Evidence for a Conformational Change in a Class II Major Histocompatibility Complex Molecule Occuring in the Same pH Range Where Antigen Binding Is Enhanced

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Summary

Many class II histocompatibility complex molecules bind antigenic peptides optimally at low pH, consistent with their exposure to antigen in acidic endosomal compartments. While it has been suggested that a partially unfolded state serves as an intermediate involved in peptide binding, very little evidence for such a state has been obtained. In this report, we show that the murine class II molecule IE^k becomes increasingly less stable to sodium dodecyl sulfate–induced dissociation since the pH is decreased in the same range that enhances antigenic peptide binding. Furthermore, at mildly acidic pH levels, IE^k binds the fluorescent dye 1-anilino-naphthalene-8-sulfonic acid (ANS), a probe for exposed nonpolar sites in proteins, suggesting that protonation produces a molten globule-like state. The association of IE^k with a single high-affinity peptide had only a small effect in these two assays, indicating that the changes that occur are distal to the peptide-binding groove. Circular dichroism analysis shows that a pH shift from neutral to mildly acidic pH causes subtle changes in the environment of aromatic residues but does not grossly disrupt the secondary structure of IE^k. We propose a model in which perturbations in interdomain contacts outside the peptide-binding domain of IE^k occur at acidic pH, producing a partially unfolded state that facilitates optimal antigen binding.

Class I and II molecules of the MHC are heterodimeric cell-surface proteins that present antigenic peptides to T cells. This way, MHC molecules serve to alert the immune system to the presence of a pathogen. Antigenic peptides associate with MHC molecules in a membrane-distal superdomain that contains a deep groove between two antiparallel α-helices that lie on a β-sheet floor (1, 2). The peptide-binding groove is formed by the first domains of both chains (α_1 and β_1) in class II molecules, and by the first two domains of the heavy chain (α_1 and α_2) in class I. Class I MHC molecules present peptide fragments of viral as well as cellular proteins, which are synthesized in the host cell's cytoplasm to T cells expressing the CD8 coreceptor (reviewed in reference 3). These endogenous antigens are transported into the ER, where together with β_2-microglobulin, they bind and stabilize the class I heavy chain, allowing cell surface expression (4, 5). In contrast, class II MHC molecules present peptide fragments of exogenous proteins, such as bacterial proteins, which are derived from endocytosed extracellular antigens. Newly synthesized class II MHC molecules associate with the invariant chain, a chaperone that targets them to a late endosomal compartment (6, 7). During transit, it seems likely that the invariant chain occupies the peptide-binding groove and prevents premature binding of endogenous antigens (8). Once in the acidic endosome, proteolytic degradation releases the invariant chain and exogenously derived antigenic peptides bind the class II molecule by a process that is not yet understood. Then on the surface, they present peptide to T cells expressing the CD4 coreceptor (reviewed in reference 3).

As first suggested by the work of Jensen (9), peptide binding to class II molecules is, in most cases, enhanced at endosomal pH (10–17). In the case of the soluble form of IE^k used in this report, we have shown a 40-fold increase in the association rate of peptides at pH 5.0 vs pH 7.3 (12). In this context, kinetic and structural studies (18–21) have suggested that class II MHC molecules may exist in multiple states. Treatment of these proteins with heat (50–65°C) or low pH levels (<5.0) can induce the formation of a "floppy" form (21). This form migrates with a larger apparent mass than the "compact" heterodimer on nondenaturing SDS-PAGE gels, and it is an intermediate in the dissociation of the proteins to free α and β chains (21). Studies by Sadegh-Nasseri and Germain (15) have shown that the binding of peptide affects the structure of the class II heterodimer and that the formation of the "compact" state is associated with the acquisition of peptides at acidic pH levels. Nevertheless, no physical evidence exists for a conformational change occuring at mildly acidic pH levels (pH 5–6)

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where peptide binding to many MHC alleles is enhanced. Thus, the nature of the peptide-receptive state remains unknown. The data presented here suggest that weakly acidic conditions destabilize the heterodimer by disruption of tertiary and/or quaternary interdomain contacts outside the peptide-binding domain. Since these changes occur in the same pH range where antigenic peptide binding to IE^k is sharply enhanced, it suggests that they may regulate the behavior of the peptide-binding groove.

