mutagenesis experiments in the WT protein to be an important DM contact site (40), and we speculated that the increased susceptibility to DM and increased DM affinity induced by the α F54C mutation were due to increased tendency to adopt the alternate conformation (39).

Evidence for increased lability in solution for α F54C and not just in crystals was obtained by hydrogen-deuterium exchange mass spectrometry (39). In this experiment, borrowed from the arsenal of tools used to investigate protein folding, peptide bond amide NH groups exchange with D₂O at different rates depending on their exposure to solvent and their participation in hydrogen-bonding interactions (Fig. 7A). We incubated pre-formed MHCII-peptide complexes in D₂O for various times, before transfer to cold acidic buffer to stop further exchange and strip the peptide from MHC for analysis by multi-dimensional mass spectrometry. The location of individual deuterated amide NH groups was determined by electron transfer dissociation (ETD) fragmentation (39). We found that amide bonds flanking the P1 pocket exchanged very slowly, implicating in particular the strong H-bonds involving the MHCII Asn β 82 side chain and Ser α 53 main chain (Fig. 7B). A much earlier study evaluating the effects of mutation of H-bonding residues on MHC-peptide dissociation revealed essentially the same phenomenon as a striking difference in the roles of Asn α 69 and Asn β 82 (41). For the HLA-DR1 α F54C mutation, faster exchange relative to WT HLA-DR1 was noted at the very stable P2 amide NH hydrogen bond involving Asn β 82 (Fig. 6B). Since the α F54C crystal structure retained the intact canonical hydrogen bond network, we interpreted this difference in H-D exchange rates as evidence for dynamic flexibility in the α F54C peptide binding groove, in particular around the P1 site.

Conformational changes in MHCII induced by peptide binding and release

No crystal or NMR structure has been reported for any MHCII protein in the absence of peptide, but biophysical and biochemical studies have shown that the peptide-free or 'empty' form of MHCII has significant conformational changes relative to the canonical peptide-bound form. For HLA-DR1 at least, peptide-free species can be obtained by refolding purified α and β subunits expressed separately in *E. coli* (42). Peptide binding stabilizes the protein against SDS-induced chain dissociation and thermal denaturation (42), but even in the absence of the peptide HLA-DR1 is thermally stable ($T_m \sim 70^\circ\text{C}$) and folded correctly as shown by CD spectroscopy and conformation-dependent antibody binding (42, 43). Peptide binding induces changes in hydrodynamic behavior consistent with ~ 10 – 15% reduction in hydrodynamic radius, as observed by gel filtration, dynamic light scattering, and analytical ultracentrifugation (42), together with alterations in the CD spectrum consistent with a small increase in helical content. Together these were interpreted in terms of local conformational changes and/or small domain reorientations (43). Several monoclonal antibodies specifically recognize the empty protein (44, 45). Epitopes for these antibodies have been mapped to two regions, one including residues β 53–67 near the β

subunit helical kink, and the other at one including residues β 182-190 at the end of the β 2 Ig domain (44). Because of the way these antibodies were raised, only β subunit specificities were obtained. Evidence for peptide-induced conformational changes in the α subunit was obtained by a differential chemical modification approach, which identified Lys α 67 and Arg α 50, in addition to Lys β 98 and Arg β 189, as sites of peptide-induced changes in reactivity or accessibility (46). Overall, these studies provide clear evidence for peptide-driven conformational changes in HLA-DR1. Although these studies provide only relatively low resolution information, they appear to implicate the same regions for which conformational variation was observed in the crystal structure, *viz.* the α subunit 3_{10} helix, the β 1 domain helical kink, and β 2 Ig domain.

