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Transmembrane Domain-Mediated Colocalization of HLA-DM and HLA-DR Is Required for Optimal HLA-DM Catalytic Activity

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HLA-DM catalyzes peptide loading and exchange reactions by MHC class II molecules. Soluble recombinant DM, lacking transmembrane and cytoplasmic domains, was observed to have 200- to 400-fold less activity compared with the full-length protein in assays measuring DM-catalyzed peptide dissociation from purified HLA-DR1 in detergent solutions. Additional studies with truncated soluble DR1 demonstrated that transmembrane domains in DR1 molecules are also required for optimal activity. The potential requirement for specific interaction between the transmembrane domains of DM and DR was ruled out in experiments with chimeric DR1 molecules containing transmembrane domains from either DM or the unrelated protein CD80. These results suggested that the major role of the transmembrane domains is to facilitate colocalization of DM and DR in detergent micelles. The latter conclusion was further supported by the observation that HLA-DM-catalyzed peptide binding to certain murine class II proteins is increased by reducing the volume of detergent micelles. The importance of membrane colocalization was directly demonstrated in experiments in which DM and DR were reconstituted separately or together into membrane bilayers in unilamellar liposomes. Our findings demonstrate the importance of membrane anchoring in DM activity and underscore the potential importance of membrane localization in regulating peptide exchange by class II molecules. The Journal of Immunology, 2001, 167: 5167–5174.

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uman histocompatibility leukocyte Ag-DM plays a critical role in the MHC class II Ag presentation pathway through its function in catalyzing peptide loading of class II molecules (1). Class II αβ heterodimers initially assemble with the chaperone protein, invariant chain (II), in the endoplasmic reticulum of APCs. II stabilizes the newly formed MHC heterodimers through multiple interactions involving sites both inside and outside the peptide-binding groove. A flexible region of II encoded by exon 3 is believed to act as a surrogate peptide, occupying the peptide-binding groove in αβI complexes. A targeting signal in the cytoplasmic domain of II directs transport and localization of the complexes to compartments in the endosomal pathway, where II is released through sequential proteolytic cleavage events. However, the II segment that occupies the peptide-binding groove, class II-associated II chain peptide (CLIP), is inaccessible and remains associated with class II αβ, blocking peptide loading (2). HLA-DM functions to accelerate the rate of dissociation of CLIP, freeing the binding site and promoting the loading of MHC class II molecules with heterogeneous peptides present in the endosomal environment (3–5).

Crystallographic studies demonstrated that the structure of HLA-DM is very similar to that of conventional MHC class II molecules (6, 7). However, the peptide-binding groove is largely closed, with only a single pocket remaining that is too small to accommodate CLIP or other peptides in a conventional manner. DM promotes CLIP dissociation and peptide exchange through a mechanism involving direct interaction with MHC class II peptide (3–5). The specific nature of the binding interaction (8, 9) remains unknown but mutational studies and other lines of evidence suggest that DM may interact with the lateral surface of class II molecules below the end of the peptide-binding groove that accommodates the N terminus of bound peptide (3, 10, 11). It was initially thought that DM might selectively recognize structural features of class II-CLIP complexes. However, subsequent studies showed that it can accelerate the dissociation of peptides with sequences unrelated to CLIP (12–14). Indeed, current evidence suggests that DM increases the rate of dissociation of all peptide complexes such that the rate observed in the presence of a given concentration of DM is approximately proportional to the intrinsic peptide dissociation rate observed in the absence of DM (12). Newly synthesized MHC class II molecules have a relatively brief exposure to DM in endosomal compartments during trafficking to the cell surface. During this brief exposure, less stable complexes, such as those bearing CLIP, are much more likely to dissociate than are complexes with greater stability. Thus, DM appears to edit the repertoire of peptides presented by class II molecules by catalyzing multiple rounds of peptide exchange, favoring survival of the most stable complexes.

