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Hydrogen Bond Integrity Between MHC Class II Molecules and Bound Peptide Determines the Intracellular Fate of MHC Class II Molecules

Lynne S. Arneson, John F. Katz, Michael Liu, and Andrea J. Sant

MHC class II molecules associate with peptides through pocket interactions and the formation of hydrogen bonds. The current paradigm suggests that the interaction of side chains of the peptide with pockets in the class II molecule is responsible for the formation of stable class II-peptide complexes. However, recent evidence has shown that the formation of hydrogen bonds between genetically conserved residues of the class II molecule and the main chain of the peptide contributes profoundly to peptide stability. In this study, we have used I-A\(^k\), a class II molecule known to form strong pocket interactions with bound peptides, to probe the general importance of hydrogen bond integrity in peptide acquisition. Our studies have revealed that abolishing hydrogen bonds contributing by positions 81 or 82 in the \(\beta\)-chain of I-A\(^k\) results in class II molecules that are internally degraded when trafficked through proteolytic endosomal compartments. The presence of high-affinity peptides derived from either endogenous or exogenous sources protects the hydrogen bond-deficient variant from intracellular degradation. Together, these data indicate that disruption of the potential to form a complete hydrogen bond network between MHC class II molecules and bound peptides greatly diminishes the ability of class II molecules to bind peptides. The subsequent failure to stably acquire peptides leads to protease sensitivity of empty class II molecules, and thus to proteolytic degradation before export to the surface of APCs. *The Journal of Immunology*, 2001, 167: 6939–6946.
interactions between MHC and peptide, hydrogen bonds are formed between conserved amino acids in the class II molecule and the main chain of the bound peptide. In both human and mouse, the class II residues that participate in this hydrogen bond network are generally highly conserved across class II alleles, suggesting the importance of the resulting hydrogen bond network formed between the class II molecule and the bound peptide.

Previous studies from our laboratory suggest that the loss of a single hydrogen bond in the hydrogen bond network formed between class II and bound peptides can have a profound effect on stable peptide acquisition (8, 12, 13). However, these studies used the I-Ak class II molecule, which has been shown to bind peptides without apparently strong pocket interactions (11). Thus, I-Ak may rely to an atypically high degree on the formation of the hydrogen bond network to achieve stable peptide binding. To understand in a general way the importance of the full integrity of the hydrogen bond network, we initiated a set of studies using a different allelic form of class II molecule, I-Aα, that engages peptides through strong pocket interactions (reviewed in Ref. 16). We found that the loss of one or more hydrogen bonds results in a severely decreased ability to acquire peptides, resulting in intracellular degradation of the class II molecule. Although the addition of a high-affinity peptide, by either endogenous or exogenous routes, can protect hydrogen bond-deficient class II molecules from degradation, the presence of the chaperone molecule DM does not prevent degradation, suggesting that high-affinity peptides that can sustain binding in the absence of hydrogen bond integrity may be rare within the normal peptide milieu of APCs. Together, these data indicate that disruption of the hydrogen bond network formed between MHC class II and bound peptides greatly diminishes the ability of the class II molecule to bind peptides, and that such failure to stably acquire peptides leads to increased protease sensitivity of empty class II molecules, and thus to proteolytic degradation before export to the surface of APCs. Thus, it appears that hydrogen bond integrity is a critical structural element for stable peptide binding by class II molecules.

