Tapasin Is Required for Efficient Peptide Binding to Transporter Associated with Antigen Processing*

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The transporter associated with antigen processing (TAP) binds peptides in its cytosolic part and subsequently translocates the peptides into the lumen of the endoplasmic reticulum (ER), where assembly of major histocompatibility complex (MHC) class I and peptide takes place. Tapasin is a subunit of the TAP complex and binds both to TAP1 and MHC class I. In the absence of tapasin, the assembly of MHC class I in the ER is impaired, and the surface expression is reduced. To clarify the function of tapasin in the processing of antigenic peptides, we studied the interaction of peptide and TAP, peptide transport across the membrane of the ER, and association of peptides with MHC class I molecules in the microsomes derived from tapasin mutant cell line 721.220, its sister cell line 721.221 expressing tapasin, and their HLA-A2 transfectants. The binding of peptides to TAP in tapasin mutant 721.220 cells was significantly diminished in comparison with 721.221 cells. Impaired peptide-TAP interaction resulted in a defective peptide transport in tapasin mutant 721.220 cells. Interestingly, despite the diminished peptide binding to TAP, the transport rate of TAP-associated peptides was not significantly altered in 721.220 cells. After transfection of tapasin cDNA into 721.220 cells, efficient peptide-TAP interaction was restored. Thus, we conclude that tapasin is required for efficient peptide-TAP interaction.

Major histocompatibility complex (MHC)1 class I molecules present antigenic peptides to cytotoxic T cells (1–4). The processing of MHC class I-presented peptides includes degradation of cytosolic proteins, translocation of generated peptides into the lumen of the endoplasmic reticulum (ER), and the assembly of MHC class I with peptides (2, 5). The transporter associated with antigen processing (TAP) (2, 5) transports peptides across the membrane of the ER. TAP was first discovered as a heterodimeric complex composed of two proteins, TAP1 and TAP2, each consisting of C-terminal hydrophilic domains that bind ATP and peptides and N-terminal multimembrane-spanned hydrophobic domains (5). The feature of TAP1 and TAP2 proteins were revealed as members of the ABC transporter family (5). TAP1 and TAP2 bind short peptides of 7–12 amino acids and have broad specificity (6–8). The efficient binding of peptides requires expression of both TAP1 and TAP2 (8). The interaction of TAP and peptides is ATP-independent (6–9). The addition of ATP dissociates peptides from TAP and stimulates the assembly of peptide and MHC class I in the lumen of the ER indicative of translocation of TAP-released peptide across the membrane of the ER (6–9). Immunoprecipitation with anti-TAP1 antiserum demonstrated that TAP associates with MHC class I heavy chain-β2-microglobulin dimer (10, 11). The importance of the TAP-MHC class I interaction for the assembly of MHC class I and peptides was suggested by the finding in which deficiency in MHC class I surface expression was found in a cell line, 721.220, lacking interaction of MHC class I and TAP (12). With anti-TAP1 antiserum, a 48-kDa protein (tapasin) was coprecipitated, and this protein was missing in 721.220 cells, indicative of requirement of tapasin in the interaction of MHC class I and TAP (13). cDNA cloning of tapasin revealed a type I membrane protein with a cytoplasmic tail containing a double lysine motif known to maintain membrane proteins in the ER (14, 15). Immunoprecipitation with anti-TAP1 or anti-tapasin antisera demonstrated a consistent and stoichiometric association of tapasin and TAP1 and TAP2 (14). The importance of tapasin in MHC class I antigen presentation was demonstrated by restored MHC class I surface expression and class I-TAP interaction in 721.220 cells after transfection with tapasin cDNA (15), suggesting that either tapasin directly regulates the assembly of MHC class I and peptide or the association of MHC class I with TAP enhances peptide loading. The importance of MHC class I-TAP interaction in peptide loading was opposed by recent findings, in which the ER luminal domain of tapasin (soluble tapasin) was expressed in 721.220 cells. Soluble tapasin associated with class I but did not interact with TAP (16). In the absence of interaction with TAP, soluble tapasin restored surface expression of MHC class I and the presentation of viral peptides to CTL (16). The conclusion from this study was that tapasin-MHC class I interaction, but not tapasin-TAP interaction, was required for peptide loading (16). However, there are conflicting reports concerning the importance of the tapasin-MHC class I interaction in promoting peptide loading. It has been reported that HLA-B27 and HLA-A2 could assemble with peptide in tapasin mutant cells, although HLA-B27 and HLA-A2 could interact with tapasin in wild type cells (17, 18). In addition, a murine mutant MHC class I Dd (E222K) was discovered having a Glu to Lys mutation at residue 222 of the heavy chain. This mutation caused deficiency in interaction with tapasin (19). Significant peptide loading onto Dd (E222K) was observed (19). These results indicate that the interaction of tapasin and MHC class I is not essential for peptide loading.