In the absence of experimental high-resolution structural information on the peptide-free MHCII conformation, several groups have used molecular dynamics and other computational approaches to gain insight into peptide-dependent conformational changes (47–50). The simulations all showed changes throughout the peptide-binding groove upon removal of peptide (47, 49, 50). A normal mode analysis of the peptide-free protein revealed similar motions (48). In one study, we removed the peptide from a HLA-DR1-peptide crystal structure and ran molecular dynamics simulations to explore conformations accessible to the peptide-free form (49). This simulation revealed conformational changes near the α subunit 3_{10} helix and β subunit helical kink. The α 51-59 change was a refolding of the extended strand region from the side of the binding groove into the position usually occupied by peptide (Fig. 8A). Strikingly, these alterations in structure maintained the general shape and electrostatic environment of the entire peptide binding groove, in particular, with the α F54 side chain engaging the P1 pocket in a similar manner as the original peptide P1 side chain (Fig. 8B). In addition, the canonical P-1 to P4 hydrogen bonding network was recapitulated between the conserved residues of the β chain and the reoriented α 51-59 region. Experimental support for this model was generated using monoclonal antibodies and a bacterial superantigen that bound epitopes on HLA-DR1 predicted to have altered accessibility due to the conformational changes induced upon peptide removal. This study also revealed a reorientation of the α subunit 3_{10} helix and a rigid body motion around the β 1 domain helical kink (Fig. 8A). Other molecular dynamics simulations of peptide-free MHCII are in agreement with a conformational change in the β kinked region as well as with more lability in the α subunit 3_{10} region, although these studies did not find the same degree of reorientation of the α 51-59 strand region (47, 48, 50).

Detailed kinetics studies from several groups have showed that peptide-free MHCII proteins can adopt (at least) two forms that differ in their ability to bind peptide (43, 51, 52), providing evidence for some degree of structural lability even in the absence of peptide. These experiments show a time-dependent decrease in MHCII peptide-binding capacity after removal of a bound peptide and have been interpreted as showing that a peptide-receptive form is generated immediately upon peptide release, which slowly and reversibly converts to a peptide-averse state over a few minutes (52). A role for DM in converting between these species has been suggested, but this is controversial (53, 54). Although there is ample evidence for distinct kinetic species, structural correlates of the peptide-receptive and peptide-averse forms have not been defined. An attractive model for the peptide-averse form is the molecular dynamics-derived structure described above with the α 51-59 region folded into the peptide binding site. In that scenario, engagement of the P1 pocket by the α F54 side chain and involvement of the conserved MHC hydrogen bonding residues would occlude the N-terminal end of the groove and interfere with peptide binding. However, this idea remains to be tested experimentally.

Nonclassical MHCII: HLA-DM and HLA-DO

Crystal structures for the nonclassical MHCII protein HLA-DM and its murine counterpart H-2M were first reported in 1998 (55, 56), with a higher resolution DM structure in 2006 (57). The protein fold overall is similar to classical MHCII, but with a distinctly collapsed upper domain (Fig. 9). DM is not known to bind peptides or other ligands, and no binding cleft is apparent in the upper domain [a small 'vestigial' pocket in the vicinity of MHCII P4 region is apparent, but is believed not to have functional role (55)]. In this aspect, DM is like some nonclassical MHCI proteins, which do not appear to bind peptide or other ligands and which also have closed binding sites (58). However, unlike these nonclassical MHCI proteins, DM does not interact at the cell surface with binding partners on other cells but instead acts to facilitate MHCII-peptide exchange by interacting with classical MHCII peptide complexes in the same membrane (59). The pattern of mutations known to disrupt DM-MHCII interaction (40, 60, 61) and studies of tethered complexes (62, 63) support a side-by-side orientation of DM in its interaction with MHCII. How this interaction leads to facilitated peptide binding and release is still a mystery, despite extensive efforts in many laboratories, and several mechanisms have been proposed. A mechanism in which HLA-DM mediates peptide catalysis by disruption of one or more hydrogen bonds between the peptide and the MHCII has been the focus of a number of studies (8, 64, 65). However, recent mutagenesis studies suggest that none of the individual hydrogen bonds that extend from the peptide to the MHCII side chains are responsible for the increased rate of peptide catalysis by HLA-DM (66, 67). Other work suggesting a compare-and-exchange mechanism in which DM mediates exchange of free peptide with MHC-bound peptide through a two-peptide intermediate state has been put forth (68). A number of studies have focused on the ability of HLA-DM to bind to alternate conformers of MHCII. One study suggests HLA-DM recognizes a 'floppy' P1 pocket (69). In addition, HLA-DM has been proposed to recognize MHCII that has a vacant P1 pocket (60). The HLA-DR1 α F54C mass spectrometry work from our lab suggests that HLA-DM preferentially binds MHCII with conformational flexibility in the 3_{10} helical region, leading to binding of HLA-DM to conformers of MHCII regardless of the peptide occupancy (39). A recent report showing that peptide-loaded MHCII isomers can be substrates for HLA-DM could be interpreted such that conformational heterogeneity of the MHCII peptide complex, rather than the occupancy of the P1 pocket, is a crucial determinant for HLA-DM peptide catalysis (70).