Materials and Methods

Reagents and cell lines

Mutations in I-Ak class II molecules were made at positions 81 or 82 in the β-chain by site-directed PCR mutagenesis (14). Amino acid 81 was mutated from His to Asn, whereas 82 was mutated from Asn to Ser. Following sequencing to verify that only the intended mutations were introduced, Ltk+ cells were stably transfected with I-Ak cDNAs with the selectable marker pSV2neo. Cells that survived drug selection were subcloned, and clones expressing the hydrogen bond-deficient variant, the class II molecule used has been shown to bind peptide in the absence of strong pocket interactions (11) and thus may rely to an unusual degree on the hydrogen bond network for stable peptide binding. Therefore, we extended these studies to gain a more generalized understanding of the relative importance of hydrogen bond interactions between peptides and MHC. To do this, we have examined the effects of the formation of the hydrogen bond network on peptide acquisition by I-Ak, an allelic form of class II molecule that uses strong pocket interactions to facilitate stable peptide binding (12, 16). We mutated I-Ak such that one or more hydrogen bonds formed between class II and bound peptides were lost. The solved crystal structure of I-Ak, HE9-48-61 complex indicates that 12 of the 26 nitrogen atoms present in the bound peptide directly form hydrogen bonds with I-Ak, whereas nine associate with the class II molecule by forming hydrogen bonds with water, which in turn form extended hydrogen bond networks with I-Ak. Both amino acids 81 and 82 in the β-chain of I-Ak directly form hydrogen bonds with the bound peptide (10). PCR site-directed mutagenesis was used to mutate either β81 from His to Asn or β82 from Asn to Ser to abrogate the formation of either one (Asnβ81) or two (Serβ82) hydrogen bonds (Fig. 1), and the hydrogen bond-deficient class II molecules and wild-type I-Ak were expressed in L cell fibroblasts (Table I).

SDS stable dimer analysis

Cells were harvested by brief exposure to trypsin, and they were counted, pelleted, and lysed in 1% 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid lysis buffer (150 mM NaCl, 50 mM Tris, pH 7.6, and 5 mM EDTA) on ice for 30 min. Postnuclear supernatants were incubated with 10.2-16 prebound to protein A-Sepharose (100 μl of culture supernatant per immunoprecipitation) and were rotated at 4°C for 4 h. Material bound to protein A-Sepharose was eluted with SDS sample buffer for 30 min at room temperature with frequent mixing. The protein A-Sepharose beads were pelleted and the supernatant was split evenly into two tubes. One tube was boiled for 3 min, whereas the other tube was not. The supernatant was fractionated on a 10% acrylamide gel overnight, with the current never exceeding 20 mAmps per gel. Protein was transferred onto nitrocellulose and the nitrocellulose was incubated with 5% milk in TBST and NaN3 (“Blotto”) for 1 h. The blot was then incubated with 10.2-16 in Blotto overnight, washed, then incubated in peroxidase-linked goat antimouse at 1/10,000 for 1 h. Following extensive washing, the blot was exposed to film with chemiluminescence.

Results

Previous work from our laboratory used a mutation at position 81 in the β-chain of I-Ak to determine the effect of the loss of a single hydrogen bond on stable peptide acquisition (8, 12, 13). Although these studies determined that the loss of a single hydrogen bond resulted in dramatically decreased peptide binding efficiency, which in turn leads to endosomal degradation of the hydrogen bond-deficient variant, the class II molecule used has been shown to bind peptide in the absence of strong pocket interactions (11) and thus may rely to an unusual degree on the hydrogen bond network for stable peptide binding. Therefore, we extended these studies to gain a more generalized understanding of the relative importance of hydrogen bond interactions between peptides and MHC. To do this, we have examined the effects of the formation of the hydrogen bond network on peptide acquisition by I-Ak, an allelic form of class II molecule that uses strong pocket interactions to facilitate stable peptide binding (12, 16). We mutated I-Ak such that one or more hydrogen bonds formed between class II and bound peptides were lost. The solved crystal structure of I-Ak, HE9-48-61 complex indicates that 12 of the 26 nitrogen atoms present in the bound peptide directly form hydrogen bonds with I-Ak, whereas nine associate with the class II molecule by forming hydrogen bonds with water, which in turn form extended hydrogen bond networks with I-Ak. Both amino acids 81 and 82 in the β-chain of I-Ak directly form hydrogen bonds with the bound peptide (10). PCR site-directed mutagenesis was used to mutate either β81 from His to Asn or β82 from Asn to Ser to abrogate the formation of either one (Asnβ81) or two (Serβ82) hydrogen bonds (Fig. 1), and the hydrogen bond-deficient class II molecules and wild-type I-Ak were expressed in L cell fibroblasts (Table I).
To study the impact of hydrogen bond loss on the structural integrity and peptide binding ability of the class II molecule, pulse-chase analysis was used to assess the folding, assembly, and intracellular fate of the wild-type and hydrogen bond-deficient class II molecules. Cells expressing wild-type I-A$k$ or I-A$k^{81H}$ were pulsed for 1 h with medium containing $[^{3}H]$leucine to label newly synthesized protein, and were then chased in complete medium for 0, 1, or 4 h to allow the labeled protein to transit through the secretory pathway. At the end of each chase point, the cells were lysed and class I and class II molecules were serially immunoprecipitated from the postnuclear lysate. Fig. 2 shows that the mature hydrogen bond-deficient class II molecule (I-A$k^{81H}$) persisting following a 4-h chase was similar to that seen with wild-type I-A$k$, indicating that the trafficking and half-life of these molecules is similar. Coseparation of class II $\alpha$-chain by the $\beta$-chain-specific Ab indicated that both wild-type I-A$k$ and I-A$k^{81H}$ assembled into correctly folded class II heterodimers that transited through the Golgi apparatus as indicated by the apparent increase in m.w. due to N-linked sugar maturation. Similar results were seen with I-A$k^{82H}$ in the absence of coexpressed invariant chain (Fig. 3).

