Binding of H-2K\textsuperscript{b}-specific Peptides to TAP and Major Histocompatibility Complex Class I in Microsomes from Wild-type, TAP1, and \(\beta_2\)-Microglobulin Mutant Mice\*  

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Intracellular vesicles are expressed on the cell surface of almost all nucleated mammalian cells. They consist of a highly polymorphic membrane-spanning heavy chain (HC) that is noncovalently associated with a light chain, \(\beta_2\)-microglobulin (\(\beta_2m\)) (1). MHC class I molecules transport and present antigen in the form of short peptides, derived from intracellularly degraded proteins, to CD\textsuperscript{8}\ T cells (2). MHC class I-presented peptides are usually 8–11 amino acids in length (3). A majority of them are generated in the cytosol by proteolytic degradation (2–4). After proteolysis, peptides are translocated into the lumen of the endoplasmic reticulum (ER), a process that is largely dependent on the transporter associated with antigen processing (TAP), where they take part in the assembly of the MHC class I heterotrimERIC complex (2, 5). Both \(\beta_2m\) and peptide are necessary for formation of a stable and properly conformed class I complex. In the absence of peptide and/or \(\beta_2m\), incompletely assembled class I complexes are retained in the ER, resulting in severely reduced levels of class I molecules at the cell surface (1, 6).

Significant achievements have been made with respect to the understanding of TAP1/2-mediated peptide translocation in human, rat, and mouse cells (reviewed in Refs. 5, 7, and 8), in particular with respect to the substrate specificity of the TAP1/2 transporter (9–15). Two types of assays have provided useful in these studies. One has involved studies with lymphoid cells permeabilized with the bacterial toxin streptolysin O, which allows the delivery of peptides to the cytosol and from there to the lumen of the ER (13, 14). The other assay has involved translocation of peptides across microsomal membranes prepared from rat and mouse livers (9, 15). Available data from these systems have suggested a dependence of ATP hydrolysis (9, 13–15) as well as a high degree of peptide specificity in the peptide translocation process (7–15). Furthermore, efficient peptide transport requires expression of both the TAP1 and TAP2 subunits (13–15). Recent data have indicated that peptide translocation may be proceeded by a step involving peptide binding to TAP (16–20). A physical association between TAP1 and MHC class I has also been demonstrated (21, 22). These observations have led to the suggestion that TAP may directly facilitate peptide binding to class I (17, 21, 22).

Formation of intact MHC class I molecules involves proper folding of the class I subunits, their assembly, and interaction with peptide (6). It is likely that several proteins, including TAP (7–9) and calnexin (23), contribute to this process. MHC class I assembly may in principle follow two different pathways: (i) the binding of peptide to preformed class I HC/\(\beta_2m\) heterodimers or (ii) the binding of peptide to free class I HC followed by association with \(\beta_2m\) (6, 27). Both pathways have been demonstrated \textit{in vitro} in cellular lysates (see Refs. 24–26; reviewed in Ref. 27).
Under these conditions, β2m associated immediately after translation with class I HC. This was followed by binding of exogenous peptide. However, less is known about the order of assembly during physiological conditions. In TAP-deficient cells, complexes between HC and β2m are readily detectable after short labeling periods (24, 25, 34, 35), suggesting that “empty” class I molecules may be a physiological intermediate of an intact class I heterotrimer. This notion has been supported by recent studies in human cells indicating that folding and assembly of MHC class I heterodimers in the ER may preceede binding of peptide (36). However, studies of class I folding and assembly in mouse β2m−/− cells have indicated that the alternative pathway may operate as well (37).

In this study, we conjugated a cross-linker (ANB-NOS) to the e-aminogroup of the lysine residue of an H-2Kb-binding ovalbumin (OVA) peptide (residues 257-264, SIINFEKL) and substituted the isoleucine at position 3 with tyrosine to allow for iodination. These modifications allowed photo-cross-linking of the OVA peptide to TAP and MHC class I in intact or permeabilized microsomes and thus enabled us to detect the stepwise processes involving peptide binding to TAP, peptide translocation into the ER lumen, and peptide binding to class I molecules in purified microsomes from wild-type, TAP1−/−, and β2m−/− as well as TAP1/β2m−/− mice.

