Tapasin Interacts with the Membrane-spanning Domains of Both TAP Subunits and Enhances the Structural Stability of TAP1-TAP2 Complexes*

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The transporter associated with antigen processing (TAP) proteins are involved in transport of peptides from the cytosol into the endoplasmic reticulum. Two subunits, TAP1 and TAP2, are necessary and sufficient for peptide binding and peptide translocation across the endoplasmic reticulum membrane. TAP1 and TAP2 contain an N-terminal hydrophobic membrane-spanning region and a C-terminal nucleotide binding domain. Tapasin is an endoplasmic reticulum resident protein that has been found associated with the TAP subunits and shown to increase expression levels of TAP. Here we investigated TAP-tapasin interactions and their effects on TAP function in insect cells. We show tapasin binding to both TAP1 and TAP2 and to the corresponding nucleotide binding domain-exchanged chimeras as well as to a truncated TAP1-TAP2 complex containing just the membrane-spanning regions of TAP1 and TAP2. However, tapasin interactions with either the truncated TAP construct containing just the nucleotide binding domain are not observed. Tapasin is not required for high affinity peptide binding to TAP1-TAP2 complexes, and in fact, the presence of tapasin slightly reduces the affinity of TAP complexes for peptides. However, at near physiological temperatures, both tapasin and nucleotides stabilize the peptide binding site of TAP1-TAP2 complexes against inactivation, and enhanced thermostability of both TAP subunits is observed in the presence of tapasin. The enhanced structural stability of TAP1-TAP2 complexes in the presence of tapasin might explain the observations that tapasin increases TAP protein expression levels in mammalian cells.

Major histocompatibility complex (MHC) class I molecules are a complex of a heavy chain, a light chain (β2-microglobulin), and a short peptide. Peptides bound within a groove of the MHC class I heavy chain are presented to CD8 T lymphocytes (1) for immune surveillance against intracellular pathogens as well as some exogenous pathogens. Many class I-associated peptides are derived from proteasome-mediated proteolysis of cytosolic proteins. These peptides are translocated across the endoplasmic reticulum (ER) membrane by the transporter associated with antigen processing (TAP). Subsequently, the peptides become bound to MHC class I heavy chain-β2-microglobulin complexes (1). The transport of peptides is an essential step, and hence, TAP is a primary component of the antigen presentation pathway (2, 3). Assembly of peptides with newly synthesized class I molecules is assisted by many proteins. These include calreticulin (a chaperone), ERp57 (a thiol-dependent oxidoreductase), and tapasin. These components together with TAP and the MHC class I subunits, constitute a large complex called the “MHC class I peptide-loading complex” (4). Upon binding to peptides, class I molecules are released from the assembly complex and transit to the cell surface (1).

TAP comprises two subunits, TAP1 and TAP2, both of which are required for the transport function. Each subunit has one C-terminal nucleotide binding domain (NBD) and one N-terminal transmembrane region with several membrane-spanning domains (MSRs) (for review, see Refs. 2 and 3). TAP catalyzes transport of peptides across the ER membrane in an ATP-dependent manner. A broad range of peptides (9–15 amino acids in length) is translocated by TAP (2, 3). The functions of TAP can be broken down as (i) sequestration of antigenic peptides on the cytosolic face of the ER membrane and (ii) binding and hydrolysis of ATP, which powers translocation of peptides across the ER membrane. At low temperatures, peptide binding to TAP does not require exogenous ATP (5–7), but nucleotides are essential for maintaining TAP complex stability at physiological temperatures (8). ATP is essential for peptide translocation by TAP, and non-hydrolyzable ATP analogs do not support peptide translocation (9).

Tapasin is an ER-resident 48-kDa transmembrane glycoprotein found associated with TAP1-TAP2 complexes (10–12). Tapasin binds to TAP through its transmembrane and/or cytosolic domains (13, 14). The presence of tapasin in mammalian cells increases expression levels of the TAP proteins (14), thus quantitatively increasing the amount of peptide translocated by TAP complexes (13). Tapasin also interacts with MHC class I molecules via residues in its N terminus (13). Optimal peptide loading of many MHC class I molecules requires the presence of

T2M, residues 1–432 of human TAP2 with an N-terminal AU5 epitope tag; T1Ctr, residues 472–748 of human TAP1 with a C-terminal hexahistidine tag; T2Ctr, residues 435–686 of human TAP2 with a C-terminal Myc epitope tag; TAP1-eGFP, a TAP1 and enhanced green fluorescent protein fusion construct.

Received for publication, July 16, 2002, and in revised form, August 18, 2002
Published, JBC Papers in Press, September 3, 2002, DOI 10.1074/jbc.M207128200
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Printed in U.S.A.