Previous work has indicated that the hydrogen bond network has a profound effect on class II peptide binding, which occurs in endosomal compartments by coexpression of invariant chain. Cells expressing either wild-type I-A$k$ or I-A$k^{81H}$ plus invariant chain were pulsed with $[^{3}H]$leucine for 1 h and chased in the absence of radiolabel for 0, 1, or 4 h. Following each chase point, the cells were lysed, and class I and class II molecules were serially immunoprecipitated from the detergent lysate. Wild-type I-A$k$ that is directed to endosomal compartments by association with invariant chain persists in a mature state after a 4-h chase, as indicated by the presence of mature $\beta$-chain (Fig. 2). Mature wild-type I-A$k$ $\alpha$-chain is also present in the 4-h chase point, but is less visible due to the formation of multiple glycosylation states. In contrast, both I-A$k^{81H}$ (Fig. 2) and I-A$k^{82H}$ (Fig. 3) fail to associate with invariant chain in the absence of radiolabeling.

Table I. Flow cytometry analysis of surface class II expression

<table>
<thead>
<tr>
<th>Class II Molecule</th>
<th>Ab</th>
<th>A$k^{wt}$</th>
<th>A$k^{81}$</th>
<th>A$k^{82}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.4.4S (neg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11</td>
<td>12</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>10.2.16</td>
<td>542</td>
<td>305</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>40L</td>
<td>505</td>
<td>246</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>40M</td>
<td>407</td>
<td>213</td>
<td>104</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Cells expressing wild-type or the hydrogen bond-deficient class II molecules were stained with the Abs indicated in the left column. Shown are the mean fluorescence intensity obtained with each Ab.

<sup>b</sup>Negative Ab 14.4.4S.
I-A^Kβ82H^{-} (Fig. 3) class II molecules are greatly diminished and almost undetectable after 4 h of chase when targeted to endosomes. Thus, the hydrogen bond-deficient class II molecules have a decreased half-life as compared with wild-type I-A^K (Fig. 2). These data suggest the loss of one or more hydrogen bonds formed between class II and bound peptide results in premature intracellular degradation of the class II molecule when targeted to endosomal compartments by association with invariant chain.

Studies using class II molecules engineered to be transited to the cell surface in a peptide-free state indicated that empty class II molecules are protease sensitive, a property reversible by stable peptide binding (8). We have observed a similar protease sensitivity of the A^K hydrogen bond variants (not shown). These results, together with our studies of the decreased intracellular half-life of the hydrogen bond-deficient class II molecules, led us to hypothesize that the hydrogen bond-deficient class II molecules are unable to efficiently bind peptide, resulting in an increased population of empty class II molecules that undergo intracellular proteolytic degradation. To test this hypothesis, we used the invariant chain molecule to efficiently load a high-affinity peptide into the peptide-binding groove of I-A^Kβ82H^{-} class II molecules. The CLIP region of invariant chain was replaced with amino acids 48–61 from HEL (15), a peptide previously shown to bind to wild-type I-A^K with high affinity (Refs. 16 and 21). Cells expressing I-A^Kβ82H^{-} were supertransfected with the mutated invariant chain construct (II-HEL) and the intracellular fate of class II in the presence of the high-affinity HEL_{48-61} peptide was assessed by pulse-chase analysis. Cells were pulsed for 1 h with [3H]leucine and were chased for 0, 1, or 4 h in the absence of radiolabeled amino acids. Following each chase point, the cells were lysed and class I and class II molecules were immunoprecipitated from the lysate. As indicated above, I-A^Kβ82H^{-} class II molecules targeted to endosomes are degraded rapidly, as shown by the very low amount of mature class II following a 4-h chase. However, the coexpression of the invariant chain construct containing the HEL peptide results in the presence of mature I-A^Kβ82H^{-} class II molecules after 4 h of chase (Fig. 3), suggesting that the high-affinity interaction of HEL_{48-61} and I-A^Kβ82H^{-} results in formation of class II peptide complexes that persist, leading the molecule to maintain protease resistance.

