Peptide-bound Major Histocompatibility Complex Class I Molecules Associate with Tapasin before Dissociation from Transporter Associated with Antigen Processing*

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Major histocompatibility complex (MHC) class I molecules present antigenic peptides to CD8+ T cells. The peptides are generated in the cytosol, then translocated across the membrane of the endoplasmic reticulum by the transporter associated with antigen processing (TAP). TAP is a trimeric complex consisting of TAP1, TAP2, and tapasin (TAP-A) as indicated for human cells by reciprocal coprecipitation with anti-TAP1/2 and anti-tapasin antibodies, respectively. TAP1 and TAP2 are required for the peptide transport. Tapasin is involved in the association of class I with TAP and in the assembly of class I with peptide. The mechanisms of tapasin function are still unknown. Moreover, there has been no evidence for a murine tapasin analogue, which has led to the suggestion that murine MHC class I binds directly to TAP1/2. In this study, we have cloned the mouse analogue of tapasin. The predicted amino acid sequence showed 78% identity to human tapasin with identical consensus sequences of signal peptide, N-linked glycosylation site, transmembrane domain and double lysine motif. However, there was less homology (47%) found at the predicted cytosolic domain, and in addition, mouse tapasin is 14 amino acids longer than the human analogue at the C terminus. This part of the molecule may determine the species specificity for interaction with MHC class I or TAP1/2. Like human tapasin, mouse tapasin binds both to TAP1/2 and MHC class I. In TAP2-mutated RMA-S cells, both TAP1 and MHC class I were coprecipitated by anti-tapasin antiserum indicative of association of tapasin with TAP1 but not TAP2. With crosslinker-modified peptides and purified microsomes, anti-tapasin coprecipitated both peptide-bound MHC class I and TAP1/2. In contrast, anti-calreticulin only coprecipitated peptide-free MHC class I molecules. This difference in association with peptide-loaded class I suggests that tapasin functions later than calreticulin during MHC class I assembly, and controls peptide loading onto MHC class I molecules in the endoplasmic reticulum.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AJ106278.

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† The abbreviations used are: MHC, major histocompatibility complex; TAP, transporter associated with antigen processing; ER, endoplasmic reticulum; β2m, β2-microglobulin; tapasin, TAP-associated glycoprotein; HC, heavy chain; OVA, ovalbumin.
With anti-TAP1 antisera, several proteins were coprecipitated with TAP1/2 (13, 18). One of the coprecipitates was identified as a 48-kDa glycoprotein (tapasin) and was found in complex with TAP1/2, calreticulin, and MHC class I (19). Tapasin is a type I transmembrane glycoprotein with a double lysine motif that mediates the retrieval of proteins back from the cis-Golgi and thus maintains membrane proteins in the ER (20, 21). Analysis of .220 cells showed that they lacked the expression of tapasin (19). Transfection of tapasin into .220 cells restored complex association and association of peptide with MHC class I (21). These pieces of evidence indicate that tapasin mediates the interaction of MHC class I HC-β2m with TAP, and this interaction is essential for peptide loading onto MHC class I HC-β2m. Moreover, the stability of the complex of tapasin and TAP1/2 was analyzed in comparison with the association of MHC class I and TAP in both normal and β2m-deficient cells (20). Tapasin was stably present in immunoprecipitates in roughly stoichiometric amounts with TAP1/2. However, MHC class I was rapidly dissociated from TAP (20). A similar stable association of tapasin with TAP1/2 was also obtained in β2m-deficient cells (20). This indicates that TAP1,-2, and tapasin form a trimeric complex. Tapasin serves as a docking site on the TAP complex specific for interaction with MHC class I HC-β2m. Interaction of tapasin with peptide in the presence of ATP suggested the function of tapasin in the loading of peptide onto MHC class I (20). More recently, studies with transfection of soluble tapasin into .220 cells indicated that association of tapasin with MHC class I is sufficient to facilitate peptide loading and assembly of MHC class I molecules (22). The lack of evidence for a tapasin analogue in mouse has suggested a direct interaction of mouse MHC class I with TAP (23).