Studies with soluble recombinant DM demonstrated that micromolar concentrations were required for half-maximal activity in assays measuring peptide dissociation or exchange with purified HLA-DR class II molecules (4). By contrast, similar activity was observed with nanomolar concentrations of detergent-solubilized full-length DM purified from B cell lines (3, 5). It was subsequently shown that substoichiometric concentrations of HLA-DO copurify with DM isolated from B cells (15). The function of HLA-DO remains controversial. It clearly has a capacity to inhibit
DM function in a variety of experimental conditions (15–18) but it also has been reported to promote the peptide loading and chaperone functions of DM (19, 20). The possibility that contaminating complexes with Sephadex G-50 columns (Boehringer Mannheim, Indianapolis, IN) Samples were diluted 1:10 to DR1 concentrations of 0.2 μM in binding buffer and divided into smaller aliquots to which various amounts of full-length or soluble DM were added together with an excess (100 μM) of unlabeled MAT(17–31) peptide. Peptide dissociation was monitored using high performance size exclusion chromatography. At various times, 20-μl sample aliquots were removed. DR1-FITC-peptide was quantified with a BioTek PAK GFC 200 (excitation column 7.8 × 150 mm; Tosohaas, Montgomeryville, CA) and a Shimadzu Scientific Instruments (Columbia, MD) RF-10A fluorimeter with 490 nm excitation and detection at 520 nm. The column buffer was 50 mM phosphate buffer (pH 7.0) with 0.2 mM dodecyl β-d-maltoside. Peptide binding was assessed as a function of fluorescence peak height.

**Peptide binding to chimeric molecules expressed in COS-7 cells**

Cells were transfected by the DEAE-dextran method as described elsewhere (26). Briefly, 4 × 10⁶ cells were plated on 60-mm tissue culture dishes in DMEM-10% FBS 24 h before transfection. Five dishes were prepared for each transfection and pooled at the end of the experiment. The following day cells were washed twice without serum in DMEM, 10 mM HEPES, and incubated in 2 ml DMEM, 10 mM HEPES containing 500 μg DEAE-dextran, 100 μM chloroquine, and DNA: 1 μg each class II e- and d-chain constructs for 3 h at 37 °C. DNA was removed by aspiration and 2 ml 10% DMSO in DMEM and 10 mM HEPES was added to each dish for 2 min at room temperature, followed by incubation in DMEM, 10% FBS, and 10 mM HEPES at 37 °C. Cells were harvested by trypsinization at 42 h after transfection. An aliquot of cells was analyzed for surface expression of DR1 by staining with mAb L243 followed by FITC-labeled goat anti-mouse Ig (whole molecule; Sigma-Aldrich) and analyzed on a FACScan cytosensor (BD Biosciences, Mountain View, CA). The remaining cells were lysed in 1% NP40 in the presence of protease inhibitors. Lysates were cleared of cellular debris by centrifugation. To each lysate, citrate-phosphate buffer (pH 5.0), 0.2% NP40, containing protease inhibitors (5) was added. Purified DM and DR1 molecules in the lysates were captured on microtiter plates coated with L243 mAb. DR1 molecules in the lysates were quantified by sandwich ELISA as described above. Biotinylated peptide bound to DR1 was quantified with europium-streptavidin fluorescence as described previously (27).

**Peptide binding to murine MHC class II molecules**

IAα and IE e were purified from detergent-solubilized A20 B lymphoma (28) membrane preparations using mAb immunoadfinity columns: MK-D6 for IAα (29) and 14-4-4 for IE e (30) as previously described (25). 14-4-4 was also used in the purification of IE e from CH27 cells (31). Purified murine class II molecules (50 nM) were added to 10 μM biotin-labeled peptide on ice (IAα, Myo106–118; IE e, HEL104–120; IE e, MCC82–103) in 100 mM citrate-phosphate buffer (pH 5.0) with protease inhibitors in the presence or absence of purified HLA-DM (100 nM). Varying concentrations of NP40 were added to duplicate samples. After mixing, the samples were equilibrated on ice for 20 min followed by a 3-h incubation at 37 °C. After neutralization, class II-biotin-peptide complexes were captured on assay plates precoated first with goat anti-mouse Ig (H and L chains; Sigma-Aldrich) followed by the appropriate mAbs: MK-D6 (IAα) or 14-4-4–4 for IE e and IE e. Biotin-peptide complexes were quantified by europium-streptavidin fluorescence as mentioned above (27).