MATERIALS AND METHODS

Mice—The generation of β2m−/− and TAP1−/− mice has been described in detail (38, 39). For the generation of TAP1/β2m−/− double mutant mice, TAP1−/− and β2m−/− mice were crossed, and offspring were subsequently intercrossed (40). All mice, including control C57BL/6 (B6) mice, were bred and maintained at the Microbiology and Tumor Biology Center, Karolinska Institute (Stockholm, Sweden).

Peptides and Peptide Modification—All peptides were synthesized in a peptide synthesizer (Applied Biosystems Model 431A) using conventional Fmoc (9-fluorenyl)methoxycarbonyl) chemistry. Peptides were subsequently purified by HPLC and dissolved in phosphate-buffered saline. The H-2Kb-binding OVA peptide (residues 257-264, SIINFEKL) was modified by coupling a phenyl azide with a nitro group to the e-aminogroup of lysine (position 7) to allow for photoactivation and by substitution of the isoleucine at position 3 with tyrosine to allow for iodination. Modification of the OVA peptide by ANB-NOS was performed by mixing 0.5 mg of ANB-NOS dissolved in 200 μl of dimethyl sulfoxide, 100 μl of phosphate-buffered saline, and 50 μl of 0.5 mM CAPS (pH 10). The reaction was allowed to proceed for 30 min on ice. To remove excess ANB-NOS and ions, the mixture was purified by gel filtration on a Sephadex G-10 column and subsequently by HPLC. An aliquot (1 μg) of the peptide was labeled by chloramine T-catalyzed iodination (125). Peptide modification and labeling experiments were performed in the dark. This modified peptide is referred to as 125I-OVA-ANB-NOS.

Antiserum, Immunoprecipitation, SDS-PAGE, and Western Blotting—The rabbit anti-mouse H-2 antiserum R218 was raised by immunizing rabbits with recombinant H-2 proteins. The conformational specific antiserum against mouse TAP1 and 2 was kindly provided by Dr. J. J. Monaco (University of Cincinnati, Cincinnati, OH). Immunoprecipitation and SDS-PAGE analysis were performed as described (28). Protein A-Sepharose was obtained from Pharmacia (Upsala). Radioactive isotopes were from Amer sham International (Buckinghamshire, United Kingdom), and immobilized streptavidin was from Dynal (Oslo, Norway). For Western immunoblotting, aliquots of microsomal membrane lysates were analyzed by 10% SDS-PAGE. Proteins were transferred onto a nitrocellulose filter, which was probed with the anti-H-2 antiserum R218 at a dilution of 1:2000. The alkaline phosphatase-conjugated goat anti-rabbit IgG antibody (Promega, Madison, WI) was used to detect bound anti-H-2 antibodies. Detection was performed according to Kaltsoy et al. (41).

Preparation of Microsomes—Microsomes were purified from the livers of B6, TAP1−/−, β2m−/−, and TAP1/β2m−/− mice as described by Saraste et al. (42). This preparation contains both ER and Golgi fractions of microsomal membranes (42).

Photo-cross-linking—For photo-cross-linking, 100 nm 125I-OVA-ANB-NOS peptide was mixed with 50 μl of microsomes (concentration of 60 μg/ml) in RM buffer (250 mM sucrose, 50 mM trithioalamine HCl, 50 mM KOAc, 2 mM MgOAc2, and 1 mM dithiothreitol). To permeabilize microsomes and digitonin was added to the cross-linking mixture to a final concentration of 0.1%. This mixture was then kept at 26 °C for 15 min. UV irradiation was subsequently carried out at 366 nm for 5 min on ice. Microsomal membranes were then recovered by centrifugation through a 0.5 s sucrose cushion in RM buffer containing 1 μM unlabeled peptide (unlabeled peptide without ANB-NOS modification). The microsomal membranes were then washed once with cold RM buffer, lysed with 1% Nonidet P-40 lysis buffer, and subjected to immunoprecipitation. Cross-linked microsomal proteins were then analyzed by SDS-PAGE. Cross-linking reactions with 1 mM ATP, using intact microsomes, were performed as described previously (15).

Peptide Competition Assays—For peptide competition, 100 nm 125I-OVA-ANB-NOS peptide was mixed with a 10-fold molar excess of unlabeled peptide. After mixing microsomes and digitonin (concentration as above) were added, and the mixtures were incubated at 26 °C for 15 min. The membranes were pelleted by centrifugation through a sucrose cushion (see above) and UV-irradiated at 366 nm for 5 min on ice. The membrane pellets were lysed, immunoprecipitated, and subsequently analyzed by SDS-PAGE.