Recently we determined the crystal structure of the other nonclassical MHCII protein, DO, in complex with DM (data not shown). The overall fold is very much like a classical MHCII protein and distinctly different from that of DM (Fig. 9). The upper domain is not collapsed as for DM and still retains the contours of an apparent binding cleft, although density was not observed in the binding site and DO has not been reported to bind peptides or other ligands. Interestingly, large structural differences relative to classical MHCII were mostly restricted to the α subunit 3_{10} region and β 2 Ig-like domain, i.e. the same regions where MHCII proteins have conformational variability. The conformational alterations at the N-terminal side of the peptide-binding site would be expected to interfere with peptide binding, at least by analogy with MHCII, and might help explain DO's tight binding to DM.

Conclusions

Analysis of available structural information on MHCII proteins reveals three areas of conformational lability: the region around the α subunit 3_{10} helix, the β 1 domain helical kink, and the β 2 Ig domain lower loops. Several sources of information, including conformational variation observed for crystal structures of different MHCII – peptide complexes, differences between identical molecules in different crystallographic asymmetric units, biochemical studies of peptide-induced conformational changes, and computational

modeling of empty and peptide-loaded MHCII proteins, all point to structural lability in the same regions. At least for the α subunit 3₁₀ helix region, the changes appear to be consistent with a concerted motion between stable conformers, rather than a continuum of related conformations. This region, along with the distal end of the β 2 domain Ig region, has been implicated in DM catalysis (40, 60, 62). How DM works still is not clear, but most studies point to important role for peptide-MHC interaction at N-terminus of the binding site in DM function. The DR1 α F54C mutant, which exhibits greatly increased susceptibility to DM action, increased structural lability of the α subunit 3₁₀ helix, and weakened MHC-peptide hydrogen bonding around the P1 pocket, provides additional support for the importance of this region. The recent structure of HLA-DO bound to HLA-DM provides another example of conformational variation within the overall MHCII fold, with alterations in the α subunit 3₁₀ helix and adjacent regions that appear to be responsible for tight binding to DM. Although the DO structure shows larger conformational alterations than observed within the current set of classical MHCII – peptide crystal structures, the changes are in the same regions, and molecular dynamics studies suggest that similar and even larger changes in the same regions are accessible to MHCII proteins in the absence of peptide. Given the strong homology between DO and classical class II MHC proteins, is it possible that the conformational alterations in MHCII proteins described represent snapshots along a trajectory for DM-MHCII interaction and subsequent peptide exchange.

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Fig. 1. Class II MHC antigen processing pathway

(A) Newly synthesized MHCII proteins associate in the endoplasmic reticulum with invariant chain chaperone that guides transport to endosomal compartments. Endosomal proteases cleave the invariant chain to leave a small peptide (clip) in the MHCII binding site. DM acts as a catalytic peptide exchange factor to release clip and promote binding of endosomal peptides derived from self and foreign proteins brought into the compartment by endocytosis or autophagy. Peptide-loaded MHCII proteins traffic to the cell surface for interaction with CD4⁺ T cells. MHCII can also be loaded with peptide at the cell surface or after recycling. (B) Arrangement of classical (HLA-DP, HLA-DQ, HLA-DR) and nonclassical (HLA-DM, HLA-DO) human MHCII α and β subunit genes in the major histocompatibility complex (MHC) locus on chromosome 6. In most haplotypes, DRB1 and one of the DRB3, DRB4, or DRB5 genes are expressed. (C) Phylogenetic tree showing sequence similarity relationships between MHC genes. Calculated for concatenated α and β sequences. Values in parenthesis show pairwise amino acid identity with HLA-DR. Classical MHCII are highly polymorphic. Similarities were calculated for 0101 alleles of all genes except for DQB*0501, using IMGT (71), BLAST (72), and TreeDyn (73).