* This work was supported by National Institutes of Health (NIH) Grant AI44115–03 (to M. R.) and by an NIH Rheumatic Disease Core Center Grant AR48310-02 (to the University of Michigan). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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The abbreviations used are: MHC, major histocompatibility complex; ER, endoplasmic reticulum; TAP1, transporter associated with antigen-processing subunit 1; TAP2, transporter associated with antigen-processing subunit 2; NBD, nucleotide binding domain; MSR, membrane-spanning region; TIMT2C, a chimeric protein containing residues 1–541 of human TAP1 and 507–866 of human TAP2, T2MT1C, a chimeric protein containing residues 1–506 of human TAP2, residues 542–748 of human TAP1, and a C-terminal hexahistidine tag; T1M, residues 1–471 of human TAP1 with an N-terminal AU1 epitope tag;
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Tampe lab (16). Human TAP1 and TAP2 cDNAs for the other TAP encoding wild type TAP1 and TAP2 constructs were obtained from the Tapasin (Bgp48C (13)), a control anti-β2-microglobulin antibody (Roche Diagnostics), or a control antibody directed against glycoprotein H of herpes simplex virus (52-S, ATCC). Wild type TAP1 (TAP1) and wild type TAP2 (TAP2) were recognized by the anti-TAP1 and anti-TAP2 antisera respectively, which were generated against C-terminal (NBD) epitopes of TAP1 and TAP2. Tapasin is recognized by an anti-tapasin antibody (13) raised against residues in the cytosolic domain of tapasin. T1MT2C is recognized by anti-TAP1 antiserum and also by an anti-His antibody (17) due to the presence of a C-terminal histidine tag.

Preparations of Microsomes, Immunoblotting Analyses, Peptide Translocation Assays, and Fluorescent Peptide-based Binding Assays—Cells were infected with appropriate baculoviruses (TAP1, TAP2 or TAP1, TAP2, tapasin) at multiplicity of infection values of 1–90 depending on the baculovirus used. Baculoviruses encoding wild type TAP1 and TAP2 constructs were obtained from the Tampe lab (16). Human TAP1 and TAP2 cDNAs for the other TAP constructs described here were obtained from Dr. John Trowsdale. Baculovirus constructs encoding the TAP chimeras (T1MT2C and T2MT1C) were previously described and contain residues 1–541 of TAP1 and 507–896 of TAP2 (T1MT2C) and residues 1–506 of TAP2 and 543–896 of TAP2 (T2MT1C), respectively. To obtain the desired constructs, restriction enzymes specific to the TAP membrane-spanning domains were made as follows. T1M encodes residues 1–471 of TAP1, tagged with an AU1 epitope tag on the N terminus. T2M encodes residues 1–432 of TAP2, tagged with an AU5 epitope tag on the N terminus. PCR was used to engineer both constructs. The 5’ primers contained a BamHI site and a sequence encoding the AU1 site of pAcUW51 (Stratagene), sequenced, and then excised and ligated into the BamHI and BglII sites of the baculovirus transfer vector pAcUW51 (Pharminen), respectively. This vector was co-transfected with BaculoGold DNA (Pharminen) into insect cells as described in the Pharringen Baculovirus Expression Manual. Pure virus was isolated using plaque assays and further amplified by re-infection. TAP NBD constructs T1Ct (resi- dues 472–748 of TAP1) and T2Ct (residues 433–836 of TAP2) were made as described previously (18) but in a single virus. T1Ct has a C-terminal hexahistidine tag, whereas the T2Ct has a C-terminal Myc epitope tag. The T1Ct-encoding construct that had been ligated into the BamHI site of pAcUW51 was excised and re-ligated into the BamHI site of pAcUW51, which had the T2Ct-encoding construct cloned into the BgII site. Baculoviruses encoding both NBD constructs in a single virus were generated using this vector.

The constructs also contained an enhanced green fluorescent protein-tagged version of TAP1 (TAP1-eGFP). The TAP1-eGFP fusion was constructed by bridge PCR. The first PCR amplified the TAP1 portion of the fusion construct using a 5’ primer with a BamHI site followed by a sequence complementary to the 5’ end of TAP1 and a 3’ primer with the last 15 nucleotides of the TAP1 sequence and the first 15 nucleotides of the eGFP sequence. The eGFP template was obtained from the pEGFP plasmid (Clontech). The second PCR used a 5’ primer that was complementary to the 3’ primer used for TAP1 amplification and a 3’ primer complementary to the 3’ end of the eGFP sequence followed by a BamHI site. Both these PCR products were gel-extracted and used as templates for a third PCR, which used the 5’ primer of the TAP1 PCR and the 3’ primer of the eGFP PCR. This bridge PCR product was gel-extracted, ligated into pCRScript (Stratagene), and sequenced. The TAP1-eGFP fusion was then ligated into pAcUW51 (Pharminen), which was used to generate virus.