Addition of Human β2m to the Cross-link Mixture—10 μg of human β2m (Sigma) was added to the mixtures of the 125I-OVA-ANB-NOS peptide and microsomes. The microsomal membranes were then permeabili zed with 0.1% digitonin. After 15 min of incubation at 26 °C, free peptides were removed by centrifugation through a 0.5 s sucrose cushion, and the membrane pellets were UV-irradiated at 366 nm for 5 min on ice. The membrane pellets were lysed, immunoprecipitated, and subsequently analyzed by SDS-PAGE.

RESULTS

Different Glycosylation Forms of MHC Class I Molecules in Microsomes from B6, TAP1−/−, β2m−/−, and TAP1/β2m−/− Mice—To analyze the expression of MHC class I molecules in microsomes derived from C57BL/6 (B6), TAP1−/−, β2m−/−, and TAP1/β2m−/− mice, microsomes were lysed, and total class I molecules were determined by Western blotting using a broadly reactive rabbit anti-H-2 antiserum (R218). Similar amounts of class I HC were detected in microsomes from TAP1−/−, β2m−/−, and TAP1/β2m−/− mice (Fig. 1, lanes 5–16). In contrast, significantly lower levels of class I HC were detected in microsomes from wild-type mice (Fig. 1, lanes 1–4).

Most of the MHC class I HC in microsomes from B6 wild-type mice (Fig. 1, lanes 1–4) had a higher molecular mass than class I HC in TAP1−/−, β2m−/−, and TAP1/β2m−/− microsomes (lanes 5–16). This result indicated that a majority of the class I HC were terminally glycosylated (mature) in wild-type microsomes, while a majority of the class I HC in TAP1−/−, β2m−/−, and TAP1/β2m−/− microsomes were immature and thus retained in the early secretory pathway. This notion was confirmed by endoglycosidase H treatment, which showed that the fast migrating species was sensitive and the slow migrating species was resistant to endoglycosidase H digestion (data not shown).

Efficient Peptide Binding to Class I Molecules in Microsomes Requires Expression of β2m—To characterize peptide binding to TAP and the influence of a TAP complex and/or β2m on peptide binding to class I molecules, we conjugated a cross linker (ANB-NOS) to the e-aminogroup of the lysine residue at position 7 of an H-2Kb-binding OVA peptide (residues 257–264, SIINFEKL) and substituted the isoleucine at position 3 with a tyrosine to allow for iodination. This modified peptide is referred to as 125I-OVA-ANB-NOS. This strategy allowed cross-linking of the 125I-OVA-ANB-NOS peptide to TAP as well as MHC class I molecules in microsomes. Peptide-bound class I molecules were subsequently analyzed by immunoprecipitation with a broadly reactive anti-H-2 antiserum (R218) as well as a conformational specific monoclonal antibody (Y3). Labelled microsomes from β2m−/− mice were permeabilized and incubated with the 125I-OVA-ANB-NOS peptide. After cross-linking, peptide-bound class I molecules were readily recovered from B6 (Fig. 2, lane 1).
as well as TAP1−/− (lanes 3 and 9) microsomes, while essentially no peptide binding to class I was detected in β2m−/− (Fig. 2, lanes 5 and 11) or TAP1/β2m−/− (lanes 7 and 13) microsomes. These data indicated that β2m expression was necessary for efficient peptide binding to MHC class I. To confirm the requirement of β2m for efficient peptide binding to class I, exogenous β2m was added to permeabilized β2m−/− and TAP1/β2m−/− microsomes. Under these conditions, peptide-bound class I molecules were detected in both β2m−/− (Fig. 2, lanes 6 and 12) and TAP1/β2m−/− (lanes 8 and 14) microsomes. Taken together, these results indicate that efficient peptide binding to MHC class I in permeabilized microsomes requires the presence of β2m. This suggests that peptide, under physiological conditions, preferentially binds to preformed I–HC heterodimers. Furthermore, these data indicate that efficient peptide binding to MHC class I heterodimer does not require the expression of an intact TAP complex or TAP1. In B6 microsomes, similar amounts of mature (terminally glycosylated) and immature class I molecules were bound by the 125I-OVA-ANB-NOS peptide (Fig. 2, lane 1). Since most of class I molecules in B6 microsomes are mature (terminally glycosylated) (Fig. 1, lanes 1–4), these results indicate that the relative percentage of peptide-receptive molecules in B6-derived microsomes is significantly higher among the immature than among the mature class I molecules. As expected, in TAP1−/− mice, the majority of peptide-bound class I molecules were immature (Fig. 2, lane 3). Interestingly, the 125I-OVA-ANB-NOS peptide bound also to an unknown protein with a molecular mass of ~55 kDa. This protein was precipitated by both the H-2Kb-specific monoclonal antibody Y3 and the broadly reactive anti-H-2 antisera R218 (Fig. 2, lanes 2–4, 9, and 10). The nature of this protein has not yet been identified.