Fig. 2. MHCII structure

(A) Crystal structure for HLA-DR1 (PDB ID 1DLH) with HA peptide shown in stick representation, with cartoon representation for the α chain shown in sand, with the 3–10 helical region in brown, and the for β chain shown in cyan. (B) Top view of the peptide binding groove shown in surface representation with HA peptide shown. The N-terminus of the peptide is at the left and the α 1 helix is at the top. (C) 90° rotation of the surface representation shown in panel (B) cutaway to show the P1, P4, P6 and P9 side chain specificity pockets.

Fig. 3. Hydrogen bonding network

(A) Top view of the peptide binding groove shown in cartoon representation with HA peptide backbone shown in stick representation, colored as in Fig. 2. MHCII conserved residues that contribute to the peptide-MHC hydrogen bonding network are shown in stick representation. Dashed lines indicate conserved hydrogen bonds. (B) Chemical diagram of the peptide-MHC hydrogen bonding. MHCII residues are noted and the positions of the peptide side chains are indicated in red.

Fig. 4. Conformational variability in MHCII structures

MHCII structures in the PDB (Table 1) were aligned to the peptide binding domain of 1DLH (residues α 1-81 and β 1-90). (A) End view of the aligned structures looking down the bound peptide from N- to C-termini, with variable region around the α subunit 3–10 helix near the peptide N-terminus boxed. (B) Closeup of 3–10 helical region. Outliers from an otherwise tightly grouped cluster of structures are shown in red (HLA-DQ, 3PL6), blue (HLA-DQ, 1UVQ), green (HLA-DQ, 1SV9), orange (HLA-DQ, 4DP8), magenta (HLA-DQ, 2NNA), cyan (HLA-DR1 F54C, 3QXD, chains d–f), and purple (I-Ag7, 1F3J). (C) MHCII were aligned to the β 2 Ig domain 1DLH (residues 91–180). After domain alignment structural heterogeneity is restricted to the lower loops.

Fig. 5. Conformational variability in the β chain helical region

(A,B) MHCII structures were aligned as in Fig. 4. (A) Top view of the aligned structures with peptide N-terminus at left and variable region around the β subunit helical kink at residues 62–70 boxed. (B) Outliers tilting distal to the peptide binding groove indicated by red arrow: red (HLA-DR5b, H15), cyan (HLA-DR5b, 1ZGL). Outliers tilting inward towards the peptide binding groove indicated by open arrow: blue (I-Ag7, 3CUP), slate (I-Au, 1U3H), chocolate (I-Au, 2P24), purple (I-Ag7, 1F3J), salmon (I-Ag7, 1ES0), yellow (I-Au, 2PXY), magenta (I-Ag7, 3MBE), orange (I-Ag7, 2Z31), and wheat (I-Ak, 1IAK). (C) Ribbon diagram of two molecules in the asymmetric unit of 1H15 showing reorientation in the β subunit helical kink region (arrow) induced by a crystal contact (green) at residue Asp β 66. (D) Top view of the two molecules shown in panel C.

Fig. 6. HLA-DR1 α F54C mutant structure showing conformational change between two molecules in the asymmetric unit

Two molecules in asymmetric unit are shown in green and salmon. (A) Top view with residues that undergo conformational changes shown in stick representation. (B) 90° rotation of the top panel showing the altered pitch of the 3–10 alpha helix and rotamer changes

Fig. 7. Hydrogen-deuterium exchange mass spectrometry provides information on stability of MHCII-peptide hydrogen bonds