In this study, we used reporter peptides in which the e- amino

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1 The abbreviations used are: MHC, major histocompatibility complex; TAP, transporter associated with antigen processing; ER, endoplasmic reticulum; tapasin, TAP-associated glycoprotein; PAGE, polyacrylamide gel electrophoresis; FACS, fluorescence-activated cell sorting; MP, membrane peptide; OVA, ovalbumin; ANB-NOS, N-5-azido-2-nitrobenzoxoysuccinimide.

2 The abbreviations used are: MHC, major histocompatibility complex; TAP, transporter associated with antigen processing; ER, endoplasmic reticulum; tapasin, TAP-associated glycoprotein; PAGE, polyacrylamide gel electrophoresis; FACS, fluorescence-activated cell sorting; MP, membrane peptide; OVA, ovalbumin; ANB-NOS, N-5-azido-2-nitrobenzoxoysuccinimide.
group of lysine was covalently modified by coupling a chemical cross-linker (ANB-NOS), and the tyrosine was labeled by iodination (125I). These modifications allowed photocross-linking of the reporter peptides to TAP and MHC class I and enabled us to monitor the peptide binding to TAP, peptide translocation, and assembly of peptide and MHC class I in purified microsomes derived from tapasin mutant cells, 721.220, and its sister cells, 721.221, as well as their HLA-A2 transfectants. Our results clearly indicate that tapasin is required for efficient peptide-TAP interaction.

**MATERIALS AND METHODS**

**Cells—**721.220 and 721.221 cell lines and 721.220-HLA-A2 and 721.221-HLA-A2 cell lines were kindly provided by Drs. J. C. Solheim and T. Elliott, respectively. The 721.174 cell line was a gift from Dr. S. Kvist. The cell lines were cultured in RPMI 1640 medium (Life Technologies, Inc.) and supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/ml penicillin, 100 mg/ml streptomycin, and 2 mM glutamine at 37 °C in a 5% CO₂ atmosphere.

The cDNA encoding wild type human tapasin was constructed into pCEP4 expression vector (CLONTECH). The constructs were transfected into 721.220 cells by electroporation. Transfected cells were selected in hygromycin-containing medium (Life Technologies, Inc.) and then cloned by limiting dilution. Expression of transfected tapasin was measured by immunoblotting with anti-human tapasin antiserum.

**Antibodies**—The monoclonal antibody BB7.2, specific for HLA-A2, and rabbit antiserum to human MHC class I (R425) were kindly provided by Dr. S. Kvist. Rabbit antisera against human TAP1 and tapasin were described previously (9, 14). The polyclonal antibodies were affinity-purified before use.

**Metabolic Labeling, Immunoprecipitation, and Immunoblotting—**Cells were washed twice with phosphate-buffered saline and incubated for 15 min at 37 °C in methionine-free RPMI 1640 medium containing 3% dialyzed fetal bovine serum. NaN₃ (125I)methionine (Amersham Pharmacia Biotech) was added, and the incubation was continued for 60 min. At the end of labeling, cells were washed three times with ice-cold phosphate-buffered saline and lysed in 1% digitonin (Sigma) or 1% Nonidet P-40 lysis buffer containing 0.15M NaCl, 25 mM Tris-HCl, pH 7.5, 1.5 mM MgOAc₂, and a mixture of protease inhibitors (2 μM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 30 μg/ml aprotinin, 10 μg/ml pepstatin). The cleared lysates were added to antibodies previously bound to protein A-Sepharose beads (Amersham Pharmacia Biotech, Uppsala, Sweden). After washing, the immunoprecipitates were analyzed by SDS-PAGE. Western blotting and FACS analysis were performed as described previously (9).