**Liposome experiments**

A lipid solution containing dipalmitoyl phosphatidyl choline (C16:0; Sigma-Aldrich) and cholesterol in an 8:2 molar ratio in chloroform was dried to form a thin film in glass tubes under a stream of nitrogen. The lipid was solubilized on ice in 1% n-octyl-β-D-glucopyranoside in the presence of purified full-length HLA-DM or HLA-DR1 to form solutions containing 1 nM liposome-bound DR1 and/or 1 μM DR and/or 88 nM DM. Samples were dialyzed for 24 h at 4 °C against three changes of PBS using dialysis membranes with a molecular mass cutoff of 12,000 Da to remove detergent and generate unilamellar liposomes (32, 33). Peptide binding to DR1 in liposomes was measured by mixing equal volumes of liposomes containing DR1 and liposomes containing DM or equal volumes of liposomes containing DR1 and liposomes containing DM and DR1. Binding of DR1 peptide containing both DR1 and liposomes was measured by mixing equal volumes of liposomes containing DR1 and liposomes containing DM or equal volumes of liposomes containing DR1 and liposomes containing DM and DR1. Liposomes containing conjugated nM DR1 and 11 nM DM (as indicated) were incubated for 3 h at 37 °C in 100 mM citrate-phosphate (pH 5) with 1 μM biotin-MAT(17–36). The samples were placed in ice and
liposomes were solubilized in buffer containing 1% NP40, 0.1% Tween 20, 0.1 M NaCl, 0.4 M Tris (pH 8), and 1 mg/ml BSA. Biotin-peptide DR1 complexes were quantified with a europium fluorescence immunoassay as described above. Control samples were solubilized in 1% NP40 before incubation with biotin-peptide at pH 5, neutralization, and assayed for peptide-DR1 complexes.

Results
Quantitative contribution of transmembrane or cytoplasmic domains
In our initial studies, soluble recombinant HLA-DM lacking both transmembrane and cytoplasmic domains (truncated DM) (Fig. 1) was observed to have little or no activity in assays measuring enhanced binding by purified DR1 molecules in detergent solution. The recombinant protein appeared to be properly folded as judged by reactivity in immunoassays using mAbs with specificity for nonoverlapping conformational determinants in DM (data not shown). Subsequent experiments demonstrated that peptide binding was efficiently catalyzed in the presence of high (micromolar) concentrations of the soluble truncated protein. Full-length detergent-solubilized recombinant DM was generated using a baculovirus expression system to investigate this phenomenon further. Since the full-length protein requires the presence of detergent for solubilization, experiments were done in standard assay buffer containing 0.2% NP40. The full-length recombinant protein was observed to be as potent as DM purified from B cells in assays measuring peptide binding to purified DR1 molecules. The potency of the recombinant full-length DM vs truncated DM was compared in assays measuring enhancement of dissociation of CLIP from preformed DR1-CLIP complexes. An approximately 400-fold greater concentration of truncated DM was required to induce the same degree of CLIP release during a 3-h incubation at pH 5 (Fig. 2a). Remarkably, a very similar dose relationship was observed when comparing the capacity of full-length DM to enhance the dissociation of CLIP from full-length vs truncated DR1

FIGURE 1. Transmembrane and cytoplasmic domains truncations of HLA-DM and HLA-DR generate soluble heterodimers. Both α- and β-chain transmembrane and cytoplasmic domains were exchanged between HLA-DM and HLA-DR, respectively. Since B7.1 is a homodimer, both α- and β-chain transmembrane and cytoplasmic domains of DR were exchanged with the same corresponding fragment of B7.1.