Human tapasin cDNA was obtained from Dr. Ping Wang (10). PCR was used to introduce BamHI sites on the 5’ and 3’ ends. The modified cDNA was then inserted into pCRScript, sequenced, and cloned into the BamHI site of the pAcUW51 vector. Recombinant baculoviruses were generated as described above.

Metabolic Labeling and Co-immunoprecipitation Analyses—Cells were infected with multiple baculoviruses at multiplicity of infection levels between 1 and 80, established to optimize expression levels of TAP subunits in the presence or absence of tapasin, the multiplicity of infection values were typically lower for the TAP viruses when co-infected with the tapasin virus compared with that used in the TAP virus infections alone. Protocols for microsome preparations were similar to those described by Meyer et al. (16). Tapasin and TAP expression in the microsomes was verified by immunoblotting analyses of the microsome preparations (6). For this analysis, membranes were incubated in 15 ml of antibody buffer containing 50 μl of 148.3 hybridoma supernatant (16), 10 μl of 433.5 ascites fluid (20), and 7 μl of anti-tapasin antiserum (13). Ligated peptide-based translocation experiments with the model peptide RRRK/(I)Y/NASTKEL were carried out as described in the methods section (6). The peptide binding assays with fluorescent peptides first described by Neumann and Tampe (21) were carried out using procedures established in our laboratory with the model fluorescent peptide RRYQKC(6)TVC(6)TEL. (6) Peptide Binding Assay by Inhibition Analyses—Different concentrations of the inhibitor peptides (5 nm to 500 μM) were mixed with a known concentration (40 nm) of RRYQKC(6)TVC(6)TEL, and the fluorescence emission signal was allowed to stabilize. 20 μl of microsomes expressing either TAPI/TAP2 or TAP1/TAP2-tapasin were added, and the decrease in fluorescence signal was monitored until the signal stabilized. The magnitude of quenching was calculated as described (6). Fluorescence quenching was also monitored in the absence of added inhibitor peptide. The rate of quenching was determined from the slope of the line fitting the logarithm of the inhibitor peptide concentration. EC50 values were obtained by fitting the curve to a one-site competition equation using GraphPad Prism software.

Thermostability of the TAP Peptide Binding Site—Microsomes expressing TAPI/TAP2 or TAP1/TAP2-tapasin were incubated for 1 h at 34 or 4 °C after which both sets of microsomes were incubated for 15 min on ice. Both sets of microsomes were tested for their ability to quench the fluorescence of 40 nm RRYQKC(6)TVC(6)TEL by estimating the quenching amplitudes. Similar sets of analyses were carried out in the presence of added exogenous nucleotide, or in the presence of 1 mM ATP, or 1 mM ADP, or 0.03 units/ml apyrase. Data were analyzed in two different ways. For each set of microsomes expressing the TAP2 or TAP1, the ratios of the quenching amplitudes in the presence of ATP relative to those obtained in the presence of no nucleotide, ADP, and apyrase were calculated. Second, for each nucleotide condition, the ratio of the quenching amplitude obtained at 34 °C was calculated relative to that obtained at 4 °C.
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**RESULTS**

**Interactions of Tapasin with TAP Subunits, Chimeras, and Truncated TAP Constructs**—Metabolic labeling experiments were carried out to assess complex formation between TAP subunits or chimeras and tapasin. We infected S21 cells with baculoviruses encoding TAP subunits (TAP1, TAP2) or chimeras T1MT2C (which encodes the MSR of TAP1 and NBD of TAP2) and T2MT1C (which encodes the MSR of TAP2 and NBD of TAP1) in combination with tapasin. To establish complex formation between TAP subunits and tapasin, digitonin lysates of metabolically labeled cells were immunoprecipitated with antibodies directed against the TAP subunit, tapasin, or an irrelevant antibody control. Cells were lysed in 1% digitonin because interactions between TAP and tapasin are weak and are unstable in other detergents (12). Proteins were immunoprecipitated from lysates using appropriate antibodies and separated by SDS-PAGE, and associating proteins were visualized. TAP-tapasin interactions could be best visualized by immunoprecipitations with an antiserum directed against residues in the cytoplasmic domain of tapasin (13) (Fig. 1, A–D, lane 3). In general, the anti-TAP antisera-based immunoprecipitations were more difficult to assess due to the presence of nonspecific bands that co-migrated in the vicinity of tapasin (Fig. 1, A–C, lane 5; highlighted with asterisks).