**FIGURE 4.** Expression of II-HEL construct leads to egress of A^K-HEL \_48-61 at the cell surface, but does not confer SDS stability to the hydrogen bond-deficient variant of I-A^K. A. Expression of the I-A^K/HEL_{48-61} complex at the cell surface. Wild-type A^K or A^Kβ82H^{-} cells expressed without invariant chain (left two panels) or with the II-HEL construct (right two panels) were stained with the class II-specific Ab 10.2-16 (solid line) or its negative control mouse Ab 14-4-4S (dotted line), shown in the left-most panels of each group. Alternatively, the cells were stained with the rat mAb C4H3 that detects the I-A^K/HEL_{48-61} complex (solid line) or its rat Ab control 2.43 (dotted line) shown in the right panels of each group. B, SDS stability of I-A^K wild type vs I-A^Kβ82H^{-}. Shown is SDS-stable dimer analyses of A^K wild type or A^Kβ82H^{-} expressed either without invariant chain (p31), or with the invariant chain construct (p31), or with the invariant chain construct containing the HEL_{48-61} cassetted into the CLIP region of II (HEL). Cells were lysed and samples were immunoprecipitated with the class II mAb 10.2-16. Immunoprecipitated material was eluted at room temperature, split into two equal aliquots, either boiled (+) or left at room temperature (−), and fractionated by SDS-PAGE. Proteins were transferred to nitrocellulose and were probed in a Western Blot for class II β-chains using 10.2-16. Indicated on the left of the figure are the positions of SDS-stable αβ dimers or free β-chains.
To show that the I-A\(^{\k}\) wild-type and \(\beta 2 H^{-}\) class II molecules that emerged to the cell surface were carrying the HEL\(_{48-61}\) peptide within their peptide binding pockets, we examined the cells for reactivity with a mAb (C4H3) that detects the I-A\(^{\k}/\)HEL\(_{48-61}\) (18). Fig. 4A shows that upon expression of the li-HEL gene construct, both wild-type I-A\(^{\k}\) and the I-A\(^{\k}/\)\(\beta 2 H^{-}\) class II molecules gain readily detectable expression of this mAb epitope, indicating that a significant fraction of the class II molecules displayed by the transfectants bear HEL\(_{48-61}\) within the class II peptide binding pocket. Shown in Fig. 4B is an analysis of SDS-stable dimers within the wild-type and I-A\(^{\k}/\)\(\beta 2 H^{-}\) cells. These analyses indicated that despite the presence of the HEL\(_{48-61}\) peptide within its Ag-binding pocket, the I-A\(^{\k}/\)\(\beta 2 H^{-}\) class II molecules do not gain SDS stability. This result is consistent with the data suggesting that accessibility to SDS at the amino-terminal end of the MHC class II peptide binding pocket, rather than peptide binding per se, determine the phenotype of SDS stability (19).