FIGURE 2. Transmembrane domains are required for efficient HLA-DM-mediated release of CLIP from DR1 molecules. a, Preformed biotin-CLIP-DR1 complexes (50 nM) were incubated for 3 h at pH 5 with various concentrations of full-length or soluble HLA-DM. b, Preformed biotin-CLIP complexes were generated with full-length DR1 or soluble DR1 and incubated for 3 h at pH 5 with various concentrations of full-length HLA-DM. The fraction of biotin-CLIP-DR1 complexes remaining after incubation with DM was measured by using a europium fluorescence immunoassay as described in Materials and Methods.
molecules (Fig. 1) lacking transmembrane and cytoplasmic domains (Fig. 2b). In these experiments, all components were generated as recombinant proteins, ruling out any contribution from HLA-DO or cofactors that might copurify with DM or DR from B cell lines. These results suggested that interactions involving the transmembrane or cytoplasmic domains of both DM and DR proteins were required to promote maximal DM catalytic activity.

We previously reported that DM is capable of enhancing the rate of dissociation of all DR1-peptide complexes (12). Even very stable peptide complexes rapidly dissociate in the presence of sufficiently high concentrations of DM. We were interested in determining whether the difference in potency of full-length and truncated DM noted in assays measuring CLIP dissociation would also be observed in experiments measuring the dissociation of an unrelated peptide from DR1. The rate of dissociation of the fluorescein-labeled influenza matrix protein peptide, MAT(17–31), was markedly accelerated in the presence of 1 nM DM, as measured using high performance size exclusion chromatography with an on-line fluorometer (Fig. 3a). In comparison, the effect observed with a 16-fold higher concentration of truncated DM was considerably less. A linear relationship was observed between the rate of peptide dissociation and DM concentration for both forms of DM (Fig. 3b). However, the slope of rate vs DM concentration, a measure of DM potency, was 240-fold lower for truncated compared with full-length DM. This difference is in reasonable agreement with that observed in assays measuring CLIP dissociation.

Transmembrane domain exchange experiments

A requirement for the cytoplasmic domains in DM catalytic function was excluded through analysis of L cell transfectants. Membrane preparations from L cells expressing comparable levels of full-length DM or a truncated form lacking only the cytoplasmic domains of both α- and β-chains was observed to have similar activity in assays measuring DM-catalyzed peptide binding to purified DR1 molecules (data not shown). The possibility that specific interactions between the transmembrane domains of DM and DR are required for optimal activity was addressed by generating chimeric molecules with altered transmembrane domains. We initially attempted to do this by replacing the transmembrane domains of DMα and β with corresponding domains from DR1. However, expression of the mutant DM molecules could not be detected in transiently transfected COS cells, suggesting that they did not fold properly. As an alternative approach, constructs encoding chimeric molecules were generated in which the transmembrane domains of DRα and DRβ were replaced with the corresponding domains from DM (Figs. 1 and 4). Surface expression of the mutant protein was similar to wild-type DR1 in COS cells after transient transfection as determined by flow cytometry using the DR-specific mAb L243 (data not shown). The capacity of DM to promote peptide binding to the mutant DR molecule was analyzed by incubating detergent lysates of the transfected cells with various concentrations of purified full-length DM in the presence of biotin-labeled MAT(17–31) peptide. Biotin-peptide-DR1 complexes were measured using a europium fluorescence immunoassay. DM was fully capable of catalyzing peptide binding to the mutant DR molecules with substituted transmembrane domains (Fig. 4). In the experiment shown, maximal peptide binding by the mutant DR was somewhat greater than wild type. This can be attributed to variation in the DR expression levels and it was not a consistent finding. The transmembrane domains of DM and DR are reasonably similar. To fully exclude a requirement for specific interactions between these domains, the transmembrane domains of DRα and β were each replaced with a sequence from the unrelated molecule, CD80 (B7-1). This mutant heterodimer was also expressed efficiently in COS cell transfectants and was fully susceptible to DM-catalyzed peptide loading (Fig. 4). Thus, we found no evidence for sequence-specific interactions between the transmembrane domains of DM and DR required to promote catalytic function.