Materials and Methods

Soluble IE^k. IE^k was expressed as glycosyl-phosphotidylinositol (GPI)[1] anchored chimera in Chinese hamster ovary (CHO) cells (11). Soluble protein was recovered from CHO transfectants grown on hollowfiber bioreactors using a Cell Pharm™ apparatus (Unisyn Technologies, Hopkinton, MA) by digestion with phosphotidylinositol-specific phospholipase C (PI-PLC) (0.2 U/ml). IE^k was isolated from cell culture supernatants by immunoaffinity chromatography using the 14.4.4 mAb coupled to cyanogen bromide–activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ).

Preparation of Peptide/IE^k Complexes. Moth cytochrome c peptide (residues 88–103) or hemoglobin (Hb) β^{dmin} (residues 64–76) were bound to soluble IE^k (100 μg/ml) by incubation for 3 d at 37°C in citrate/phosphate buffer, pH 5.1, (22) using a 30-fold molar excess of peptide, as described elsewhere (23, 24). Peptide-loaded IE^k was then isolated by gel filtration as described previously (23, 24).

Fluorescence Spectroscopy. 1-anilino-napthalene-8-sulfonic acid (ANS)-binding studies were performed using a recording spectrofluorimeter (SLM 8000; SLM Instruments International, Urbana, IL) at 25°C in 1-cm quartz cuvettes. The excitation wavelength was 380 nm, and emission spectra were recorded from 430 to 600 nm at 1-nm increments. For the data in Fig. 3 *A*, the concentrations of ANS and IE^k were 22 and 0.8 μM, respectively, in 20 mM Hepes/20 mM MES (2 [*N*-morpholino]ethanesulfonic acid) buffer. The data in Fig. 3 *B* were obtained using 22 μM ANS and 0.4 μM protein and 20 mM Hepes/20 mM MES buffer containing 150 mM NaCl. The pH of the samples were adjusted with concentrated NaOH and HCl. Spectra were recorded immediately after the addition of protein or the adjustment of pH.

SDS-PAGE. Concentrated citrate/phosphate buffers were used to adjust the pH of MHC samples. Citrate/phosphate buffers covering the pH range of 6.2–7.3 were prepared by combining 0.1 M dibasic sodium phosphate with 0.05 M citric acid at different ratios as described by McIlvaine (22), except that the pH of the buffers was checked with a pH electrode. 2 μl of MCC/IE^k or IE^k-only stocks (0.2 mg/ml in PBS) were diluted into 18 μl of citrate/phosphate buffer at the pH indicated. 10 μl of nonreducing SDS-PAGE sample buffer (125 mM Tris-HCl, pH 6.8, 40% glycerol, 6% SDS, 0.2% bromophenol blue) were added, and the samples were allowed to incubate for 5 min at 25°C. The final pH of these incubation mixtures was checked using a pH electrode and found not to differ significantly from the citrate/phosphate buffer. After neutralization with concentrated sodium phosphate buffer, pH 7.5, the samples were fractionated without previous heating by electrophoresis on 12% acrylamide gels and were silver stained as described elsewhere (25), except that the glutaraldehyde step was omitted. The relative amount of dissociation of the IE^k heterodimer was determined by densitometric scanning using a densitometer (Molecular Dynamics, Sunnyvale, CA).

Circular Dichroism. Circular dichroism measurements were performed using a spectropolarimeter (AVIV Associates, Inc., Lakewood, NJ) at 25°C. Spectra were recorded in the far-UV using a 1-cm quartz cuvette at a protein concentration of 30 μg/ml in 10 mM sodium phosphate buffer. The pH of the sample was adjusted using concentrated HCl. Near-UV spectra were recorded in a 0.1-cm quartz cuvette in 40 mM Hepes/40 mM MES buffer and at protein concentrations that ranged from 0.8 to 2.0 mg/ml. Spectra were averaged over four to six repetitive scans collected at 0.1-nm intervals and were buffer corrected. Far-UV data is reported according to the molar concentration of peptide bond (mean residue ellipticity) and near-UV data is expressed as molar ellipticity using a relative molecular weight of IE^k of 70,000 g/mol. IE^k concentrations were determined using an extinction coefficient of 1.41×10^5 $mol^{-1} \cdot cm^{-1} \cdot$liters at 280 nm.