We have now cloned the mouse homologue of tapasin and analyzed the interaction of mouse tapasin and TAP1/2 as well as peptide binding in both wild type and TAP2 mutant cells. Mouse tapasin is similar in both function and amino acid sequence to the human homologue. Interaction of tapasin with TAP1 and MHC class I is independent of TAP2. Tapasin associates with peptide-bound TAP1/2 and MHC class I, indicative of a functional difference between tapasin and calreticulin.

**MATERIALS AND METHODS**

**Cells**—RMA and RMA-S cell lines were kindly provided by Professor Klas Kärre (Karolinska Institute, Stockholm, Sweden). The cell lines were cultured in RPMI 1640 medium (Life Technologies, Inc.), 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.

**Antibodies**—The conformational specific antibody to H-2Kb (Y3) was obtained from American Type Culture Collection (Manassas, VA). Rabbit antisera against mouse TAP1 was kindly provided by Dr. Y. Yang (The R.W. Johnson Pharmaceutical Research Institute, San Diego). Anti-calreticulin antisera was obtained from Affinity Bioreagents, New Jersey. The anti-mouse tapasin antisera was generated by immunization with a peptide (CATAASLTIPRNSKKSQ) derived from the protein. The conformational specific antibody to H-2Kb (Y3) was obtained from American Type Culture Collection (Manassas, VA). Rabbit antisera against mouse TAP1 was kindly provided by Dr. Y. Yang (The R.W. Johnson Pharmaceutical Research Institute, San Diego). Anti-calreticulin antisera was obtained from Affinity Bioreagents, New Jersey. The anti-mouse tapasin antisera was generated by immunization with a peptide (CATAASLTIPRNSKKSQ) derived from the protein.

**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. Then, 0.2 µCi/ml of [35S]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 (re-purified from digitonin purchased from Sigma) lysis buffer containing 0.15 M NaCl, 25 mM Tris-HCl, pH 7.5, 1.5 mM iodoacetamide, and a mixture of protease inhibitors (2 mM phenylmethylsulfonyl fluoride, 10 mg/ml leupeptin, 30 mg/ml aprotinin, 10 mg/ml pepstatin). The cleared lysates were added to antibodies previously bound to protein-A-Sepharose beads (Amersham Pharmacia Biotech). After washing, the immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis as described previously (20). For immunoblotting, aliquots of cell lysates were loaded onto a 10% SDS-polyacrylamide gel electrophoresis. Proteins were transferred onto a nitrocellulose filter, which was probed with the anti-tapasin antisera at a dilution of 1:1,000. Detection was performed according to Kalttov et al. (24).

**cDNA Cloning of Mouse Tapasin**—A cDNA library constructed into a λgt10 phage vector (CLONTECH) from poly(A)-selected RNA prepared from mouse liver was screened with a probe prepared from cDNA of human tapasin. Twenty positive plaques contained overlapping inserts. Ten of these inserts were sequenced and reconstructed in a 1.8-kilobase pair fragment into a pGEM3-f vector under T7 promoter. The nucleotide sequence reported in this paper has been submitted to the EMBL (accession number AF106278).