The OVA Peptide Specifically Competes with the 125I-OVA-ANB-NOS Peptide for Binding to H-2Kb—To examine whether the cross-link modification alters the binding capacities of the OVA peptide to H-2Kb, the unlabeled native OVA peptide as well as a panel of other peptides were used in a competition experiment. The native OVA peptide competed efficiently with the reporter peptide (Fig. 3, lanes 2, 5, and 14), whereas none of a panel of competing peptides with specificity for class I molecules other than H-2Kb competed for 125I-OVA-ANB-NOS peptide binding to H-2Kb (lanes 3, 6, 15, and 16). The latter included a lymphocytic choriomeningitis virus glycoprotein peptide (residues 33–41, KAVYFNATM) specific for H-2D b molecules, an influenza A virus nucleoprotein peptide (residues 393–391, SYRWAI RTR) specific for HLA-B27, and an influenza A virus matrix peptide (residues 58–66, GILGFVFPTL) specific for HLA-A2. These data confirmed the specificity in peptide binding to class I and demonstrated that the modification of the ε-amino group of lysine as well as the substitution of the isoleucine at position 3 with a tyrosine did not significantly alter

the H-2Kb binding capacity of the OVA peptide.

Promiscuous, ATP-independent Binding of Peptides to Mouse TAP Molecules—The interaction of the 125I-OVA-ANB-NOS peptide with TAP was then examined. Both TAP1 and TAP2 molecules on intact B6 microsomes bound the 125I-OVA-ANB-NOS peptide (Fig. 4, lanes 2 and 3). In contrast, the same peptide did not bind to TAP2 in microsomes from TAP1−/− mice (Fig. 4, lanes 6 and 7). In comparison with H-2Kb binding, all four competing peptides used above (Fig. 3) efficiently competed with the 125I-OVA-ANB-NOS peptide for binding to TAP (Fig. 4, lanes 9–12). Peptide binding to TAP occurred in the absence of added ATP.

**ATP Is Required for TAP-dependent Peptide Translocation, but Not for Binding to Class I**—To characterize the ATP requirement for peptide translocation across intact microsomal membranes and subsequent binding to class I molecules, intact B6 microsomes were incubated with the 125I-OVA-ANB-NOS peptide. Under these experimental conditions, peptide-bound H-2Kb molecules were detected by the anti-H-2 antisera R218 only in the presence of ATP (Fig. 5, lane 2).
results were obtained with the H-2Kb-specific monoclonal antibody Y3 (data not shown). Moreover, the binding pattern of both mature and immature class I molecules under these conditions was similar to that with permeabilized microsomes in the absence of ATP (Fig. 5, lane 2; and Fig. 2, lane 1). These and the above-mentioned results demonstrated that peptide binding to TAP was ATP-independent and promiscuous, while TAP-dependent peptide translocation in intact microsomes was ATP-dependent. In contrast, peptide binding to class I was ATP-independent, but peptide-specific.

Sequential Interaction of Peptide with TAP and MHC Class I—To assess the stepwise interactions of peptide with TAP and class I molecules, intact B6 microsomes were incubated with the 125I-OVA-ANB-NOS peptide in the presence or absence of ATP. Incubations were terminated at different time points to determine the on-rate of peptide binding. Peptide binding to TAP was rapid and ATP-independent (Fig. 6, lanes 1–10). In contrast, peptide binding to H-2Kb molecules was detected only in the presence of added ATP (Fig. 6, lanes 1–5) and with an association rate significantly slower than that of peptide binding to TAP (lanes 1–10). Taken together, these results demonstrate a stepwise binding of peptide to TAP, subsequent translocation across the ER membrane, a step that requires ATP hydrolysis, and finally, binding of peptide to class I molecules.