Results

SDS-PAGE Analysis of IE^k. An interesting feature of class II MHC molecules is that they remain largely intact ($\alpha\beta$) during SDS-PAGE if the samples are not heated and the peptide-binding groove is occupied by peptides. In the absence of antigen, they are less stable and dissociate into α and β chains (26). These facts and the possibility that class II molecules partially unfold at low pH suggested that their stability to SDS may be pH-dependent as well. To test this hypothesis, we incubated IE^k in nonreducing SDS-PAGE buffer (2% final SDS concentration) at different pHs followed by neutralization and electrophoresis. As shown in Fig. 1 *A*, IE^k dissociates in the presence of SDS in a strikingly pH-dependent manner. Dissociation did not take place if the samples were neutralized before incubation with SDS (data not shown). While ~90% of the heterodimer remains intact at pH 7.2, most of it dissociates at mildly acidic pH (6.2). Perhaps most interesting is that this effect starts to titrate at near-neutral pH, where peptide binding is sharply effected (discussed in more detail below). It is also clear that this is a very sensitive assay, since the destabilization is measurable at mild conditions where other techniques have failed.

To determine if the presence of a single high affinity antigenic peptide affects the pH-dependent dissociation, moth cytochrome c (MCC) peptide (residues 88–103) was bound to IE^k and the complex was isolated by fast protein liquid chromatography, as described elsewhere (23, 24). Such preparations of complexes are >80% loaded with specific peptide (11, 12, 23, 24), and they are stable for several days at pH 5.0 or 7.5 because of a very slow dissociation rate of MCC (12). As shown in Fig. 1 *B*, the presence of peptide causes a characteristic sharpening of the $\alpha\beta$ band of IE^k (11), but dissociation of IE^k still occurs in a pH-dependent manner. To determine whether the peptide had any stabilizing effect on IE^k, the amount of dissociation was deter-

[1] *Abbreviations used in this paper:* ANS, 1-anilino-naphthalene-8-sulfonic acid; CHO, Chinese hamster ovary; GPI, glycosyl-phosphotidylinositol; MCC, moth cytochrome c; PI-PLC, phosphotidylinositol-specific phospholipase C.

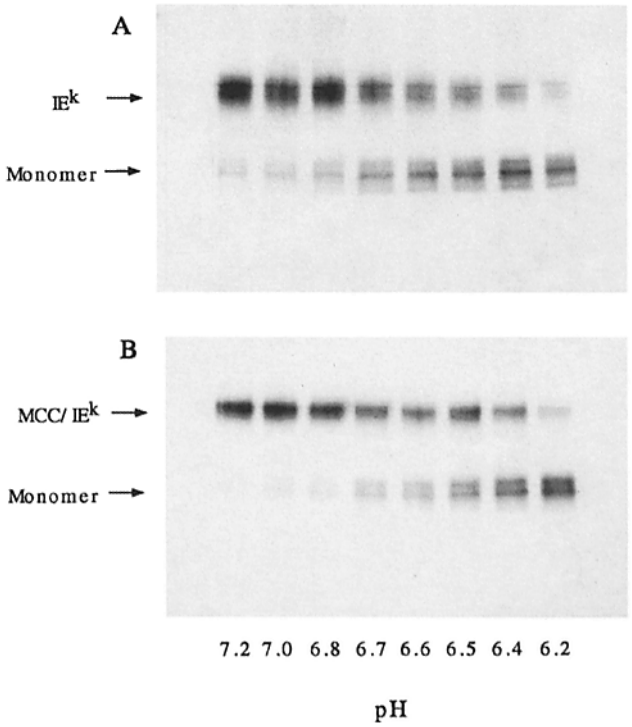

Figure 1. The pH-dependent destabilization of IE^k. Shown is SDS-PAGE analysis of IE^k (*A*) or MCC/IE^k (*B*) run under nondenaturing conditions after preincubation in the presence of 2% SDS at the pHs indicated. The two gels shown were electrophoresed simultaneously in a two-gel apparatus and were silver stained together.