**Peptides and Peptide Modification**—All peptides were synthesized in a peptide synthesizer (Applied Biosystems, Model 431A), using conventional Fmoc (N-(9-fluorenylmethoxycarbonyl) chemistry. Peptides were subsequently purified by high pressure liquid chromatography, and dissolved in phosphate-buffered saline. The H-2Kb binding peptide of OVA peptide (residues 257–264, SIINFEKL), was cotransfected with a peptide synthesizer (Applied Biosystems, Model 431A), using conventional Fmoc (N-(9-fluorenylmethoxycarbonyl) chemistry. Peptides were subsequently purified by high pressure liquid chromatography, and dissolved in phosphate-buffered saline. The H-2Kb binding peptide of OVA peptide (residues 257–264, SIINFEKL), was covalently modified by coupling a phenol azide with a nitro group to the e-amino group of lysine (position 7) to allow for photoactivation and by substitution of the isoleucine at position 3 with tyrosine to allow for iodination as described previously (10, 11). An aliquot (100 ng) of the peptide was labeled by chloramine T-catalyzed iodination (125I). The modified peptide was then kept for 10 min at 26 °C. 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, lysed by 1% digitonin, and subjected to immunoprecipitation. Crosslinked microsomal proteins were immuno-precipitated with specific antisera and analyzed by SDS-polyacrylamide gel electrophoresis. Crosslinking reactions with 1 mM ATP were performed as described previously (10, 11). For peptide competition, 100 nM of the 125I-OVA-ANB-NOS peptide was mixed with a 10-fold molar excess of unlabelled and unmodified OVA peptide before the crosslinking reaction.

**RESULTS**

**cDNA Cloning of Mouse Tapasin**—To obtain the mouse tapasin, a mouse liver cDNA library was screened with human tapasin cDNA as a probe. Ten positive clones were sequenced. A full-length clone with a 1.8-kilobase pair insert accommodated an open reading frame encoding a polypeptide of 465 amino acids as compared with the 448 amino acids of human tapasin (Fig. 1). Amino acid sequence comparison of mouse and human tapasin showed 78% identity including identical conserved sequences of signal peptide, N-linked glycosylation site and double lysine motif at the C-terminal end (Fig. 1). Both polypeptides showed the same hydrophobic profile and a similar amino acid long hydrophobic stretch of amino acids, position 395 to 427 in human and position 402 to 427 in mouse tapasin) predicted to be a transmembrane domain (Fig. 1). Despite the close homology in most parts of mouse and human tapasin, significant differences in the predicted cytosolic domains were revealed. Sequence identity between mouse and human cytosolic domains was less than 50% (Fig. 1). In addition, mouse tapasin has a longer cytosolic domain, with 35 amino acids compared with 21 amino acids of the human homologue (Fig. 1). This may indicate the species specificity of the interaction of tapasin with MHC class I or TAP1/2.

**Interaction of Tapasin with TAP1 Is TAP2-independent**—Previously, we have shown that human tapasin is a subunit of the TAP complex demonstrated by stable association of tapasin with TAP1/2 in stoichiometric amounts even after a 3 h chase. This indicates that TAP and tapasin are stably maintained in a trimeric complex, which is dependent on the interaction of tapasin with MHC class I and TAP1/2. Moreover, the stability of the complex of tapasin and TAP1/2 was analyzed in comparison with the association of MHC class I and TAP in both normal and β2m-deficient cells (20). Tapasin was stably present in immunoprecipitates in roughly stoichiometric amounts with TAP1/2. However, MHC class I was rapidly dissociated from TAP (20). A similar stable association of tapasin with TAP1/2 was also obtained in β2m-deficient cells (20). This indicates that TAP1,-2, and tapasin form a trimeric complex. Tapasin serves as a docking site on the TAP complex specific for interaction with MHC class I HC-β2m. Interaction of tapasin with peptide in the presence of ATP suggested the function of tapasin in the loading of peptide onto MHC class I (20). More recently, studies with transfection of soluble tapasin into .220 cells indicated that association of tapasin with MHC class I is sufficient to facilitate peptide loading and assembly of MHC class I molecules (22). The lack of evidence for a tapasin analogue in mouse has suggested a direct interaction of mouse MHC class I with TAP (23).