Effect of detergent concentration on HLA-DM interaction with murine MHC class II molecules

A puzzling observation obtained in peptide-binding experiments with murine class II molecules may be relevant to the experiments described above, demonstrating a role for transmembrane domains in DM function. Using standard assay buffer, containing 0.2%
NP40 detergent, purified HLA-DM was observed to have little activity in catalyzing peptide binding by certain purified full-length mouse class II molecules. However, DM activity was revealed by reducing the NP40 concentration and optimal activity was observed at concentrations approximating or below the critical micelle concentration for this detergent (Fig. 5). Variable results were obtained under conditions where no NP40 was added, probably due to protein aggregation. The assay samples also contained low concentrations of SOG (0.3 mM), the detergent used to maintain solubility of the purified protein stocks. Avva and Creswell (34) previously reported that detergents containing 8- to 10-carbon unbranched hydrocarbons, such as octylglucoside, can facilitate CLIP release from DR molecules at acidic pH. It is possible that SOG could mediate a similar effect at limiting concentrations of NP40 by promoting the dissociation of previously bound peptides from mouse class II molecules, freeing more binding sites for biotin-labeled peptide. However, several points weigh against this explanation. The detergent concentrations used in the CLIP release assays where 100-fold higher than the concentration of SOG present in our experiments. In addition, peptide binding in the absence of DM was not affected by varying NP40 concentrations (Fig. 5). Finally, reduction in NP40 concentrations below 0.2% had
little effect on DM-catalyzed peptide binding to DR1 molecules under parallel conditions. Several studies based on transfection experiments have demonstrated that HLA-DM can modulate peptide loading by mouse IA<sub>κ</sub>-d molecules (35–37). However, the possibility that there may be a partial species incompatibility between human DM and mouse class II molecules has been raised (38). One explanation for our results is that, at limiting detergent concentrations, DM and mouse class II molecules are codistributed into a smaller volume of detergent micelles and their effective concentrations are therefore increased. This effect could compensate for a partial species incompatibility. The interaction between DM and full-length DR may be sufficiently optimized so that NP40 detergent concentrations below 0.2% have little effect. Consistent with this idea, we have observed that DM activity is reduced at NP40 concentrations >1% in assays with DR1 (data not shown).

**Effect of membrane colocalization on DM activity**

The results described above support the hypothesis that the transmembrane domains of DM and DR optimize their functional interaction in detergent solutions by facilitating their colocalization in detergent micelles. However, the possibility that detergent interactions with the transmembrane domains may have some complex effect in promoting DM activity could not be excluded. DM and DR were reconstituted into lipid bilayers to directly determine the effect of membrane localization on DM-mediated peptide exchange. Purified full-length DM and DR were reconstituted together or separately into unilamellar liposomes containing phosphatidylcholine and cholesterol generated by detergent dialysis. Care was taken to ensure that all samples contained the same total concentration of DM, DR, and liposomes such that they differed only in the distribution of DM and DR molecules among the liposomes. The samples were incubated at pH 5 with biotin-MAT(17–31) peptide, followed by pH neutralization, detergent solubilization, and assay for DR1-biotin-peptide complexes. Peptide binding was only slightly above background in samples containing DM and DR independently distributed into different liposomes (Fig. 6). In contrast, peptide binding was markedly enhanced under conditions where DM and DR were localized in the same liposomes. As a control, parallel liposome samples were solubilized on ice in 0.5% NP40 detergent before incubation with labeled peptide. Under these conditions, the proteins were randomly redistributed into micelles and rapid peptide-binding activity was restored in all samples containing both DM and DR. These results demonstrate directly that membrane colocalization is a critical parameter in controlling DM-mediated peptide exchange activity.