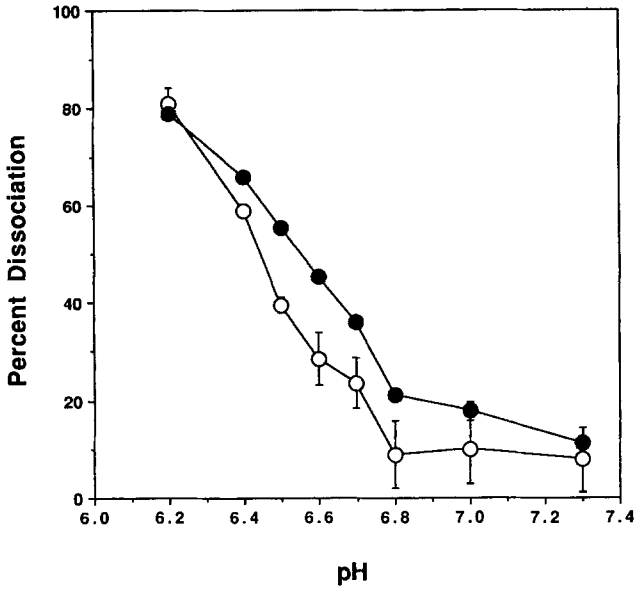

Figure 2. Stability differences for IE^k and MCC/IE^k. The degree of dissociation of IE^k (*closed circles*) or MCC/IE^k (*open circles*) was determined by densitometric scanning of the two gels shown in Fig. 1. The percent dissociation refers to the amount of remaining αβ band as a percent of the sum of αβ plus free chains. Error bars indicate the standard deviation of replicate scans of the same gels.

mined by densitometric scanning of the two gels shown in Fig 1. These gels were run in a two-gel electrophoresis apparatus (hence under identical conditions) and were stained and developed together. In each case there is clearly an inverse relationship between pH and dissociation, but the two curves are displaced from each other such that less dissociation occurs with MCC/IE^k at a given pH (Fig. 2). It cannot be determined whether this small shift occurs because the peptide contacts both the α and β chains in the peptide-binding groove, thereby stabilizing the heterodimer, or if the conformation of the peptide-bound state is slightly less responsive to the pH-induced change. Nevertheless, these data show that the presence of a high-affinity peptide (MCC) cannot prevent the response to low pH. The conformation of the peptide-binding groove of IE^k has also been examined by two-dimensional nuclear magnetic resonance of a labeled MCC peptide bound to IE^k (27). The α carbons of 11 COOH-terminal residues of MCC were held in a single conformation, and contacts were clearly evident between certain MCC side chains and residues of the IE^k molecule, yet the conformation of the peptide and, by inference, the peptide-binding groove were the same at pH 7.0 and 5.0 (27). These data can be explained by a conformational change occuring at a distance from the peptide-binding site. One possibility is that at an acidic pH, the interdomain contacts occuring between the α and β chains could loosen and destabilize the heterodimer. This hypothesis was tested by probing IE^k for exposed nonpolar sites at different pH with the fluorescent dye ANS.

ANS Binding by Soluble IE^k. A number of fluorescent probes are weakly fluorescent in an aqueous environment, but they emit brightly in nonpolar environments such as organic solvents or the interior of a protein. One such dye, ANS, binds to nonpolar pockets that are exposed in partially unfolded protein intermediates, as measured by its enhanced fluorescence and blue-shifted emission spectra (28, 29). Because of these properties, ANS binding has become a valuable probe for the study of molten globule states in proteins (30–32). To determine if low pH exposes ANS-binding sites in IE^k, the fluorescence emission spectra of the dye was measured in the presence and absence of IE^k at different pH levels. Unbound ANS in an aqueous environment is weakly fluorescent, with an emission maximum at ~520 nm (Fig. 3 *A*). In the presence of IE^k, a relatively small fluorescence enhancement and blue shift is apparent at pH 7.5 (Fig. 3 *A*), indicating that IE^k binds some ANS at this pH. When the sample is adjusted to pH 5.3, however, the fluorescence is increased approximately five- to sixfold relative to unbound ANS at this concentration, and the spectra is blue shifted with an emission maximum at 470 nm (Fig. 3 *A*). Autofluorescence of IE^k at either pH was not significant under these conditions (data not shown). To determine whether ANS binding was reversible, the sample containing bound ANS at pH 5.3 was adjusted to pH 7.1, resulting in a fluorescence emission spectra intermediate between pH 7.5 and 5.3 (Fig. 3 *A*). A small amount (<10%) of ANS remains bound after complete neutralization (pH 7.5) and during the time course of the ANS assay