We have now cloned the mouse homologue of tapasin and analyzed the interaction of mouse tapasin and TAP1/2 as well as peptide binding in both wild type and TAP2 mutant cells. Mouse tapasin is similar in both function and amino acid sequence to the human homologue. Interaction of tapasin with TAP1 and MHC class I is independent of TAP2. Tapasin associates with peptide-bound TAP1/2 and MHC class I, indicative of a functional difference between tapasin and calreticulin.
To further characterize the properties of mouse tapasin, we prepared an anti-mouse tapasin antiserum specific for the C-terminal end (see “Materials and Methods”). The antiserum was specific for mouse tapasin and did not bind to human tapasin (Fig. 2). After metabolic labeling, RMA and RMA-S cells were lysed by digitonin and precipitated with anti-mouse tapasin antiserum. Analogously to antiserum against human tapasin, anti-mouse tapasin coprecipitated both TAP1/2 and MHC class I of RMA cells (Fig. 3, lane 1). From RMA-S cells, anti-tapasin recovered tapasin, TAP1, and MHC class I (Fig. 3, lane 2). Because RMA-S has a mutation of TAP2 causing pre-mature termination of the TAP2 protein (26), the coprecipitation of tapasin and TAP1 from RMA-S indicates direct association of tapasin with TAP1. This finding is in line with the previous observation that interaction of MHC class I with TAP was TAP2-independent (12, 13). In addition to TAP1/2 and MHC class I, anti-tapasin antiserum precipitated a 65-kDa protein in RMA cells (Fig. 3, lane 1). The nature of this molecule has not yet been identified.

Association of Tapasin with Peptide-bound Class I—It has been reported that the MHC class I HC-b2m dimer interacts with both calreticulin and tapasin (19). In the absence of tapasin, assembly of peptide with MHC class I was defective, which is indicative of involvement of tapasin, but not calreticulin, in the process of peptide loading (19). To clarify whether binding of peptides can bring the interaction of tapasin, TAP1/2, and MHC class I into effect, we investigated the association of tapasin with peptide-bound MHC class I and/or TAP1/2 in the presence of peptides. As described previously, we used a crosslinker modified and 125I-labeled H-2Kb binding OVA peptide (residues 257–264, SIINFEKL) with a substitution of isoleucine at position 3 with a tyrosine to allow iodination (11). This modified peptide is referred to as 125I-OVA-ANB-NOS. For analysis of interaction of tapasin to peptide-bound MHC class I and TAP1/2, the 125I-OVA-ANB-NOS was incubated with microsomes purified from RMA cells in the absence or presence of ATP. This will allow the binding of peptides to TAP1/2 and/or MHC class I (11). After crosslinking, the microsomes were washed and lysed by 1% digitonin lysis buffer. The clear lysates were precipitated with anti-TAP1 and anti-tapasin antiserum, respectively. In the absence of ATP, peptide-bound TAP1/2, but not MHC class I, were recovered by both anti-TAP1 and anti-tapasin antiserum from RMA microsomes (Fig. 4, lanes 3 and 4). In the presence of ATP, both anti-TAP1 and anti-tapasin antiserum precipitated peptide-bound TAP1/2 and MHC class I (Fig. 4, lanes 1 and 2). In RMA-S microsomes, neither peptide-bound TAP1/2 nor MHC class I were detected by anti-TAP1 and anti-tapasin in the presence of ATP (Fig. 4, lanes 6 and 7), suggesting a defective peptide transport in RMA-S microsomes. The evidence of tapasin binding to peptide-loaded MHC class I may indicate that peptide transport and peptide loading are very transient processes and that tapasin directly facilitates peptide loading onto MHC class I. The specificity of peptide binding was indicated by competition of cold peptide for the binding of 125I-OVA-ANB-
**Interaction of Peptide Loaded MHC Class I with Tapasin**

**Fig. 4. Association of tapasin with peptide-bound TAP1/2 and MHC class I.** The 125I-OVA-ANB-NOS peptide was mixed with microsomes purified from RMA (lanes 1-3) or RMA-S cells (lanes 6 and 7) in the presence (lanes 1, 2, 5, 6, and 7) or absence (lanes 3 and 4) of ATP. In lane 5, the 125I-OVA-ANB-NOS peptide was mixed with a 10-fold molar excess of unlabeled and unmodified native OVA peptide. The mixtures were incubated for 10 min at 26 °C and then transferred to ice and exposed to UV light for 5 min to allow for crosslinking. After crosslinking, microsomes were collected by centrifugation and lysed in 1% digitonin buffer. The cleared lysates were precipitated with anti-TAP1 (lanes 2, 4, 5, and 7) or anti-tapasin (lanes 1, 3, and 6) antiserum. The immunoprecipitates were analyzed on an SDS gel. Positions of TAP1/2 and MHC class I HC are indicated.