(A) Mass spectra of HA peptide in H₂O (left), HA peptide in D₂O (center), and HA peptide bound to MHCII after incubation in D₂O for 30 seconds. With increasing time in D₂O additional exchange is observed, depending on amide hydrogen bonding and exposure. Cartoons above spectra are intended to indicate general effect of MHC-peptide hydrogen-bonding on hydrogen-deuterium exchange. (B) Summary of hydrogen-deuterium electron transfer dissociation data for amide NH groups of HA peptide bound to HLA-DR1, from Painter *et al.* (39). MHC-peptide hydrogen bond network shown by dashed lines. NH groups were characterized as having slow (red), medium (orange), or fast (green) exchange kinetics based on degree of exchange of NH at several time points: slow, less than 30% at all time points; medium, less than 80% exchanged at 128 minutes; fast, more than 80% exchanged at 2 minutes and/or unprotected. Star indicates the NH group that experienced less protection for HA bound to the αF54C mutant of HLA-DR1, where it was assigned a medium exchange rate value.

MHC II peptide Structure

Peptide-free model

Fig. 8. Comparison of crystal structure of MHCII – peptide complex and molecular dynamics model of peptide-free MHCII

(A) Peptide-binding domain of HLA-DR1 with HA peptide (1DLH, cyan) and peptide-free model from molecular dynamics simulation (49). Side chain specificity pockets are labeled P1-P9. In the peptide-free model the α chain has partially unwound and the extended strand region has moved into the peptide-binding groove. Residues $\alpha 51$ and $\alpha 59$ that define the extent of the conformational rearrangement are indicated). (B) Surface representation cutaway view of peptide binding site (same orientation as Fig. 1C) with the P1 pocket engaged by the HA peptide tyrosine in the MHCII-peptide complex or by the extended strand region $\alpha F54$ side chain in the peptide-free model.

Fig. 9. Structural similarities between classical MHCII (HLA-DR1) and nonclassical MHCII (HLA-DO and HLA-DM)
 α chains shown in brown, beta chains in cyan, and peptide (for HLA-DR1) shown in red. (A) Top view of peptide binding site. (B) Same view as top panel with surface representation. (C) 90° rotation of the top panel.

Table 1

PDB ID	Protein	Peptide	Res.
1K8I	H2-M		3.1
1HDM	HLA-DM		2.5
2BC4	HLA-DM		2.27
3LQZ	HLA-DP2	DRa	3.25
3PL6	HLA-DQ1 (A1*0102/B1*0502)	MBP	2.55
1S9V	HLA-DQ2 (A1*0501/B1*0201)	deam. gliadin	2.2
4D8P	HLA-DQ2.3 (A1*0301/B1*0201)	Gliadin	3.05
1UVQ	HLA-DQ6 (A1*0102/B1*0602)	hypocretin	1.8
1JK8	HLA-DQ8 (A1*0301/B1*0302)	human insulin	2.4
2NNA	HLA-DQ8 (A1*0301/B1*0302)	deam. glutein	2.1
3QXA	HLA-DR1	CLIP	2.71
3QXD	HLA-DR1	CLIP	2.3
3S4S	HLA-DR1	HA	2.4
3S51	HLA-DR1	HA	2.1
1AQD	HLA-DR1 (B1*0101)	endogenous	2.45
1DLH	HLA-DR1 (B1*0101)	HA	2.8
1FYT	HLA-DR1 (B1*0101)	HA	2.6
1HXY	HLA-DR1 (B1*0101)	HA	2.6
1JWM	HLA-DR1 (B1*0101)	HA	2.7
1JWS	HLA-DR1 (B1*0101)	HA	2.6
1JWU	HLA-DR1 (B1*0101)	HA	2.3
1KGO	HLA-DR1 (B1*0101)	EBV	2.65
1KLG	HLA-DR1 (B1*0101)	TPI	2.4
1KLU	HLA-DR1 (B1*0101)	TPI	1.93
1LO5	HLA-DR1 (B1*0101)	HA	3.2
1PYW	HLA-DR1 (B1*0101)	HA variant	2.1
1R5I	HLA-DR1 (B1*0101)	MAM	2.6
1SEB	HLA-DR1 (B1*0101)	endogenous	2.7
1SJE	HLA-DR1 (B1*0101)	HIV 16mer	2.45
1SJH	HLA-DR1 (B1*0101)	HIV 13mer	2.25
1T5W	HLA-DR1 (B1*0101)	synthetic	2.4
1TX5	HLA-DR1 (B1*0101)	synthetic	2.5
2FSE	HLA-DR1 (B1*0101)	collagen II	3.1
2G9H	HLA-DR1 (B1*0101)	HA	2
2IAM	HLA-DR1 (B1*0101)	TPI	2.8
2IAN	HLA-DR1 (B1*0101)	TPI	2.8
2ICW	HLA-DR1 (B1*0101)	HA	2.41
2IPK	HLA-DR1 (B1*0101)	HA variant	2.3
2OJE	HLA-DR1 (B1*0101)	HA	3
2WBJ	HLA-DR1 (B1*0101)	ENGA	3