We observed that TAP1 co-immunoprecipitated with tapasin in analyses with the anti-tapasin antibody (Fig. 1A, lane 3 (co-infection) compared with lane 2 (single infection with tapasin-encoding virus)). A nonspecific band (labeled as such) was also observed in all the immunoprecipitations with the anti-tapasin antibody. T1MT2C interaction with tapasin could also be visualized by the anti-tapasin immunoprecipitations (Fig. 1B; lane 3 (co-infection) compared with lane 2 (single infection with tapasin-encoding virus)), although the presence of a band corresponding to T1MT2C in the control lanes 1 and 6 suggests that there may be a low level of nonspecific precipitation of T1MT2C. Complex formation between TAP2 and tapasin was apparent in the analysis shown in Fig. 1C by immunoprecipitations with both anti-tapasin (Fig. 1C, lane 3 (co-infection) compared with lane 2 (single infection with tapasin-encoding virus)) and anti-TAP2 antisera (Fig. 1C, lane 4 (co-infection) compared with lane 5 (single infection with TAP2)). Likewise, complex formation between T2MT1C and tapasin was apparent in immunoprecipitations with both anti-tapasin (Fig. 1D, lane 3 (co-infection) compared with lane 2 (single infection with tapasin-encoding virus)) and anti-his antibodies (Fig. 1D, lane 4 (co-infection) compared with lane 5 (single infection with T2MT1C)).

To study the association of the MSRs or NBDs of the TAP subunits with tapasin, constructs encoding TAP membrane-spanning domains (T1M and T2M, respectively, of TAP1 and TAP2) alone or NBDs alone (T1Ctr and T2Ctr, respectively, of TAP1 and TAP2) were generated. The TAP constructs have been previously described (18). The construct with the TAP1 transmembrane regions (T1M) has an AU1 epitope at its N terminus and can be recognized by an anti-AU1 antibody. The TAP2 counterpart (T2M) has an N-terminal AU5 tag that can be recognized by an anti-AU5 antibody. Co-immunoprecipitation analyses with anti-AU1 and anti-AU5 antibodies of cells infected with a baculovirus encoding both T1M and T2M revealed the presence of 2 proteins of ~40 kDa (Fig. 2A, lanes 1 and 2). Based upon the expected size, we predicted that the larger protein was T1M. Consistent with this expectation, more of the higher molecular weight protein was visualized in the AU1 immunoprecipitations compared with the AU5 immunoprecipitations. Co-immunoprecipitation analyses suggested that both T1M and T2M associated and that T2M was expressed in excess relative to T1M (Fig. 2A, lanes 1 and 2). Thus, the membrane-spanning domains of TAP1 and TAP2 associate even in the absence of the NBD, consistent with previous observations implicating membrane-spanning residues of TAP1 and TAP2 in the TAPI-TAP2 interaction interface (22). By contrast, the TAP NBDs do not form stable complexes under the conditions of these assays as previously demonstrated (Fig. 2A, lanes 2 and 3) (18).

To investigate interactions of TAP MSRs and NBDs with tapasin, cells were co-infected with baculoviruses encoding TAP along with viruses encoding the TAP MSR (T1M/T2M) or NBD (T1Ctr/T2Ctr). Metabolically labeled cells were immunoprecipitated with anti-AU1, anti-AU5, anti-tapasin, or irrelevant antibodies for MSR interactions with tapasin or with anti-His, anti-Myc, anti-tapasin, or irrelevant antibodies for NBD interactions with tapasin. For the former set of experiments, the anti-tapasin immunoprecipitation revealed tapasin and T2M as well as a faint signal for T1M, indicative of complex formation (Fig. 2B, lane 3). The low signal for T1M compared with T2M is likely due to the reduced expression of T1M relative to T2M. The ratio of T1M/T2M intensities in the anti
...tions in the absence of tapasin. The data are representative of at least three independent analyses.

![Image](https://via.placeholder.com/150)