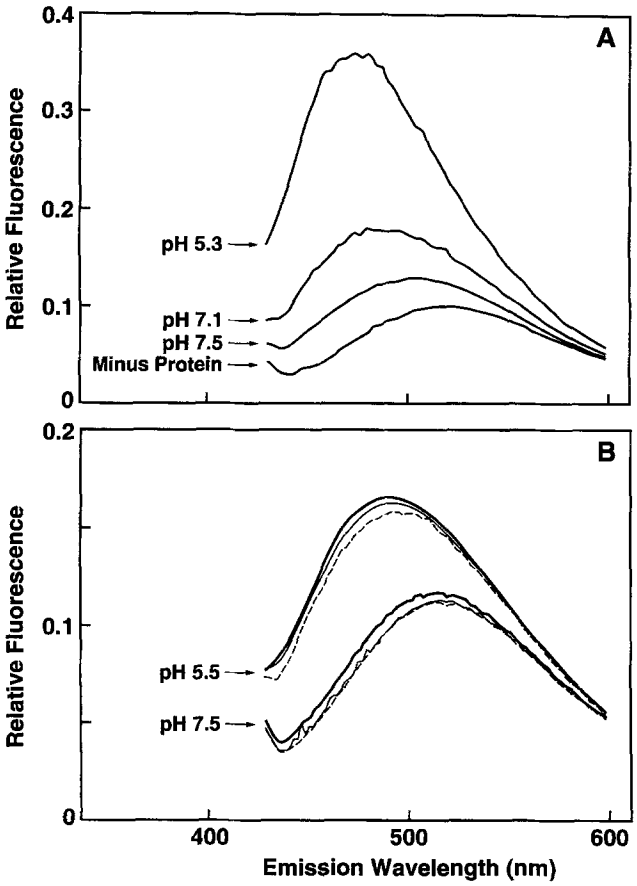

Figure 3. (*A*) ANS binding to IE^k. Shown is the fluorescence emission spectra of ANS in the presence and absence of IE^k at the pHs indicated. The emission spectra in the absence of IE^k was recorded first and is the same at pH 7.5 and 5.3. The subsequent emission spectra were recorded immediately (<5 min) after the addition of protein or pH adjustment. The pH levels of the solutions were verified using a pH electrode after the spectrophotometry. (*B*) Effect of high affinity antigenic peptides. Shown is the ANS emission spectra in the presence of IE^k only (*heavy line*), MCC/IE^k (*solid light line*), or Hb/IE^k (*dashed line*) at the pHs indicated.

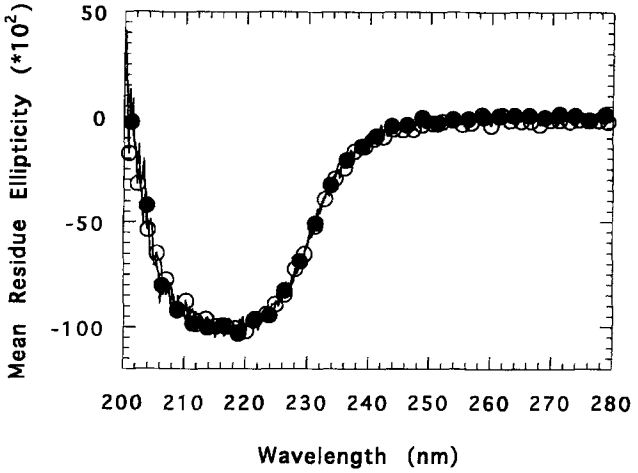

Figure 4. Far-UV circular dichroism. Shown is the circular dichroic spectra of IE^k at pH 7.3 (*open circles*) or pH 5.3 (*closed circles*). Protein concentrations were 30 μg/ml in 10 mM sodium phosphate buffer containing 10 mM NaCl. Spectra were averaged over repetitive scans in 1-cm quartz cuvettes. The data are expressed in units of mean-residue ellipticity.

(data not shown). The pH dependence of ANS binding was also studied with IE^k bound to either MCC or a hemoglobin peptide (Hb β^{dmin} 64-76) (Fig. 3 *B*). These results were similar to IE^k only, indicating that the occupation of the peptide-binding groove with either high affinity peptide does not block ANS binding to IE^k (Fig. 3 *B*). These data are consistent with the binding of ANS to nonpolar sites in IE^k that are reversibily exposed at mildly acidic pH and possibly outside the peptide-binding groove.

Circular Dichroism. The exposed hydrophobicity in IE^k could involve a disruption of its secondary, tertiary, or quaternary structure. Relative changes in a protein's secondary and higher-order structure can often be measured using circular dichroism in the far-UV and near-UV, respectively. Fig. 4 shows the circular dichroic spectra of IE^k at acidic (pH 5.3) and neutral pH levels in the far-UV. The spectrum of IE^k is characterized by a broad minima centered ~217 nm, consistent with the abundant secondary structure and high degree of β-sheet in class II MHC molecules (2, 16, 33, 34). Interestingly, the two spectra are indistinguishable at these two pHs, indicating that the changes in the structure of IE^k measured by SDS-induced dissociation and ANS binding do not involve gross alterations in secondary structure. The relatively small effect of pH on the secondary structure of class II MHC molecules has been reported elsewhere (16) as well.