**Fig. 5. Dissociation of peptide-bound MHC class I from TAP complexes.** The 125I-OVA-ANB-NOS peptide was mixed with purified microsomes from RMA cells and incubated for 10 min at 25 °C in the presence of ATP. After incubation, microsomal membranes were pelleted and free peptides were washed off. The pellets were resuspended in RM buffer in the presence of ATP for 0 (lanes 1 and 4) or 15 min (lanes 2 and 3) at 25 °C. The membranes were pelleted again and crosslinked (see Fig. 4 legend). Cleared lysates were immunoprecipitated with conformation specific antibody (Y3) (lane 2) or anti-tapasin (lanes 3 and 4), respectively. Precipitation with normal serum (lane 1) served as a control. The precipitates were then analyzed by SDS-polyacrylamide gel electrophoresis. Positions of TAP1/2, tapasin, and MHC class I are indicated.

**Fig. 6. Calreticulin associates with peptide-free MHC class I.** For detection of association of calreticulin with MHC class I, RMA (panel A, lane 1) and RMA-S cells (panel A, lane 2) were labeled for 1 h with [35S]methionine and lysed with 1% digitonin. Aliquots of the lysates were immunoprecipitated with anti-calreticulin antiserum. The positions of calreticulin and MHC class I HC are indicated. For detection of interaction of calreticulin and tapasin with peptide-bound MHC class I, the 125I-OVA-ANB-NOS peptide was mixed with microsomes purified from RMA in the presence of ATP (panel B, lanes 3-8). The mixtures were incubated for 10 min at 26 °C and then transferred to ice and exposed to UV light for 5 min to allow for crosslinking. After crosslinking, microsomes were collected by centrifugation and lysed in 1% digitonin buffer. The cleared lysates were first precipitated with anti-tapasin (panel B, lane 8) or anti-calreticulin (panel B, lane 7) antiserum. The supernatant precipitated with anti-tapasin was reprecipitated with anti-calreticulin (panel B, lane 4) and supernatant precipitated with anti-calreticulin was reprecipitated with anti-tapasin. The aliquots of the lysates were labeled for 1 h with [35S]methionine and lysed with 1% digitonin. After crosslinking, microsomes were collected by centrifugation and lysed in 1% digitonin buffer. The cleared lysates were first precipitated with anti-tapasin (panel B, lane 8) or anti-calreticulin (panel B, lane 7) antiserum. The supernatant precipitated with anti-tapasin was reprecipitated with anti-calreticulin (panel B, lane 4) and supernatant precipitated with anti-calreticulin was reprecipitated with anti-tapasin (panel B, lane 3). The immunoprecipitates were analyzed on a SDS gel. Positions of peptide-bound TAP1/2 and MHC class I HC are indicated. Panel B, lanes 5 and 6 showed peptide-bound MHC class I precipitated with anti-H-2Kb antibody (Y3) as control.