**Fig. 2. Interactions of tapasin with truncated TAP constructs.** Analyses were carried out as described in Fig. 1 but assessed the following sets of interactions. A, TAP1-TAP2 MSR and NBD associations. Cells were infected with viruses encoding T1M/T2M (MSRs of TAP1 and TAP2) (lanes 1 and 2) or T1Ctr/T2Ctr (NBDs of TAP1 and TAP2) (lanes 3 and 4). Triton lysates were immunoprecipitated (IP) with anti-AU1 (lane 1), anti-AU5 (lane 2), anti-His (lane 3), and anti-Myc (lane 4). Association of MSRs of TAP1 and TAP2 was observable (lanes 1 and 2), whereas no such association was observable with the NBDs of TAP subunits (lanes 3 and 4). B, interaction of MSRs of TAP with tapasin. Digitonin lysates from metabolically labeled cells infected with viruses encoding T1M/T2M and tapasin (lanes 1–4) were immunoprecipitated with anti-AU1 (lane 1), anti-AU5 (lane 2), anti-tapasin antiserum (lane 3), or a control antibody (lane 4). T1M/T2M-tapasin interactions were visualized in the anti-tapasin-based immunoprecipitations and to a lower extent in the anti-AU1 and anti-AU5-based immunoprecipitations. C, interaction of NBDs of TAP with tapasin. Digitonin lysates from metabolically labeled cells infected with viruses encoding T1Ctr/T2Ctr and tapasin (lanes 1–4) were immunoprecipitated with anti-His (lane 1), anti-Myc (lane 2), anti-tapasin antiserum (lane 3), or a control antibody (lane 4). T1Ctr, T2Ctr, and tapasin do not associate as seen from anti-TAP and anti-tapasin immunoprecipitations (lanes 1–3).

Tapasin immunoprecipitation was roughly in proportion to the levels at which the proteins were expressed. Tapasin-specific signals were also visualized in the anti-AU1 and anti-AU5-based immunoprecipitations. By contrast to the detectable interaction between T1M/T2M and tapasin, binding was not observable between either T1Ctr and tapasin and/or T2Ctr and tapasin in immunoprecipitations with anti-His, anti-Myc, or anti-tapasin antibodies (Fig. 2C, lanes 1–3, respectively). Our observations that T1Ctr and T2Ctr cannot be co-immunoprecipitated with tapasin do not preclude the possibility that TAP NBD residues of intact TAP1-TAP2 complexes participate in TAP-tapasin interactions; rather, our data suggest that residues contained in TAP1 and TAP2 NBD are not sufficient to mediate stable binding to tapasin under the conditions of the analyses (Fig. 2C). Because T2Ctr in particular is quite unstable in solution, they cannot exclude the formal possibility that folding constraints in the NBD account for the lack of observable interactions with tapasin.

The Peptide Binding Affinity of TAP1-TAP2 Complexes Is Slightly Reduced in the Presence of Tapasin—Tapasin-deficient 721.220 cells, when transfected with tapasin, show increased steady state levels of the TAP1 protein (14). This increase in TAP expression level quantitatively enhanced the amount of peptide translocated without increasing the intrinsic rate of peptide translocation (13). To verify this result in an insect cell-reconstituted system, we generated microsomes expressing TAP1-TAP2 alone or TAP1-TAP2-tapasin such that the TAP protein levels were approximately the same in the presence or absence of tapasin. Conditions for obtaining maximal tapasin expression were first optimized, and multiplicity of infection values for TAP virus infections were subsequently optimized. An exact correspondence of TAP expression levels in the presence and absence of tapasin was difficult to achieve, although generally, the expression levels of at least one of the TAP components was matched (Fig. 3A). Under these conditions, we observed that the presence of tapasin did not significantly affect the peptide translocation efficiency, as assessed by the established peptide translocation assay (Fig. 3B) (9, 16, 20). Using a similar translocation assay, we previously described mutant TAP complexes as well as chimeric TAP complexes that were reduced in translocation efficiency when compared with wild type (6, 17), presumably due to changes in their ATPase activities. Thus, the assay itself is sensitive enough to detect changes in translocation rates, had tapasin affected changes in the rate.

We also investigated the effects of tapasin on peptide binding...
to TAP complexes. In the absence of tapasin, TAP1-TAP2 complexes expressed in insect cells bind peptides with high affinity, an observation that suggests tapasin is not essential for high affinity peptide binding by TAP1-TAP2 complexes (6, 20, 21). Other investigations of TAP-specific peptide binding to 721.220 (tapasin-deficient) membranes compared with 721.221 (tapasin-expressing) membranes or to 721.220 membranes that were transfected with tapasin revealed a higher level of peptide cross-linking to 721.221 microsomes compared with 721.220 microsomes (14, 23), which is likely to reflect the higher expression levels of the TAP proteins in the presence of tapasin. Analysis of the ability of different concentrations of an ovalbumin-derived peptide, SIYNPEKL, to inhibit binding of a cross-linker-modified and 125I-labeled variant of the same peptide indicated that significantly lower concentrations of unlabeled peptide were required to completely inhibit photocross-linking in 721.220 microsomes compared with 721.221 microsomes (23). These results were previously interpreted as indicating a lower peptide binding affinity in the absence of tapasin (23). However, the results suggested to us that the presence of tapasin might in fact decrease rather than increase the affinities of peptide-TAP complexes. We therefore undertook a careful quantitative analysis of peptide binding affinities of TAP1-TAP2 complexes compared with TAP1-TAP2-tapasin complexes.