The disruption of tertiary and quaternary structure in a protein can affect its near-UV circular dichroic spectra by altering the environment of buried aromatic residues. The effect of pH on the near-UV circular dichroic spectra of MCC/IE^k and IE^k-only is shown in Fig. 5, *A* and *B*, respectively. In contrast to the far-UV, the near-UV circular dichroic (CD) spectra of IE^k, with or without bound MCC, is different at pH 7.5 and 5.0 (Fig. 5, *A* and *B*). A small loss of spectra, most noticeable at 265–270 nm, occurs at pH 5.0, consistent with a subtle perturbation of the tertiary or quaternary contacts of the protein. Most of the change observed at pH 5 was reversible (Fig. 5, *A* and *B*). For comparison, the near-UV spectra of IE^k-only unfolded in the presence of 8 M urea is shown (Fig. 5 *C*). Under these conditions, a near-complete loss of structure is evident. Here too, however, the pH-induced changes in the CD spectra of IE^k are not prevented by the presence of a single high affinity MCC peptide. It is also worth noting that IE^k-only shows a slightly different near-UV CD spectra than MCC/IE^k, characterized by a less positive peak at 265–270 nm, consistent with an effect of peptide on the overall structure of IE^k (Fig. 5).

Together, these data indicate that at a weakly acidic pH, protonation of IE^k causes a small disruption of its tertiary and/or quaternary structure with the hydration of some of its nonpolar interior. This partially unfolded state forms reversibly, without a gross alteration of secondary structure, and it leads to a destabilization of the heterodimer interface. As discussed below, this conformational change may occur

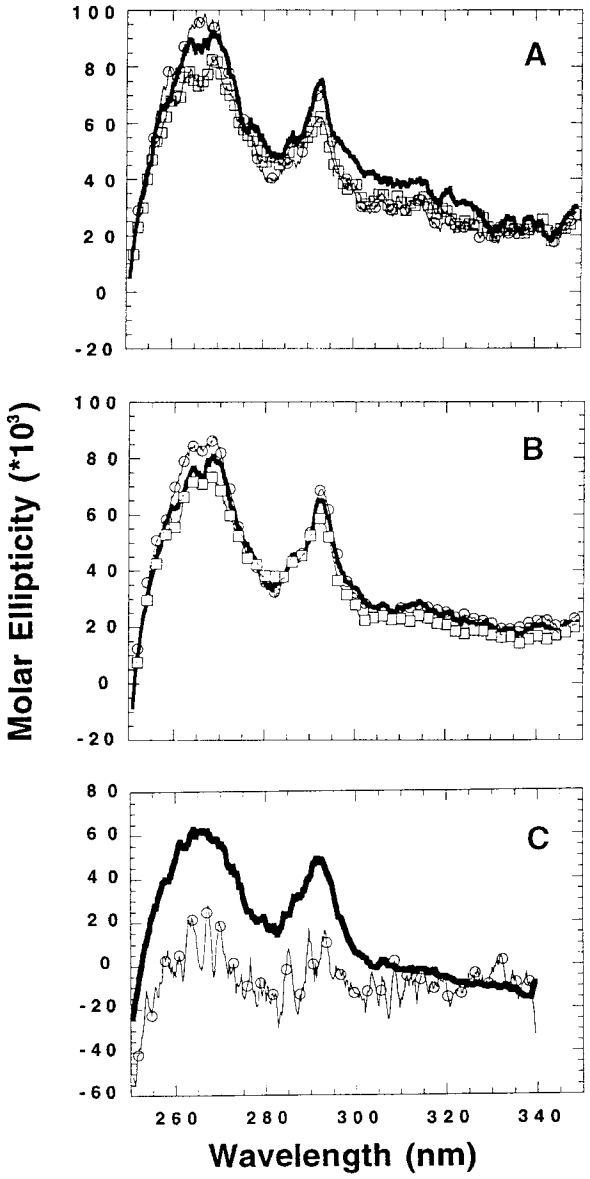

Figure 5. Near-UV circular dichroism. Shown is the circular dichroic spectra of MCC/IE^k (*A*) and IE^k only (*B*) at different pH levels. Spectra were first recorded in a buffer mixture of 40 mM Hepes and 40 mM MES at pH 7.5 (*open circles*), and subsequent spectra were recorded after adjustment of the pH to 5.0 (*open squares*) with concentrated HCl followed by readjustment to pH 7.5 (*heavy line*) with concentrated NaOH. (*C*) The spectra of IE^k-only in the presence (*open circles*) or absence (*heavy line*) of 8 M urea. Data were recorded at 0.1- or 0.25-nm increments, and repetitive scans collected during 2–3 h were averaged and then smoothed using an averaging window of 10 data points. The data are expressed in units of molar ellipticity.

outside the peptide-binding groove and may involve interdomain contacts.