**Peptides and Peptide Modification**—All peptides were synthesized in a peptide synthesizer (Applied Biosystems, model 431A), using conventional Fmoc (N-(9-fluorenylethoxycarbonyl) chemistry. Peptides were subsequently purified by high pressure liquid chromatography and lyophilized in phosphate-buffered saline. The e-amino group of lysine in peptides of influenza A virus nucleoprotein, NP383–391 (H5N1) (SRYWAHKTR), influenza A matrix protein (MP), M58–64G58YF62K (YIL-GKYVTLL), and OVA 257–264 (ISSYNEFKL) were covalently modified by a photoreactive cross-linker as described previously (7). An aliquot (1 μg) of the peptide was labeled by chloramine T-catalyzed iodination (125I). The modification and labeling experiments were performed in the dark. The cross-linker modified and 125I-labeled peptides are referred to as 125I-OVA-ANB-NOS or 125I-MP-ANB-NOS or 125I-nucleoprotein-ANB-NOS.

**Preparation of Microsomes and Photocross-linking**—Microsomes from cell lines were prepared and purified according to Saraste et al. (20). For photocross-linking, 125I-labeled and ANB-NOS-modified peptide was mixed with 20 μl of microsomes (concentration of 60 A₂₆₀/ml) to a final concentration of 100 nM, in RM buffer (250 mM sucrose, 50 mM triethanolamine-HCl, 50 mM KOAc, 2 mM MgOAc₂, 1 mM diothreitol). This mixture was then kept at 26 °C for 5 min UV irradiation was subsequently carried out for 5 min on ice at 366 nm. Microsomal membranes were recovered by centrifugation through a 0.5 M sucrose cushion in RM buffer containing 1 mM cold peptide (unlabeled peptide without ANB-NOS modification). The microsomal membranes were washed once with cold RM buffer, lyed by 1% digitonin or 1% Nonidet P-40, and subjected to immunoprecipitation. Cross-linked microsomal proteins were immunoprecipitated with specific antiserum. The precipitates were analyzed by SDS-PAGE or quantitated by a γ-counter. Cross-linking reactions with transport buffer containing 1 mM ATP were performed as described previously (7, 14). For peptide competition,
in 721.221 or 721.220 cells, 100 nM of cross-linker-modified reporter peptide, $^{125}$I-OVA-ANB-NOS, was incubated with 721.221 or 721.220 microsomes in the presence of native and unlabeled OVA peptide at different concentrations. In order to dissect the peptide binding from the peptide translocation, the assay was done in the absence of ATP as previously reported (9). Again a significant deficiency of peptide-TAP interaction was detected in 721.220 microsomes in the absence of competing peptide (Fig. 3, lane 1). The peptide binding to TAP in 721.220 microsomes was completely competed by a lower concentration (400 nM) of native peptide (Fig. 3), whereas in 721.221 microsomes, more than 1.6 μM concentration was required for completely competing away reporter peptide binding (Fig. 3). These data indicate that the affinity for peptide binding to TAP in tapasin mutant cells is much lower than in wild type cells.

**Peptide Transport Efficiency Is Not Reduced in Tapasin Mutant Cells**—After having demonstrated a lower peptide binding to TAP in tapasin mutant cells, we examined the transport efficiency by measuring the time for translocation of 50% TAP-bound peptides into the microsomes of wild type, 721.221A2, or tapasin mutant cells, 722.220A2, in the presence of ATP. After incubation of $^{125}$I-MP-ANB-NOS peptide with microsomes from 721.220A2 or 721.221A2 cells, the excess $^{125}$I-MP-ANB-NOS peptides were washed off. Peptide-loaded microsomes were then resuspended in transport buffer with 100 μM ATP and incubated for different periods of time. After incubation, the microsomes were lysed in 1% Nonidet P-40 lysis buffer and subsequently precipitated with anti-TAP1 or anti-HLA-A2 antibodies. The peptide-bound TAP or HLA-A2 molecules were quantitated by γ-counter. The presented results are from one representative experiment out of three performed.

**Peptide Transport Efficiency**

In 721.221A2 and 721.220A2 microsomes, the excess $^{125}$I-MP-ANB-NOS peptides were washed off. Peptide-loaded microsomes were then resuspended in transport buffer with 100 μM ATP and incubated for different periods of time. After incubation, the microsomes were lysed in 1% Nonidet P-40 lysis buffer and subsequently precipitated with anti-TAP1 or anti-HLA-A2 antibodies. The peptide-bound TAP or HLA-A2 molecules were quantitated by γ-counter. The presented results are from one representative experiment out of three performed.