To investigate the relative peptide binding affinities of TAP1-TAP2 complexes compared with TAP1-TAP2-tapasin complexes, we again generated two sets of microsomes expressing TAP1-TAP2 alone or TAP1-TAP2-tapasin with protein expression levels similar to that shown in Fig. 3A. We used a fluorescence quenching-based binding assay (21) in the absence of added exogenous nucleotides to compare peptide binding to TAP1-TAP2 complexes in the presence or absence of tapasin. For this assay, the model peptide RRYQKCFITCTEL-quenching signal (RRYQKC_{FITC}-TEL) was used (6). Microsome preparations containing TAP1-TAP2 or TAP1-TAP2-tapasin were added to increasing concentrations of RRYQKC_{FITC}-TEL, and the fluorescence emission signal was monitored as a function of time. Unlabeled peptide was added to the reaction mixtures, and the recovery of fluorescence was also monitored over time as the bound fluorescent peptide dissociated from TAP. The apparent binding constants (K_d) for peptide binding by TAP1-TAP2 and TAP1-TAP2-tapasin were obtained by plotting steady state fluorescence quenching signals as a function of peptide concentration (Fig. 4A).

Analyses of the relative binding affinities of peptides of TAP1-TAP2 complexes in the presence or absence of tapasin indicated that tapasin slightly reduced the peptide binding affinity of the TAP1-TAP2 complex. In parallel sets of analyses, we observed a 2-fold reduction in the affinity calculated for TAP1-TAP2-tapasin complexes (K_d = 50.4 ± 5.05 nM; average of three experiments) compared with TAP1-TAP2 complexes (K_d = 24.3 ± 24.4 nM, average of two experiments in the present analyses; we previously reported room temperature peptide binding constants of 14.4 ± 11 nM for TAP1-TAP2 complexes in the absence of exogenous nucleotides (17) and 19.4 ± 4.8 nM for TAP1-TAP2 complexes in the presence of apparyse (6), which were derived from sets of analyses independent from the data described here). Consistent with the observed affinity reduction, the calculated dissociation rates were generally higher for TAP1-TAP2-tapasin (k_d = 0.015 ± 0.002 s^{-1}) complexes compared with TAP1-TAP2 (k_d = 0.009 ± 0.002 s^{-1}) complexes. However, because the standard deviations on the previously reported k_d values for TAP1-TAP2 complexes were relatively high (6, 17), the effect of tapasin on the dissociation rate was more difficult to assess than its effect on the derived K_d value.

The observation that RRYQKC_{FITC}-TEL bound TAP with ~2-fold reduced affinity in the presence of tapasin raised the question of whether the presence of tapasin enhanced or reduced the binding affinities of other peptides, in particular with lower affinities for TAP complexes. To address this question, a variety of MHC class I binding peptides with the sequences VMAPC_{FITC}LLL (HLA-E-specific), VEITYPKPTW (HLA-B44-specific), and LLDVPTAAV (HLA-A2-specific) were cyanine-substituted at position 4 or 5 of their sequences and fluorescently labeled, and their binding to TAP was assessed by the quenching assay. The labeled sequences were VMAPC_{FITC}LLL, VEIC_{FITC}PYKPTW, and LLDC_{FITC}PTAAV. Fluorescence quenching was not observed with any of these peptides upon addition of TAP-containing microsomes, indicating that these peptides either did not bind to TAP complexes with appreciable affinity, or that the environment of the fluorophores in the bound peptides were not significantly different from that in the free peptide. To distinguish these possibilities, the ability of the corresponding unlabeled peptides to interact with TAP1-TAP2 complexes was assessed by investigating whether unlabeled peptides could inhibit binding of RRYQKC_{FITC}-TEL to TAP1-TAP2 complexes. Of all the peptides tested, only VMAPC_{FITC}LLL appeared to bind to TAP complexes with an affinity higher than 50 μM. Very high concentrations of the other peptides were required to observe a 50% reduction in RRYQKC_{FITC}-TEL-quenching signal (≥50 μM for VEICPYKPTW and ≤100 μM for LLDCPTAAV).