DISCUSSION

Using a cross-linker-modified H-2Kb-binding peptide, we have assessed peptide binding to TAP and MHC class I in purified microsomes from B6 (wild-type), TAP1−/−, β2m−/−, and TAP1/β2m−/− mice. Peptide bound to both immature and mature (terminally glycosylated) class I molecules in permeabilized microsomes from wild-type mice. Efficient peptide binding to class I in permeabilized microsomes was dependent on β2m, but occurred in the absence of TAP1 and thus an intact TAP complex. Using intact microsomes from wild-type mice, peptide binding to TAP was readily detectable. TAP-dependent peptide translocation over intact microsomal membranes was ATP-dependent, while peptide binding to TAP on intact microsomes as well as peptide binding to class I in permeabilized microsomes were ATP-independent. Kinetic studies allowed the study of the sequential binding of peptide to TAP, TAP-dependent translocation over the ER membrane, and peptide binding to class I.

MHC class I assembly may proceed through two distinct pathways: (i) class I HC association with β2m followed by peptide binding or (ii) class I HC binding to peptide followed by association with β2m (reviewed in Refs. 6 and 27). One question, which has not been fully addressed in previous studies, is what is the extent of peptide binding to β2m-free class I HC.

![Image](https://example.com/image1.png)

**Fig. 3.** The OVA peptide specifically competes with the 125I-OVA-ANB-NOS peptide for binding to H-2Kb in permeabilized microsomes. The 125I-OVA-ANB-NOS peptide was mixed with a 10-fold molar excess of different competing peptides. Mixed peptides were incubated with permeabilized microsomes derived from B6, TAP1−/−, β2m−/−, and TAP1/β2m−/− mice. After cross-linking (see legend to Fig. 2), H-2Kb molecules were immunoprecipitated with the rabbit anti-H-2 antiserum R218 and analyzed by SDS-PAGE. No competing peptide was added in the samples shown in lanes 1, 4, 7, 10, and 13. The competing peptides were (amino-terminal end to the left) as follows: OVA, residues 257–264, SIINFEKL; lymphocytic choriomeningitis virus glycoprotein; LCMV, residues 33–41, KAVYNFATM; influenza A virus nucleoprotein, residues 383–391, SRYWAIRTR; and influenza A virus matrix protein (M), residues 58–66, GILGFVFTL. The molecular masses in kilodaltons are shown to the right of the gel.

![Image](https://example.com/image2.png)

**Fig. 4.** 125I-OVA-ANB-NOS peptide binding to TAP: competition for peptide binding by H-2Kb- and non-H-2Kb-specific peptides. The 125I-OVA-ANB-NOS peptide was mixed with intact microsomes from B6 (lanes 1–3 and 9–12) and TAP1−/− (lanes 5–7) mice. After cross-linking (see legend to Fig. 2), TAP molecules were precipitated with either an anti-TAP1 (lanes 2 and 6) or an anti-TAP2 (lanes 3, 7, and 9–12) antiserum. Precipitation with normal rabbit serum (NRS) served as a negative control (lanes 1 and 5). Peptide-cross-linked H-2Kb molecules from permeabilized microsomes are shown in lanes 4 and 8. The molecular masses in kilodaltons are shown to the right of the gel. LCMV, lymphocytic choriomeningitis virus glycoprotein; NP, influenza A virus nucleoprotein; M, influenza A virus matrix protein.

![Image](https://example.com/image3.png)

**Fig. 5.** 125I-OVA-ANB-NOS peptide binding to class I in intact microsomes. The 125I-OVA-ANB-NOS peptide was mixed with intact microsomes from B6 mice in the absence (lane 1) and presence (lane 2) of ATP. After cross-linking (see legend to Fig. 2), H-2Kb molecules were immunoprecipitated with the rabbit anti-H-2 antiserum R218 and analyzed by SDS-PAGE. The molecular masses in kilodaltons are shown to the right of the gel.
Fig. 6. Sequential interaction of the $^{125}$I-OVA-ANB-NOS peptide with TAP and class I molecules in intact microsomes from B6 mice. The $^{125}$I-OVA-ANB-NOS peptide was mixed with intact microsomes from B6 mice in the presence (lanes 1–5) or absence (lanes 6–10) of ATP for different time periods as indicated. After termination of the incubation, microsomal membranes were pelleted and cross-linked (see legend to Fig. 2). Cleared lysates of each aliquot were subsequently divided into two fractions, and immunoprecipitation was carried out with an anti-TAP2 antiserum and the rabbit anti-H-2 antisera R218, respectively. The precipitates were then analyzed by SDS-PAGE. The molecular masses in kilodaltons are shown to the right of the gel.