**Discussion**

In this study the mechanism responsible for the enhanced activity of full-length HLA-DM as compared with soluble truncated molecules lacking the cytoplasmic and transmembrane domains was investigated. Our findings confirm the results of Busch et al. (21) demonstrating that in solutions efficient DM-mediated peptide exchange requires the transmembrane/cytoplasmic domains of both DM and the substrate MHC class II molecule. A role for the cytoplasmic domains was excluded based on the activity of truncated DM molecules lacking the cytoplasmic domain but retaining the transmembrane regions. The potency of soluble DM, lacking transmembrane domains, was observed to be 200-to 400-fold reduced as compared with full-length DM in assays measuring the dissociation of two different peptides from DR1. A very similar result was obtained when the capacity of full-length DM to catalyze the release of CLIP from full-length vs truncated DR1 was compared. Thus, transmembrane domains in both molecules are required for optimal activity and their contribution is substantial.

It is possible that the transmembrane domains of DM or DR somehow affect the conformation of these molecules promoting functional interaction. This possibility is supported by a previous observation that membrane localization can alter the pH dependence of peptide binding by DR molecules (39). In addition, specific sequences in the transmembrane domain of HLA-Cw6 were reported to be required for functional recognition by inhibitory receptors on natural killer cell clones through a mechanism that might involve alterations in membrane mobility or conformation (40). However, the fact that transmembrane domains in both DM and DR are required makes it unlikely that these domains indirectly facilitate functional interaction between the molecules by subtly altering the conformation or conformational flexibility of both molecules.

Experiments with chimeric DR1 molecules containing transmembrane sequences from DM or a completely unrelated molecule, CD80, ruled out the possibility that a specific interaction between the transmembrane domains (41) promotes the functional interaction of DM with DR. The mutant DR1 protein was efficiently assembled and expressed in transiently transfected COS cells, indicating that the DMα and β transmembrane regions readily substitute for the corresponding sequences in DR1 molecules. It is interesting that the converse was not true. We were unsuccessful in expressing chimeric DM molecules containing transmembrane sequences from DR1. The observation that truncated soluble DM or DR molecules can be efficiently expressed and secreted by insect cells (at 26°C) demonstrates that there is no obligate requirement for interactions between the α and β transmembrane domains for pairing or formation of stable molecules. However, assembly of IA<sub>A</sub> MHC class II heterodimers in COS cell transfectants was previously reported to involve interactions between the transmembrane domains (42). It is possible that transmembrane domain interactions play a role in assembly of DM heterodimers at physiological temperatures in mammalian cells. Notably, Calafat et al. (43) demonstrated that the transmembrane and cytoplasmic domains of MHC class II have the potential to

**FIGURE 6.** Requirement for colocalization of DM and DR in lipid bilayers. Unilamellar liposomes containing purified DR1 and/or DM were generated as described in Materials and Methods. Samples containing identical quantities of DM and DR1 distributed together or separately into liposomes were incubated for 3 h at pH 5 with 1 μM biotin-MAT(17–31) peptide. After chilling on ice, liposomes were solubilized in 1% NP40 and biotin-MAT-DR1 complexes were quantified by europium fluorescence immunoassay (■). As a control, parallel samples were solubilized in detergent before incubation with peptide to redistribute DM and DR together in micelles (□).
specifically induce multilaminar morphology in lysosomal compartments of human embryonal kidney cells, similar to MHC class II compartment peptide loading compartments of professional APC. Thus, in addition to membrane anchoring, the transmembrane/cyttoplasmic domains in MHC class II molecules have the potential to influence the organization of endosomal compartments.