Because the fluorescence of VMAPC_{FITC}LLL was not quenched by TAP binding, we used an inhibition-based assay to
study the effect of tapasin on the binding of unlabeled VMA\textsubscript{PTCLLLL} to TAP complexes. Representative inhibition plots, one each for TAPI-TAP2 and TAPI-TAP2-tapasin complexes, are shown in Fig. 4B. \textit{EC}_{50} (concentration of VMA\textsubscript{PTCLLLL} required to bring about 50\% inhibition of the RRYQKC\textsubscript{PTTL}TEL-quenching signals) values were obtained by fitting the data to a one-site competition equation using Graph Pad Prism software. The \textit{EC}_{50} values derived for TAPI-TAP2-tapasin were again about 2-fold higher than that of TAPI-TAP2 when present alone. The mean \textit{EC}_{50} values from multiple independent experiments are estimated to be 1504 ± 185 nM for TAPI-TAP2 complexed with 3009 ± 304 nM for TAPI-TAP2-tapasin complexes. \textit{K}_D calculations using the formula \textit{K}_D = \textit{EC}_{50}/(1+\textit{[RRYQKC\textsubscript{PTTL}TEL]}/[\textit{D}_{2}\textit{RRYQKC\textsubscript{PTTL}TEL}]) (24) indicated that the affinity for VMA\textsubscript{PTCLLLL} binding to TAPI-TAP2 (average \textit{K}_D = 915 nM) was about 4-fold higher than for VMA\textsubscript{PTCLLLL} binding to TAPI-TAP2-tapasin (average \textit{K}_D = 3700 nM). Taken together, these observations emphasize that tapasin increases the affinities of TAP complexes for peptides.

Effect of Tapasin on TAP Complex Stability in the Absence and Presence of Nucleotides—Tapasin increases TAP expression levels in mammalian cells that could arise from its effect on TAP complex stability at physiological temperatures (14, 25). We wanted to use an \textit{in vitro} assay to investigate the effect of tapasin on TAP complex stability at near physiological temperatures. TAPI-TAP2 complex formation is required to create a high affinity peptide binding site, and the structural integrity of the peptide binding site can thus be used as a measure of the structural integrity of the TAP complex. It has been shown that human TAP proteins undergo structural changes when incubated to 37 °C that lead to a complete loss of peptide binding function. The presence of nucleotide diphosphates or triphosphates was found to stabilize the TAP complex against inactivation of peptide binding function at 37 °C (8). We studied the effect of tapasin in stabilizing the TAP heterodimer against heat-induced inactivation and compared the effects of tapasin and nucleotides in rendering stability to TAPI-TAP2 complexes (Fig. 5).

Microsomes expressing TAPI-TAP2 or TAPI-TAP2-tapasin were incubated at 34 °C for 1 h followed by incubation for 15 min on ice. These microsomes as well as untreated microsomes (4 °C treated microsomes) were tested for their ability to bind peptides using the fluorescence quenching-based assay. To illustrate the effects of nucleotides on maintaining the stability of TAPI-TAP2 and TAPI-TAP2-tapasin complexes, the quenching signals obtained in the presence of no added nucleotide, ADP, or apyrase were normalized relative to the signals that were obtained in the presence of ATP (Fig. 5A). Both ATP and ADP stabilized TAP complexes against inactivation of their peptide binding functions at 34 °C as previously described (7, 8), with ATP being slightly more optimal under some conditions. Setting the quenching signals obtained in the presence of ATP at 1, the relative signals obtained in the presence of ADP, apyrase, or no exogenous nucleotides were compared. For TAPI-TAP2 complexes stored at 4 °C, the relative signals were 0.72 ± 0.25 if no exogenous nucleotide was added, 0.94 ± 0.19 in the presence of ADP, and 0.54 ± 0.16 when microsomes were treated with apyrase. After the 34 °C incubation, however, the signals for TAPI-TAP2 complexes were 0.04 ± 0.04 if no exogenous nucleotide was added, 0.72 ± 0.18 in the presence of ADP, and 0.16 ± 0.06 when microsomes were treated with apyrase. For TAPI-TAP2-tapasin complexes stored at 4 °C before the peptide binding assays, the relative signals were 1.03 ± 0.21 if no exogenous nucleotide was added, 1.16 ± 0.29 in the presence of ADP, and 0.56 ± 0.10 when treated with apyrase. For TAPI-TAP2 complexes incubated at 34 °C before the peptide binding assays, the relative signals were 0.54 ± 0.07 if no exogenous nucleotide was added, 0.82 ± 0.22 in the presence of ADP, and 0.28 ± 0.10 when microsomes were treated with apyrase.