Discussion

It is now clear that many class II MHC molecules show optimal peptide binding at an acidic pH (9–17), mimicking conditions of the endosomal compartment where antigen binding occurs. A number of proteins that encounter endosomal compartments exhibit conformational changes that are related to their function (reviewed in reference 35). While it has been suspected that a conformational change at endosomal pH accounts for the enhanced binding of peptide in vitro and contributes to efficient antigen loading in vivo, direct evidence for such a change has been lacking.

There is evidence that antigenic peptides influence the structure and stability of class II proteins. Class II heterodimers isolated from cells and containing bound endogenous peptides migrate as intact molecules ($\alpha\beta$) during nondenaturing SDS-PAGE, despite the fact that the α and β chains are noncovalently associated. The study of "empty" class II molecules from cells defective in their ability to process protein antigen (36) or from certain soluble expression systems (26) indicates that SDS stability requires a bound peptide. Upon heating (50–65°C) or treatment at low pH levels (<5.0), conditions that could release peptides, detergent solubilized class II molecules first form a slowly migrating "floppy" state, followed by dissociation to free α and β chains at more harsh conditions (21). Sadegh-Nasseri and Germain (15) showed that peptide has a role in determining the structure of class II proteins, and that both peptide binding and low pH levels are necessary to generate the stable "compact" state.

One possibility is that the "floppy" form is an intermediate involved in peptide binding at low pH . Dissociation of the $\alpha\beta$ heterodimer competes with peptide binding at a low pH (17, 37) and the "floppy" form is produced as an intermediate in dissociation reactions (21). Generation of the "floppy" form of IE^k, however, requires significantly lower pH than necessary for optimal peptide binding to IE^k (21), and this may cause irreversible denaturation. IE^k binds antigenic peptides maximally at ~pH 5.0, but shows a steep dependence on either side of this pH (11, 13, 14). Some class II molecules have more alkaline pH optima (pH 5.0–6.5) for peptide binding (9, 14). In our system (11), peptide binding to IE^k improves by ~50% when the pH is reduced from 7.5 to 6.0, but decreases sharply by 80% from pH 5.0 to 4.0, where the "floppy" form is generated. Thus, the formation of the "floppy" form does not adequately explain the pH dependence of peptide binding to IE^k, and its nature and relevance, in general, to peptide binding remains unclear.

In addition to SDS-PAGE analyses, spectroscopic studies have been performed on class II molecules. Far-UV circular dichroism measurements (16) have been performed on IA^d and IE^d at different pHs. As described above, conformational changes were only detected at or below the pH optima for peptide binding to these molecules and not at mildly acidic pH (16). Two-dimensional nuclear magnetic resonance analyses (27) of a labeled MCC peptide bound to IE^k have also failed to show a conformational change occuring between pH 5 and 7.5. To date, no physical evidence exists for conformational changes occuring in class II molecules at mildly acidic pH, and the nature of the intermediate responsible for enhanced peptide binding is unknown.

The fact that bound peptides can retard SDS-induced dissociation in class II molecules (15, 26, 36, 37) and that peptide binding is enhanced at low pH levels suggests that SDS-stability may be pH dependent as well. Indeed, as shown here, dissociation of IE^k by SDS is very sensitive to pH. By this approach, a conformational change in IE^k is detected at very mildly acidic conditions, and it indicates that an equilibrium between the native state and a destabilized state of IE^k titrates at near-neutral conditions. Although the stabilizing effect of the MCC peptide is evident in this assay, it is also clear that the conformational change responsible for destabilization still occurs in the MCC/IE^k complex.

Because destabilization of a multimeric protein can occur by disruption of interdomain contacts resulting in the exposure of buried amino acids, we probed IE^k for exposed hydrophobicity with the dye ANS. The enhanced binding of the dye to IE^k at low pH is consistent with the exposure of nonpolar interior portions of the protein. Another possibility that cannot be excluded from these data is that the ANS binding site(s) in IE^k at neutral pH levels becomes more hydrophobic at acidic pH. The lack of an effect of MCC on ANS binding indicates that the bulk of the ANS binding lies outside the peptide-binding groove. This is perhaps not surprising, since ANS can often bind to very specific sites and at low stoichiometry, even in the case of molten globules (38). The exposure of nonpolar regions outside the peptide-binding domain also would be compatible with the minimal effect of peptide at stabilizing IE^k against low pH–induced SDS dissociation.