**Table:**

| Antiserum          | Y3 | Tapasin |
|--------------------|----|---------|
| Incubation time (min) |    |         |
| 0                  | 15 | 15      |
| 15                 | 0  |         |

**Dissociation of Peptide-bound MHC Class I from Tapasin—Earlier, we have shown (20) that tapasin binds constitutively to TAP1/2 but transiently to MHC class I. To explore the kinetics of the interaction between peptide-loaded MHC class I and tapasin, 125I-OVA-ANB-NOS peptide was mixed with RMA microsomes in the presence of ATP. After incubation for 15 min at 25 °C, the free peptides were washed off, and microsomes were resuspended in ATP containing RM buffer (see “Materials and Methods”). The resuspended microsomes were incubated for 0 or 15 min at 25 °C (Fig. 5). After incubation, the microsomes were pelleted again and lysed with 1% digitonin buffer. The cleared lysates were precipitated with antibody to MHC class I (Y3) (Fig. 5, lane 2) or tapasin (Fig. 5, lanes 3 and 4). The 15 min chase resulted in dissociation of peptide-bound MHC class I from tapasin (Fig. 5, lane 3). These results demonstrated that MHC class I molecules were loaded with peptides when they were part of the TAP complex and then released from this complex.

**Calreticulin Interacts Only with Peptide-free Class I—**Both calreticulin and tapasin bind to MHC class I HC-β2m dimer (19). Moreover, a complex of calreticulin, tapasin, TAP1/2, and MHC class I was also found (19, 21). For investigation of binding of calreticulin to peptide-loaded MHC class I, 125I-OVA-ANB-NOS was incubated with microsomes derived from RMA cells in the presence of ATP. After incubation, microsomes were lysed with 1% digitonin and precipitated with anti-calreticulin antibody or anti-tapasin antiserum. The anticalreticulin was reprecipitated with anti-tapasin and the supernatant precipitated with anti-calreticulin was reprecipitated with anti-tapasin. The results showed again that peptide-bound MHC class I interacted with tapasin but not calreticulin (Fig. 6B, lanes 3 and 4). To confirm the binding of calreticulin to peptide-free MHC class I, RMA and RMA-S cells were metabolically labeled and lysed with 1% digitonin. The cleared lysates were precipitated with anti-cal-
reticulin antibody. A significant amount of MHC class I molecules was coprecipitated with calreticulin from both cell lines (Fig. 6A, lanes 1 and 2), which suggested that calreticulin interacted with peptide-free MHC class I molecules.

**DISCUSSION**

As a peptide transporter, TAP functions to translocate peptides from the cytosol into the lumen of the ER (3–5). In addition to translocating peptides, interaction of MHC class I with TAP has been found to be important in the assembly of peptide and MHC class I (12, 13). Recently, it has been discovered that tapasin, a subunit of the TAP complex, mediates the association of MHC class I and TAP. Cells lacking tapasin have a deficient expression of surface MHC class I as a result of reduced assembly of MHC class I and peptides in the ER (17, 19). Transfection of tapasin restored the assembly and surface expression of class I (21). Human tapasin has been cloned and the amino acid sequence showed a type I transmembrane glycoprotein with a strong ER retention signal at the C terminus (20, 21). Because a mouse analogue of tapasin had not been identified from coprecipitated TAP complex, it was suggested that mouse MHC class I may not need tapasin for interaction with TAP (23). We have now cloned mouse tapasin. The predicted amino acid sequence reveals 78% identity to human tapasin with identical signal peptide, N-glycosylation site, transmembrane domain, and double lysine motif at the C-terminal end, indicative of a similar function of human and mouse tapasin. An interesting feature of the sequence of mouse tapasin is the predicted cytosolic domain, which showed less than 50% homology with the human protein. In addition, mouse tapasin has 14 extra amino acids at the C terminus compared with human tapasin, suggestive of species specificity of the tapasin function. In line with the sequence results, it was recently found that the intracellular maturation and surface expression of HLA-B4402 in murine cells required co-expression of human tapasin but not human TAPI/2 (37). The cytosolic domain of tapasin may determine its interaction with MHC class I or TAP1.

In studies of the interaction between MHC class I and TAP, it was found that TAP1, but not TAP2, is required for the association of TAP with class I molecules (12, 13). Because tapasin is essential for the association of MHC class I to TAP (19), tapasin may directly interact with TAP1. We have now shown that tapasin interacts with TAP1 and MHC class I in RMA-S cells, which have a mutation that causes premature termination of the TAP2 protein. Thus, the interaction between TAP1 and MHC class I appears to be mediated by the tapasin. From these results, the predicted order of interaction between different molecules in the TAP complex is TAP2 to TAP1, TAP1 to tapasin, and tapasin to MHC class I. By these linked molecules, the translocation and loading of peptides are rapidly and efficiently processed in the same microenvironment.