The li-HEL construct used in the previous experiment enhanced the efficiency of HEL peptide loading onto the hydrogen bond-deficient class II molecules before arrival in endosomal compartments. In a more physiological setting, the class II molecules would encounter the high-affinity HEL peptides within endosomal compartments in the presence of other peptide Ags. It has been shown that cocculture of H-2\(^{b}\) splenocytes with HEL Ag increases life span and the steady-state expression of the I-A\(^{k}\) molecule (20, 21). To determine whether the high-affinity HEL peptide can be loaded onto the hydrogen bond-deficient class II molecules following invariant chain proteolysis and CLIP removal, cells expressing either wild-type I-A\(^{k}\), I-A\(^{k}/\)\(\beta 81 H^{-}\), or I-A\(^{k}/\)\(\beta 2 H^{-}\) and that coexpressed invariant chain were pulsed for 8 h with \(\left[{\text{H}}\right]\)leucine in the presence or absence of 8 mg/ml exogenous HEL protein. Following the pulse, the cells were lysed and class I and class II molecules were serially immunoprecipitated from the cell lysate. During the 8-h pulse, class II molecules were continually being synthesized and transported through the cell. Fig. 5 shows that at the end of the pulse, significant levels of wild-type I-A\(^{k}\) were present in the cell, and these levels were not altered appreciably by incubation with exogenous HEL. This result suggests that the quantity of peptides able to bind to the wild-type A\(^{k}\) molecule spontaneously are not limiting in the endosomal compartments of the transfectants. In contrast, both I-A\(^{k}/\)\(\beta 81 H^{-}\) and I-A\(^{k}/\)\(\beta 2 H^{-}\) undergo rapid degradation during the pulse due to targeting to endosomal compartments by coexpressed invariant chain, resulting in very low levels of mature class II molecules remaining at the end of the pulse. Incubation of cells expressing these hydrogen bond-deficient class II molecules with exogenous HEL results in an increase in the mature class II present in the cells following the 8-h pulse (Fig. 5). These results indicate that high-affinity peptides from exogenous sources can associate with hydrogen bond-deficient class II molecules, an event that leads to protection of the class II molecules from proteolytic degradation.

The hydrogen bond-deficient class II molecules undergo premature degradation when targeted to an endosomal compartment by association with invariant chain, suggesting that degradation is occurring in endosomes. To test this hypothesis, we incubated cells with ammonium chloride, a lysosomotropic agent that neutralizes the pH in endosomes, thus decreasing the proteolytic capacity of these organelles. Cells expressing either wild-type I-A\(^{k}\), I-A\(^{k}/\)\(\beta 81 H^{-}\), or I-A\(^{k}/\)\(\beta 2 H^{-}\) and invariant chain were pulsed for 8 h with \(\left[{\text{H}}\right]\)leucine and were incubated in the presence or absence of 20 mM ammonium chloride. Following the pulse, the cells were lysed and class I and class II were immunoprecipitated from the cell lysate and analyzed by SDS-PAGE for levels of mature class II remaining at the end of the 8-h pulse (Fig. 5). Incubation in the presence of ammonium chloride resulted in increased levels of labeled class II molecules following the overnight pulse, indicating that degradation of the hydrogen bond-deficient class II molecules indeed occurred in acidic proteolytic compartments, most likely endosomes.

We next determined the effect of DM coexpression on the fate of the hydrogen bond-deficient class II molecules directed to endosomal compartments. If high-affinity peptides reside in endosomal compartments in the fibroblasts transfected with class II, yet the hydrogen bond-deficient class II molecules are rapidly degraded before loading can occur, the presence of DM might rescue the class II molecules by increasing the efficiency of peptide loading. However, if peptides of sufficiently high affinity are rare in the APC, the addition of DM would have no effect on the degradation of the hydrogen bond-deficient class II molecules. Such a prediction, of course, assumes that the hydrogen bond-deficient variants

![FIGURE 5](image-url) High-affinity peptides derived from exogenous Ag rescue the hydrogen bond-deficient class II molecules from endosomal degradation. Cells expressing invariant chain and either I-A\(^{A}\), I-A\(^{A}/\)\(\beta 81 H^{-}\), or I-A\(^{A}/\)\(\beta 2 H^{-}\) were pulsed with \(\left[{\text{H}}\right]\)leucine for 8 h. During the pulse, the cells were incubated in either complete medium, or medium modified by the addition of either 8 mg/ml HEL or 20 mM ammonium chloride. Following the pulse, the cells were lysed and class II (A) and class I (B) molecules were serially immunoprecipitated from the postnuclear lysate with 16-1-11N or 10.2-16, respectively. Mature \(\alpha\) and \(\beta\) class II chains and the class I \(\alpha\) chain are indicated.