**The Binding of Peptides to HLA A2 in Microsomes**

In 721.221A2 and 721.220A2 cells, 50% of translocated peptides were recovered by anti-HLA-A2 antibody at the same time point in both 721.220A2 and 721.221A2 microsomes, although a much lower amount of peptide-bound A2 was obtained in 721.220A2 microsomes (Fig. 4, lower panel). These results indicated that mutation of tapasin greatly affected peptide interaction with TAP but affected the peptide translocation much less or not at all.

**Fig. 3. Low affinity of peptide-TAP binding in tapasin mutant 721.220 cells.** Cross-linking was carried out with microsomes and cross-linker-modified and $^{125}$I-labeled OVA reporter peptide, $^{125}$I-OVA-ANB-NOS, in the absence or presence of various concentrations of native and cold OVA peptide as indicated. After cross-linking, the microsomes were lysed in 1% Nonidet P-40 and subsequently precipitated with anti-TAP1 antiserum. The precipitates were analyzed on SDS-PAGE.

**Fig. 4. Kinetics of peptide binding to TAP and HLA-A2 in 721.220As and 721.221A2 microsomes.** Microsomes of 721.221A2 or 721.220A2 were incubated with cross-linker-modified and $^{125}$I-labeled HLA-A2 binding peptide in the absence of ATP. After removing the excess peptide, the peptide-loaded microsomes were resuspended in transport buffer in the presence of ATP (see “Materials and Methods”). Aliquots of incubated microsomes were pelleted at different time points as indicated, irradiated under UV, and lysed by 1% Nonidet P-40 lysis buffer. The peptide-cross-linked TAP and HLA-A2 molecules were recovered by anti-TAP1 or anti-HLA-A2 antibody. The precipitates were quantitated by γ-counter. The presented results are from one representative experiment out of three performed.
of native and unlabeled MP peptide was added in the reaction. Micro-
cipitates were analyzed on SDS-PAGE. In
The cross-linker-mod-
ified HLA-A2 binding peptide, MP, was incubated with microsomes
derived from 721.221A2 or tapasin mutant 721.220A2 in the presence of
ATP. After cross-linking, the cleared lysates were precipitated by anti-
TAP1 (lanes 1–3) and anti-HLA-A2 (lanes 4–7), respectively. The pre-
cipitates were analyzed on SDS-PAGE. In lanes 5 and 7, a 10-fold excess
of native and unlabeled MP peptide was added in the reaction. Micro-
somes of 721.214 were used as a negative control (lane 1).

The cleared lysates were aliquoted and precipitated with anti-
TAP1 and anti-HLA-A2, respectively. Consistent with the results
shown in Fig. 1, anti-TAP1 recovered peptide-bound TAP
and HLA-A2 in 721.221A2 microsomes (Fig. 5, lane 3), but only
a weak peptide cross-linked TAP band was detected in
721.220A2 microsomes (Fig. 5, lane 2). With anti-HLA-A2 ant-
ibody, less peptide-cross-linked HLA-A2 was recovered in
721.220A2 microsomes (Fig. 5, lane 6) in comparison with
721.221A2 microsomes (Fig. 5, lane 4). In addition, a weak
peptide-cross-linked tapasin was also detected (Fig. 5, lane 4),
which corresponds to our previous findings (14, 21). The less
peptide-cross-linked HLA-A2 in 721.220A2 microsomes (Fig. 5,
lane 6) was correlated with reduced HLA-A2 surface expression
(Fig. 6). However, in 721.220A2 microsomes, anti-HLA-A2
could still precipitate a significant amount of peptide-bound A2
(Fig. 5, lane 6), which was compatible with the amount of
peptides bound to TAP (Fig. 5, lane 2) and with the amount of
HLA-A2 expressed on the cell surface (Fig. 6).

These results suggest that the reduced surface expression
and translocated peptides in the ER of 721.220A2 cells result
from deficient peptide-TAP interaction. Moreover, despite the
deficiency of peptide-TAP interaction in 721.220A2 cells, TAP
still can transport peptides in the presence of ATP, which
is compatible with previous findings (12).

Interaction of Peptide-loaded HLA-A2 with Tapasin—Previ-
ously, it was reported that the assembly of peptide and MHC
class I in tapasin mutant cells was defective (8). We have
previously demonstrated that murine tapasin associated with
peptide-bound H-2K\(^\text{b}\) (21). To further confirm the interaction of
peptide-bound MHC class I with human tapasin, purified mi-
Croplodes from 721.220A2 and 721.221A2 were incubated with
125\text{I}-MP-ANB-NOS peptide in the presence of ATP. Anti-tapa-
sin antiserum precipitated peptide-bound HLA-A2 and TAP in
721.221A2 but not in 721.220A2 microsomes (Fig. 7, lanes 1
and 2). This result clearly indicates that tapasin does not
exclusively bind to peptide free MHC class I.