Previous reports have indicated the existence of several ER chaperones, which interact with MHC class I (4, 19, 27–30). Among them, calnexin and calreticulin were best characterized (4, 31). By using conformation specific antibodies and a β2m mutant cell line, a distinct difference between calreticulin and calnexin in their mode of association with MHC class I was found (19, 29, 32). Calnexin binds only to β2m-free heavy chain in human cells (29, 33), whereas, calreticulin binds only to the MHC class I HC-β2m dimer (19, 32). Similarly to calreticulin, interaction of tapasin with MHC class I is β2m-dependent (19, 20), despite the fact that calreticulin and tapasin are quite different molecules. Like calnexin, calreticulin is an ER chaperone with lectin-like activity, and it binds to several other glycoproteins in the ER besides MHC class I (34, 35). The binding is regulated by glucose trimming of nascent N-linked oligosaccharides (34, 35). Tapasin so far was found only in the complexes where MHC class I and/or TAP1/2 are present (19, 20). Because tapasin is also an N-linked glycoprotein, it has not yet been proven whether tapasin can directly bind to calreticulin. In addition, tapasin is a subunit of the TAP complex, but calreticulin does not directly associate with TAP (19, 20). Assembly of MHC class I and peptides was defective in the absence of tapasin, and the presence of calreticulin (19), indicating that either calreticulin is not required for the loading of peptides to MHC class I or both tapasin and calreticulin are essential for the association of peptide with MHC class I. In this study, a distinct difference in association of these two molecules with peptide-bonded MHC class I molecules was found. Tapasin, but not calreticulin, bound to peptide-loaded MHC class I before this complex dissociated from TAP. This may indicate that tapasin directly catalyzes the loading of peptides onto MHC class I, and calreticulin controls the conformation of β2m-heavy chain dimer before it interacts with TAP. Lehner et al. (22) have shown that TAP1/2-free soluble tapasin can restore MHC class I expression in tapasin-negative cell line 220. This finding, together with the demonstrated interaction between tapasin and peptide-loaded MHC class I, suggests that tapasin functions not only to form a bridge between TAP1/2 and MHC class I but also directly to facilitate the assembly of MHC class I with peptides.

The interaction of TAP with MHC class I molecules was found to be polymorphic (36). Some HLA-B alleles associate less or not at all with TAP (36). The binding of different peptides to TAP-free MHC class I may differ from that of TAP associated MHC class I molecules. Polymorphism in mechanisms of peptide loading has been found recently in studies of HLA-B2705, which can present antigenic peptides in the absence of tapasin (37). In comparison, expression of B*4402 was tapasin-dependent (37), although tapasin mediated interaction of both B2705 and B4402 with TAP1/2. The question is whether the difference in peptide loading is dependent on the peptides selected by these alleles or on the native structure of different alleles resulting in different stability of the MHC class I HC-β2m dimer in the ER. It is important now to determine the molecular structure of the binding between MHC class I and tapasin as well as between tapasin and TAP1. This will form a basis for understanding both the function of tapasin and the selectivity of peptide assembly with different MHC class I.

Based on our results and the data previously published, we conclude that mouse tapasin is similar to the human analogue both in amino acid sequence and function. The difference between the mouse and human molecules in the cytosolic domain may indicate a species specificity of tapasin. Tapasin interacts with TAP1 and MHC class I in the absence of tapasin, raising the possibility of sequential interaction of TAP2-TAP1-tapasin-MHC class I in the TAP complex. Interaction of peptide-bound MHC class I with tapasin, but not calreticulin suggests that tapasin may directly influence the loading of peptides onto MHC class I.

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