![FIGURE 6](image-url) Coexpression of DM does not result in efficient peptide loading of hydrogen bond-deficient class II molecules. Cells expressing I-A\(^{A}/\)\(\beta 2 H^{-}\) class II were supertransfected with either p31 invariant chain, p31HEL, or p31 and DM \(\alpha\)- and \(\beta\)-chains. These cells were pulsed with \(\left[{\text{H}}\right]\)leucine for 1 h and were then chased in unlabeled complete medium for either 0 or 4 h. After each chase point, one-half of the cells were lysed, and class I (B) and class II (A) molecules were serially immunoprecipitated with 16-1-11N or 10.2-16, respectively. Mature \(\alpha\) and \(\beta\) class II chains are indicated, as are p31, p31HEL, and the class I \(\alpha\) chain.
can interact productively with DM, an issue that we currently have no data on. Nevertheless, we sought to determine what effect DM has on degradation of these class II molecules. Therefore, we supertransfected cells expressing I-A<sup>β</sup>82H<sup>+</sup> and invariant chain with genes encoding murine DM α- and β-chains. Cells expressing DM were pulsed for 1 h, and they were then lysed immediately or chased for 4 h to assess the half-life of the class II molecule. Class I and class II molecules were then serially immunoprecipitated from postnuclear cell lysates. Coexpression of DM had no appreciable effect on the apparent half-life of the I-A<sup>β</sup>82H<sup>+</sup> class II molecule (Fig. 6), thus supporting the conclusion that the ability of the hydrogen bond-deficient class II molecule to stably acquire peptide is severely compromised.

**Discussion**

MHC class II molecules bind peptides through two types of interactions, the insertion of peptide side chains into pockets in the class II binding groove, and the formation of hydrogen bonds between conserved class II residues and the main chain of the bound peptide. Although the current paradigm indicates that pocket interactions are responsible both for peptide specificity and the formation of stable class II peptide complexes, recent work and this paper suggest that the hydrogen bond network formed between class II and bound peptides is crucial for efficient peptide binding (8, 12, 13, 22). In this study, we demonstrate that a type of class II molecule that generally associates with bound peptides through strong peptide side-chain class-II pocket interactions also requires the formation of the hydrogen bond network for efficient peptide binding. In the absence of complete hydrogen bond network formation, these class II molecules are susceptible to degradation in endosomal/lysosomal compartments. The addition of a high-affinity peptide results in peptide binding, despite the disruption of the hydrogen bond network, resulting in stabilization of the class II-peptide complex and resistance to internal degradation. However, coexpression of DM does not result in decreased class II proteolysis, indicating that the loss of one or more hydrogen bonds results in class II molecules that are profoundly compromised in their ability to stably acquire peptides.

MHC class II molecules undergo peptide exchange during their lifetime, for instance, associating with CLIP throughout the secretory pathway, which is released in endosomal compartments and exchanged for a peptide derived from either self- or foreign-internalized protein. Following expression at the cell surface and internalization, class II may exchange a previously bound peptide for a new one and return to the cell surface to display the new peptide for T cell surveillance. For peptide exchange to occur, the interactions resulting in the formation of stable class II-peptide complexes must be reversible. Thus, it seems likely that class II molecules have evolved to rely less on static pocket interactions and more on a cooperative interaction between pocket association and hydrogen bond formation in which these interactions are dependent on the integrity of different but nearby bonds. This cooperative interaction of nearby hydrogen bonds thus allows these bonds to be formed and dissolved relatively easily, allowing reversible peptide binding by class II molecules.

Although both class I and class II molecules bind and present peptides at the cell surface, their intracellular trafficking differs. Class I molecules associate with peptide in the ER shortly after synthesis, and peptide association is required for egress from the ER. In general, class I molecules are not typically thought to exchange bound peptides during their lifetime in APCs. Class II molecules do not bind antigenic peptide until late in their intracellular trafficking pathway. To prevent polypeptide binding in the ER, class II molecules associate with invariant chain, a part of which, CLIP, occupies the peptide binding groove. In endosomes, CLIP is removed from the class II molecule and is replaced with a peptide from an endogenous or internalized protein. The class II molecule presents these peptides at the cell surface, and may exchange bound peptides later following internalization from the cell surface. We speculate that features in the class II molecule responsible for stable peptide binding may respond to changes in pH found in endosomes, allowing exchange of associated peptides and may rely most extensively on cooperative binding interactions that allow stable yet reversible peptide binding.