DISCUSSION

The function of TAP to mediate peptide transport into the ER
is well established (2, 5, 8). TAP interacts with peptides at its
cytosolic part and with MHC class I at its luminal part (6–9).
The importance of a direct interaction between peptide and
TAP for peptide translocation was clearly demonstrated by the
findings in which the herpes simplex virus ICP47 protein in-
hits the MHC class I antigen presentation pathway by occu-
pying the peptide binding site on TAP (22–24). The association
of MHC class I and TAP is mediated by tapasin (13–15, 18, 21).
Tapasin is a type I ER membrane protein and bridges the
association of MHC class I with TAP (13, 14). Cells with mu-
tated tapasin have a defective surface expression of MHC class
I (12), and this defect can be corrected by transfection of wild
type tapasin cDNA (13). Tapasin was suggested to function by
promoting the peptide loading onto MHC class I (8). In the
present study, we systematically examined the peptide inter-
action with TAP, the peptide translocation into the ER, and
the peptide assembly with MHC class I in microsomes purified
from tapasin mutant cells and wild type cells as well. A severe
defect in peptide-TAP interaction was revealed in the tapasin
mutated cell 721.220. In contrast, the peptide translocation
across the membrane of the ER was intact, as indicated by the
same off rate of TAP-associated peptides in tapasin mutated
and wild type microsomes in the presence of ATP. The trans-
located HLA-A2-binding peptide could bind to HLA-A2 mole-
cules in both tapasin mutated and wild type microsomes, al-
though the amount of peptide-associated HLA-A2 in
721.220A2 cells was much less than that in its sister cell line
721.221-A2 expressing wild type tapasin. This reduction was
due to the deficient interaction between peptide and TAP.

Tapasin is a subunit of the TAP complex as indicated by the
consistent and stoichiometric association of tapasin with TAP1
and TAP2 (14). Previously, it was reported that peptide trans-
port in tapasin mutant cells was not altered (12). In that study,
translocation of peptides in 721.220 and wild type cells was
analyzed by recovery of a glycosylated reporter peptide in
streptolysin O-permeabilized cells. Since TAP-dependent
peptide translocation was intact in tapasin mutant cells, it was
conceivable that accumulation of translocated peptides in the
ER might result from the decay kinetics of transported pep-
tides in tapasin mutant cells. Restored peptide-TAP binding
and peptide translocation in tapasin-transfected 721.220 cells

![Fig. 5. Reduced peptide assembly in 721.220A2 is correlated with deficiency of peptide-TAP interaction. The cross-linker-modified HLA-A2 binding peptide, MP, was incubated with microsomes derived from 721.221A2 or tapasin mutant 721.220A2 in the presence of ATP. After cross-linking, the cleared lysates were precipitated by anti-TAP1 (lanes 1–3) and anti-HLA-A2 (lanes 4–7), respectively. The precipitates were analyzed on SDS-PAGE. In lanes 5 and 7, a 10-fold excess of native and unlabeled MP peptide was added in the reaction. Microsomes of 721.214 were used as a negative control (lane 1).](Image 381x461 to 482x572)

![Fig. 6. Reduced surface expression of HLA-A2 in 721.220A2 cells. The cells of 721.220A2 or 721.221A2 were incubated with BB7.2 antibody and subsequently with fluorescein isothiocyanate-conjugated goat anti-mouse Ig antibody. After washing, the BB7.2-labeled cells were analyzed by flow cytometry on a FACScan.](Image 368x629 to 495x729)
clearly demonstrated that tapasin is required for efficient peptide binding to TAP. In agreement with our results from tapasin-transfected 721.220, a study of 721.220B8 tapasin transfected also revealed a more than 4-fold increase in peptide translocation after expressing tapasin in 721.220B8 cells (16). In addition, it has recently been reported that the C-terminal region of tapasin was identified as a binding site of TAP (25). Transfection of the C-terminal region of tapasin enhanced the function of TAP in tapasin mutant cells (25).