Using a photo-cross-linkable peptide and permeabilized microsomes, we could not detect any peptide binding to class I HC in the absence of $\beta 2m$. The addition of exogenous $\beta 2m$ to permeabilized $\beta 2m$-deficient microsomes led to efficient peptide binding to class I. These results strongly suggest that class I HC-$\beta 2m$ heterodimer formation is required for efficient peptide binding to class I under physiological conditions, arguing for the first pathway as the principal route of class I assembly in vivo. However, these results do not exclude that a small fraction of peptide may bind to class I HC prior to association with $\beta 2m$. Indeed, it has been reported that in the absence of $\beta 2m$, a limited number of functional class I HC-peptide heterodimers are formed (37, 43, 44).

The TAP complex has recently been shown to form a physical complex with class I molecules (21, 22). This interaction appears to be mediated by the TAP1 subunit (17, 21, 22). This has led to the suggestion that an intact TAP complex, or the TAP1 subunit itself, may directly facilitate peptide loading to class I (17, 21, 22). In this study, we readily observed peptide binding to class I in permeabilized microsomes from TAPI/− mice, indicating that efficient peptide binding to class I in the ER can occur even in the absence of an intact TAP complex or TAP1. However, our data do not argue against the idea that an association of TAP with class I might form a favorable microenvironment that may facilitate peptide binding to class I.

Using microsomes from wild-type mice, our data demonstrate the presence of peptide-receptive class I molecules in the early as well as late secretory pathways. In contrast, peptide-receptive molecules were only detected in the early secretory pathway of TAPI/− mice. This observation is in line with the results of Day et al. (45). At first sight, this may appear to be a paradox. In the presence of a peptide transporter, peptide-accessible class I molecules are readily detectable in the late secretory pathway. However, at least some of these complexes may have arisen from class I-$\beta 2m$ heterodimers that, in the course of intracellular transport, have lost their peptide cargo. This is in concordance with the observation that the expression of free class I HC may be more abundant on the cell surface of $\beta 2m$-positive cells than on the cell surface of $\beta 2m$-deficient cells (46).

The processes of peptide binding and peptide translocation over the ER membrane can be distinguished using a cross-linkable peptide. Our results, based on competition experiments, indicate a promiscuous binding of peptide to TAP provided that both the TAPI and TAP2 subunits are expressed. In the absence of TAPI, no peptide binding to TAP2 was observed. These data support the notion that TAPI and TAP2 form a functional complex: in the absence of either of the subunits, peptide cannot efficiently bind and be translocated. These results are in line with recently published observations indicating that elements of both TAPI and TAP2 contribute to the formation of a peptide-binding site (16–18). The first attempts to identify the nature of the peptide-binding site on TAP molecules have recently been described (20).

It is commonly accepted that peptide translocation over the ER membrane is TAP-dependent and requires hydrolysis of ATP (7–15). However, different results have been obtained in other experimental models (7–15, 30–33). Our results, based on studies with a cross-linkable H-2Kb-binding peptide, support the notion that TAP-dependent translocation of peptide is dependent on ATP hydrolysis. Taken together, these results demonstrate that in the absence of ATP, no peptide translocation is observed, but peptide can still be cross-linked to TAP. This conclusion is well in agreement with that of Androlewicz et al. (16, 17). In the presence of ATP, peptide can be translocated over the ER membrane in a TAP1/2-dependent manner. Thus, ATP hydrolysis is required for the translocation process itself, rather than for peptide binding to the translocator.

This study has demonstrated a stepwise interaction of peptide with TAP and class I by the assessment of the on-rates of peptide binding to TAP and class I in the same intact microsomes. While peptide binding to TAP occurred momentarily, an increased binding of peptide to class I was observed with time. Overall, this process was rapid, and peptide binding to class I was readily observed within minutes. Taken together, the available data support a scenario in which peptide binds to TAP, a process that is independent of ATP. This is followed by an ATP-dependent translocation across the ER membrane, a process with a certain degree of peptide specificity. This process is followed by an ATP-independent, but highly peptide-specific binding to MHC class I.

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