Similar to class II peptide association, the formation of class I peptide complexes is mediated both by pocket interactions and the formation of hydrogen bonds between the class I molecule and the main chain of the bound peptide. However, unlike class II peptide complexes in which hydrogen bonds form throughout the length of the bound peptide, the hydrogen bonds formed in class I peptide complexes are clustered near the amino and carboxyl termini of the bound peptide. In fact, the amino and carboxyl groups at the first and last peptide residues participate in extensive hydrogen bond networks, which likely contributes substantially to the stability of the class I peptide complex (23) and to the closing of the peptide binding pocket at the periphery. Substitution of the terminal amino or carboxyl peptide groups with methyl groups (24), or rearrangement of the MHC molecule to facilitate binding of a 10-mer peptide (25), resulting in the loss of one or more hydrogen bonds between the class I molecule and the bound peptide, results in destabilization of the class I peptide complex. Thus, although both class I and class II molecules have important hydrogen bonding interactions with peptides, the precise role of the hydrogen bonds is likely distinct in these two molecules.

Our data indicates that the loss of a single hydrogen bond between a class II molecule and the bound peptide results in inefficient peptide binding and thus susceptibility to endosomal degradation. DM has previously been shown to enhance the loading of peptide onto class II molecules, in addition to removing CLIP from the class II peptide binding site (5). Therefore, DM may additionally function to protect class II molecules from premature degradation in endosomes by maintaining class II in a continuously peptide loaded state. Based on this theory, we would expect the coexpression of DM with the hydrogen bond-deficient class II molecules trafficked to endosomes to enhance peptide loading, resulting in protection from endosomal degradation. However, the coexpression of DM is not sufficient to promote peptide binding within the APC, suggesting that the hydrogen bond loss variants have severely compromised ability to stably acquire peptides. Although the model cell system used is not a professional APC, previous data have shown that this cell type is capable of presenting, most if not all, Ags used (reviewed in Ref 5). Therefore, it seems unlikely that the repertoire of peptides generated in L cells is more limited in abundance or diversity than would be found in B cells or freshly isolated APCs, and therefore, that the type of peptide that can bind to the hydrogen deficient class II molecules is very rare. This in turn suggests that the integrity of the hydrogen bond network is essential for stable association of most peptides with class II molecules.

An alternative or additional explanation for the inability of DM coexpression to result in promotion of peptide binding is that DM may be unable to associate with the hydrogen bond-deficient class II molecules. Random mutagenesis of HLA-DR molecules has identified a region of class II in close proximity to the amino terminus of the bound peptide that is required for productive interaction with DM (26). The class II molecules used in our studies lack one or more hydrogen bonds near the amino terminus of the
bound peptide, and therefore, any conformational changes resulting from the loss of these hydrogen bonds may affect the efficiency or consequences of DM interaction. We do not yet have any biochemical evidence that the hydrogen bond-deficient variant can interact productively with DM. Finally, it is conceivable that the levels of DM expressed within the transfectants are insufficient for promoting peptide binding.

Loading of the hydrogen bond-deficient class II molecules with either endogenous or exogenous HEL peptide protects the class II molecule from proteolysis. This change in susceptibility to proteolysis may result from a conformational change in the class II molecule induced by peptide binding. Previous studies have shown that class II molecules undergo a conformational change when they associate with peptide (28, 29). The formation of a compact class II dimer associated with a peptide that is resistant to SDS denaturation is also consistent with a peptide-induced conformational change in the class II molecule that results in inability of other molecules, either proteases or SDS, to act on the complex (30, 31). Finally, this data suggests that the initial proteolytic sites are sequestered by this conformational change, and then become accessible in the absence of peptide binding.

The finding that the hydrogen bond-deficient class II molecules are degraded is consistent with the view that full integrity of the hydrogen bond network is required for stable peptide binding, an event that allows escape from the proteolytic environment of endosomal compartments. This view is supported by our own in vitro peptide binding studies, which have shown that the loss of a single hydrogen bond between class II molecules and associated peptide results in accelerated dissociation of peptides (12). Hydrogen bonds are formed between the class II molecule and the main chain of the bound peptide along its full length, with sets of symmetrical hydrogen bonds formed at opposite ends of the peptide (βHis181Asn82 vs αHis68Asn69). Recent work has demonstrated that in I-Aβ class II molecules, these different sets of hydrogen bonds contribute disproportionately to peptide-MHC stability. The loss of hydrogen bonds near the amino terminus of the bound peptide, as previously demonstrated, resulted in substantial acceleration of peptide dissociation, and there-