In previous studies (14, 21) as well as the present studies (Fig. 5, lane 4), we found a weak interaction of peptides and tapasin in the presence of ATP but not in the absence of ATP. Since the peptide-TAP interaction is independent of ATP, it is not likely that tapasin is involved in the peptide binding site on the TAP complex. Both TAP1 and TAP2 are required for efficient peptide binding, certain peptides binding preferentially to TAP1 and others to TAP2 (10). Although peptide binding to human TAP is relatively promiscuous, the difference of binding affinity of various peptides can be as great as 3 orders of magnitude, depending on both the terminal and the internal sequence of the peptides (7, 26). Among three peptides tested in this study, similar deficiency in their ability to bind to TAP of 721.220 cells suggested that the requirement of tapasin for peptide-TAP interaction is not based on the sequence of the peptides.

Searching for peptide-binding sites, the regions of the TAP subunits to which the photoreactive peptides bind were mapped by proteolysis of the TAP proteins after photocross-linking and immunoprecipitation with antisera specific for distinct hydrophilic regions (27, 28). The results suggest that the binding site is composed of multiple regions of TAP1 and TAP2. Interaction of the same photoreactive peptide to multiple regions of TAP1 and TAP2 may indicate a large binding pocket (27, 28). This study also suggested that the binding regions are very close to or part of the transmembrane domains. The interaction of tapasin with TAP may largely regulate the peptide binding site. Recently, the ER luminal domain of tapasin was expressed as a soluble form of tapasin in 721.220 cells (16). Soluble tapasin bound to MHC class I, but not to TAP. The peptide transport was measured in 721.220, 721.220 transfected with soluble tapasin, and 721.220 with wild type tapasin. Results showed that wild type tapasin increased the efficient peptide translocation in 721.220 cells, but soluble tapasin transfectants retained their deficient peptide transport (16). Taken together, these results and our own indicate that the interaction of tapasin and TAP is critical for efficient peptide transport. Tapasin may function to stabilize the peptide-binding site of TAP.

Since tapasin directly interacts with MHC class I heavy chain and β2-microglobulin dimer, it was suggested that this interaction enhances peptide loading onto class I molecules, possibly due to a high local concentration of peptides or to the requirement of tapasin itself for peptide-MHC class I assembly. Support for the importance of MHC class I-TAP interaction was evidenced by the finding of a deficient TAP-dependent peptide assembly of a mutant HLA-A2.1 (29, 30). A point mutation of threonine 134 to lysine resulted in HLA-A2.1 incapable of interacting with TAP (29, 30). Moreover, the deficient assembly of HLA-A2.1 (T134K) was corrected by a direct delivery of peptide to the ER in a TAP-independent manner. Therefore, it was concluded that the interaction of TAP and MHC class I is essential for peptide loading. Recently, this notion has been called into question by the finding that a soluble tapasin restored the surface expression of HLA-B8 in 721.220B8 cells, despite the lack of interaction between MHC class I and TAP (16). Notably, soluble tapasin-MHC class I interaction was unstable in detergent lysates and required chemical cross-linking for their detection. Since soluble tapasin lacks the motif of the ER retrieval signal present in the wild type tapasin, the increased surface expression can be explained as a lack of control of MHC class I retention in the ER. Tapasin may regulate the MHC class I release from the ER rather than directly load peptides on MHC class I. Recently, it was found that HLA-B27 and HLA-A2 presented peptides to cytotoxic T cells in tapasin mutant 721.220B27 or 721.220A2 cells (17, 31), although both alleles were found to associate with tapasin in wild type cells. Furthermore, a mutant H2-Dd with a Glu to Lys mutation at residue 222 (Dd E222K) was shown not to be substantially impaired in its ability to present peptides (18) despite its loss of ability for interaction with tapasin. Although there is a lack of direct evidence for the involvement of tapasin in peptide loading onto MHC class I, a recent study of the HLA-A2 T134K mutant indicates that the interaction of MHC class I with the TAP complex may be important for the optimization of MHC class I assembly (32). The T134K mutant did not bind to tapasin or calreticulin, and it failed to present endogenous viral peptides to T cells (32). However, the mutant class I had the ability to bind peptides in vitro (32). Therefore, it was suggested that the TAP complex is involved in a quality control stage during MHC class I assembly. This view is supported by the study of green fluorescence protein-tagged MHC class I (33), which shows that peptide-loaded MHC class I can also be retained in the ER for optimizing peptide loading. In the present study, most of the peptide-loaded HLA-A2 was found to be associated with tapasin in 721.221A2, which is in line with our previous finding of an association of peptide-loaded mouse MHC class I with tapasin (21) and indicates that tapasin may also regulate the transport of assembled MHC class I to the cell surface.

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