Our findings are most compatible with the conclusion that the transmembrane domains of DM and DR are required to facilitate colocalization in membrane or detergent micelles. The role of membrane colocalization was clearly illustrated in experiments involving reconstitution of full-length DM and DR molecules into lipid bilayers. DM and DR were observed to cooperate efficiently when localized together in unilamellar liposomes, whereas little or no activity was observed when the two proteins were segregated into separate liposomes incubated together with labeled peptide Ag. Full activity was recovered in the latter samples when DM and DR were randomly redistributed into micelles by detergent solubilization of the liposomes. Thus, DM-DR interactions occur efficiently in cis- but not trans-conformation. In addition, we observed that the capacity of purified full-length HLA-DM to enhance peptide binding by several mouse class II molecules was increased by reducing the concentration of NP40 detergent in the assays. There are a number of potential explanations for this. For example, the conformation of some or all murine class II might be differentially affected by exposure to detergent, indirectly influencing interactions with DM. Alternatively, by reducing detergent concentration, the volume of micelles is reduced with a concomitant reduction in the effective volume in which the transmembrane-containing protein is distributed. The predicted effect is to increase the effective concentration of DM and class II, increasing their rate of interaction. In contrast to the results with the mouse class II proteins examined (IEβ, IAβ, IAδ), reducing the concentration of NP40 below 0.2% had little effect on DM-catalyzed peptide binding to DR1. However, high concentrations of NP40 (≥1%) partially inhibit DM activity on DR1. It is possible that there is a partial species incompatibility between human DM and mouse class II molecules that accounts for this difference (38). Notably, HLA-DM can efficiently catalyze peptide binding to purified IAβ molecules in 0.2% NP40 (44). It is interesting to consider the possibility that polymorphisms may influence the ability of HLA-DM to interact with mouse class II molecules. The effect of varying detergent concentrations has not yet been studied with IAβ. Detergents can influence peptide binding by MHC class II molecules through a variety of potential mechanisms, including direct effects on peptide release or exchange (34). In our studies, however, the concentration of NP40 selectively modulated DM activity with no direct effect on peptide binding in the absence of DM. Thus, we believe that the best explanation for the effect of detergent concentration in our experiments relates to its effect on the volume of micelles into which DM and DR partition.

The effect of transmembrane anchoring on DM-DR interaction provides an important example of how membrane localization can affect the kinetics of protein-protein binding and enzymatic reactions. Soluble proteins in solution are freely tumbling with random orientation, whereas transmembrane proteins have relatively constrained orientations with respect to the plane of the membrane. It is likely that productive interactions between DM and DR occur through a lateral interface in both molecules (10) and that this topology is favored by colocalization in lipid bilayers or micelles. The high local concentration of interacting molecules in biological membranes promotes interactions through mass action whereas conventional concentrations are defined by total solution volume. Volume exclusion by irrelevant integral membrane proteins has also been proposed to contribute toward enhanced association of membrane-associated molecules (45). This effect is not likely to be important in our experiments with purified molecules. Grasberger et al. (45) reported model calculations for the contributions of these parameters, estimating that these factors can increase the likelihood of forming dimers by six orders of magnitude as compared with soluble proteins in solution. Under the experimental conditions used in the present study, with purified proteins variably partitioned into detergent micelles through transmembrane domains, we empirically measured an enhancement of somewhat greater than two orders of magnitude based on relative DM functional potency.

Our findings underscore the potential importance of the local distribution of DM and substate class II molecules in the membranes of peptide loading compartments. Schafer et al. (46) estimated that the ratio of DR:DM was 23:1 in a B cell line. This was reduced to 5:1 in isolated subcellular fractions enriched for MHC class II compartment peptide loading vesicles. DM-catalyzed peptide loading occurs in a series of late endosomal compartments enriched for both class II molecules and DM (MIIC). These endosomes have complex internal membrane structures including vesicles or sheets. DM has been reported to be enriched in the limiting membrane of these compartments, whereas DR is distributed in both limiting and internal membranes (47, 48). Of relevance, it has been reported that the cytoplasmic tail of HLA-DOβ can influence the distribution of both DM and DR in multivesicular MIIC by preferentially localizing them to the limiting membrane (49). Through mechanisms such as this, extrinsic factors might influence DM peptide exchange function by controlling DM-DR colocalization. Of further interest is the potential role of tetraspan proteins such as CD82 which coprecipitate with DM, DR, and other proteins (47, 48). These molecules may form scaffolds that regulate protein interactions. In contrast to DM, tetraspan proteins have been reported to be enriched in internal membranes (47, 48). The limiting and internal membranes also differ in the distribution of lipids, defining specialized domains (50). Clearly, the local distribution of DM and DR and dynamics of trafficking in these complex structures could have a major impact on the regulation of peptide loading and editing in the MHC class II Ag presentation pathway.

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References