PDB ID	Protein	Peptide	Res.
3L6F	HLA-DR1 (B1*0101)	MART-1	2.1
3PDO	HLA-DR1 (B1*0101)	CLIP	1.95
3PGC	HLA-DR1 (B1*0101)	CLIP (Flipped)	2.66
3PGD	HLA-DR1 (B1*0101)	CLIP	2.72
1FV1	HLA-DR2a (B5*0101)	MBP	1.9
1H15	HLA-DR2a (B5*0101)	EBV	3.1
1ZGL	HLA-DR2a (B5*0101)	MBP	2.8
1HQR	HLA-DR2a (B5*0101)	MBP	3.2
1BX2	HLA-DR2b (B1*1501)	MBP	2.6
1YMM	HLA-DR2b (B1*1501)	MBP	3.5
1A6A	HLA-DR3 (B1*0301)	CLIP	2.75
3T0E	HLA-DR4 (B1*0401)	MBP	4
1D5M	HLA-DR4 (B1*0401)	pep mimetic	2
1D5X	HLA-DR4 (B1*0401)	pep mimetic	2.45
1D5Z	HLA-DR4 (B1*0401)	pep mimetic	2
1D6E	HLA-DR4 (B1*0401)	pep mimetic	2.45
1J8H	HLA-DR4 (B1*0401)	HA	2.4
2SEB	HLA-DR4 (B1*0401)	Collagen II	2.5
2XN9	HLA-DR4 (B1*0401)	HA	2.3
3O6F	HLA-DR4 (B1*0401)	MBP	2.8
2Q6W	HLA-DR52a (B3*0101)	platelet integ.	2.25
3C5J	HLA-DR52c (B3*0301)	UNP	1.8
1LNU	I-A ^b	3K (Ea. variant)	2.5
1MUJ	I-A ^b	CLIP	2.15
3C5Z	I-A ^b	3K	2.55
3C60	I-A ^b	3K	3.05
3C6L	I-A ^b	3K	3.4
1IAO	I-A ^d	Ova	1.6
2IAD	I-A ^d	HA	2.4
3RDT	I-A ^b	3K	2.7
1ES0	I-Ag7	GAD65	2.6
1F3J	I-Ag7	HEL	3.1
3CUP	I-Ag7	GAD	3.09
3MBE	I-Ag7	HEL	2.89
1D9K	I-A ^k	Conalbumin	3.2
1IAK	I-A ^k	HEL	1.9
1JL4	I-A ^k	Ovotransferrin	4.3
1K2D	I-A ^u	MBP	2.2
1U3H	I-A ^u	MBP	2.42
2P24	I-A ^u	MBP	2.15
2PXY	I-A ^u	MBP	2.23

PDB ID	Protein	Peptide	Res.
2Z31	I-A ^u	MBP	2.7
1FNE	I-E ^K	HB	1.9
1FNG	I-E ^K	HB	1.9
3QIU	I-EK	MCC	2.7
3QIW	I-EK	MCC	3.3
1I3R	IE-k	Hemoglobin b2	2.4
1IEA	IE-k	HB	2.3
1IEB	IE-k	HSP70	2.7
1KT2	IE-k	MCC	2.8
1KTD	IE-k	PCC	2.4
1R5V	IE-k	artificial pep.	2.5
1R5W	IE-k	artificial pep.	2.9
3QIB	IE-k	MCC	2.7