Far-UV circular dichroism analysis demonstrates that the gross secondary structure of IE^k is the same at pH 5.3 and 7.3, consistent with the data discussed above for IA^d and IE^d (16). In retrospect, the retention of secondary structure with a loosening of the interior contacts in IE^k may be an ideal mechanism to promote peptide binding. The peptide-binding domains of MHC molecules are formed by a complex series of β-sheet and α-helices and loss of part of this structure could abolish peptide binding. In contrast to far-UV circular dichroism, the spectra of a protein in the near-UV often provides information about higher order structure and the environment of aromatic residues. Using this assay, minor changes are observed (Fig. 5), indicating that the environment of aromatic residues in the protein is perturbed at pH 5.0, but remains largely intact.

These data suggest that class II MHC molecules at low pH levels may be in a molten globule-like state. Molten globules are protein states with exposed hydrophobicity, contain native-like secondary structure, remain compact, but are destabilized relative to the native state because of a near complete loss of tertiary structure (reviewed in reference 39). Molten globules were once thought to be formed by only a few proteins under very specific conditions. They have now been described for a large number of proteins and have been generated by treating proteins with heat, dilute acid, base, or by mutagenesis or cofactor dissociation, and may function as intermediates, general to protein-folding reactions (reviewed in reference 39). Thus, at low pH levels, IE^k fulfills these criteria, as evidenced by its destabilization, exposed nonpolar interior sites, and native-like secondary structure, but differs by its greater retention of tertiary and quaternary structure. It is possible that a more gross alteration of higher-order structure occurs at more acidic pH levels where IE^k begins to dissociate into free α and β chains (19, 21). In vivo, such a state could be stabilized by interchain interactions that are absent in our soluble construct (i.e., transmembrane domains) or by association with the invariant chain or invariant chain peptides. In the absence of invariant chain, class II molecules remain in the endoplasmic reticulum complexed to BiP (40), grp94, and p72 (41), which are members of the heat shock family of proteins. In at least one case (GroEL), a heat shock protein has been shown to have specificity for molten globule intermediates (42, 43).

As to what parts of IE^k are involved in the conformational change, we suspect that critical parts are outside of the peptide-binding domain. One possibility is that a destabilization of interdomain contacts occurs in the lower domains (α_2 and β_2) or between them and the floor of the peptide-binding groove. The rigidity of Ig domains (44) indicates that this possibility may be more likely than a disruption of the tertiary structure within the individual α_2 or β_2 domains. A role for lower domains in peptide binding to class II proteins is not unreasonable given that the α_2 domain is in close contact with the floor of the peptide-binding groove of DR1 (33). The best evidence for this are the data with class I MHC molecules, where it is clear that peptide-binding properties are strongly affected by the presence of β_2-microglobulin (the α_2-equivalent domain) (5, 45–47). Additionally, site-directed mutagenesis studies indicate that the low pH enhancement of peptide binding to IE^k may involve residues in the α_2 domain (Wettstein, D. A., and M. M. Davis, manuscript in preparation).

We propose that protonation of IE^k at weakly acidic pH produces a partially unfolded state that is characterized by a loosening and hydration of nonpolar interdomain contacts. At least some of the disrupted contacts may lie outside but adjacent to the peptide-binding domain and regulate its binding properties. A study of the electrostatic contributions to binding of antigenic peptides to IE^k also showed that nonionic interactions are more dominant at acidic pH levels, supporting the exposure of nonpolar pockets in the peptide-binding domain (24). A corollary to our model is that when occupied by a high-affinity peptide, the binding domain of IE^k may be more rigid and less responsive to the protonation of lower domain residues. This possbility is supported by the lack of conformational change detected by two-dimensional nuclear magnetic resonance analysis of MCC/IE^k (27) at pH 5.0 vs neutral pH , and it may explain the results of Reay et al. (12), which show a low pH enhancement of the association rate, but not the dissociation rate, for MCC binding to IE^k.

Runnels et al. (in this issue) have obtained results similar to those reported here with several alleles of detergent solubilized protein, indicating that these conclusions may be general to class II MHC molecules. The reversible forma-

tion of a partially unfolded state, possibly stabilized by the invariant chain or invariant chain peptides, could be a unique mechanism to obtain a specific function (peptide binding) while trafficking through an acidic endosomal compartment.

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