To examine the stabilizing effect of tapasin, the ratios of quenching signals obtained after 34 °C incubations were expressed as a ratio relative to the signal obtained with untreated (4 °C incubated) microsomes for each of the conditions (no exogenous nucleotide, ATP, ADP, or apyrase) (Fig. 5B). In the absence of added nucleotide, microsomes that express TAPI-TAP2 alone lose their ability to bind peptides after incubation at 34 °C. Thus the average ratio (34/4) was 0.06 ± 0.04 over many independent experiments. However in the presence of tapasin, TAP complexes retained their ability to bind peptides after incubation at 34 °C. The average ratio (34/4) of...
quenching amplitudes for the TAP1-eGFP-TAP2 complexes were in the range of 0.29 ± 0.09. In the presence of ATP, the observed ratio for the TAP1-TAP2 complex was 0.36 ± 0.11, whereas it was 0.62 ± 0.09 for TAP1-TAP2-tapasin complexes. In the presence of ADP, the ratio was 0.38 ± 0.07 for TAP1-TAP2 compared with 0.56 ± 0.1 for TAP1-TAP2-tapasin. Under conditions of depleted nucleotides (apprase treatment), the ratio was 0.09 ± 0.05 for TAP1-TAP2, whereas the presence of tapasin increased this ratio by nearly 2-fold (0.21 ± 0.06). The data shown in Fig. 5 represent average ratios from many independent experiments, each done in duplicate. Within individual experiments, consistently higher 34/4 ratios were obtained (under all nucleotide conditions) with microsomes expressing TAP1-TAP2-tapasin compared with microsomes expressing TAP1-TAP2 alone, but ratio differences were most pronounced under the conditions in which neither exogenous nucleotide nor apprase was present. Under these conditions, low levels of endogenous nucleotides might be present that synergize with tapasin to enhance TAP complex stability.

We investigated the thermolability of the peptide binding site (Fig. 5) to determine whether it was accompanied by TAP1-TAP2 dissociation and whether tapasin was still associated with the TAP proteins upon incubation of the proteins at 34 °C. We used metabolic labeling and co-immunoprecipitation analyses to address these questions. TAP1 and TAP2 are not well resolved by SDS-PAGE; however, the TAP1 fusion protein, TAP1-eGFP, was well separated from TAP2. Insect cells were infected with viruses encoding the TAP1-eGFP-TAP2 combination or the TAP1-eGFP-TAP2-tapasin combination and metabolically labeled 60-h post-infection. 72 h post-infection, the labeled cells were centrifuged and incubated in peptide binding assay buffer (lacking nucleotides and Mg2+) for 1 h at 4 or 34 °C. At these late time points post-infection, most of the cells are permeable, and thus, the TAP proteins were exposed to the “no-added nucleotide” conditions of Fig. 5. Cells were lysed, and TAP and tapasin were immunoprecipitated with anti-GFP, anti-TAP2 antisera, and anti-tapasin. Proteins were separated by SDS-PAGE, and radiolabeled bands were visualized by phosphorimaging analyses (Fig. 6). TAP1 and TAP2 could be co-immunoprecipitated after a 34 °C incubation (Fig. 6A, middle panel, lane 2) even in the absence of tapasin, although the expression of both proteins were significantly reduced. Under the infection conditions used for the analysis, TAP2 appears to be expressed in greater than 3-fold excess relative to TAP1 based upon intensities of the TAP1-eGFP and TAP2 bands; thus, most of the TAP1 would be expected to be in complex with TAP2. Nevertheless, TAP1 signals were reduced after the 34 °C incubations (Fig. 6A, top and middle panels; lane 1 compared with lane 2), indicating that TAP proteins in TAP1-TAP2 complexes were susceptible to inactivation/degradation upon 34 °C incubation. However, the 34 °C incubation resulted in TAP1-TAP2 intensity ratios approaching stoichiometric levels (Fig. 6A, middle panel, lane 1 compared with lane 3), indicating that TAP1-TAP2 complexes were less susceptible to thermal inactivation compared with the free subunits. It is thus possible that the thermolability of the peptide binding site is coincident with a more rapid dissociation of TAP1-TAP2 complexes at higher temperatures.

Tapasin could be co-immunoprecipitated with both subunits after the 34 °C incubation (Fig. 6A, bottom panel; second lane). Thus, consistent with enhanced thermostability of the peptide binding site of TAP1-TAP2 complexes in the presence of tapasin, tapasin association with the TAP subunits was maintained upon 34 °C incubation. We quantified levels of thermostable TAP1 and TAP2 in the presence or absence of tapasin, as assessed by immunoprecipitations with anti-GFP and anti-TAP2, respectively. The analysis revealed a significant increase in the levels of thermostable TAP1 and TAP2 in the presence of tapasin; however, the effect on TAP1 was more pronounced than that on TAP2 (Fig. 6B, first and second bars). We also quantified levels of thermostable TAP1 and TAP2 in the anti-tapasin based immunoprecipitation (Fig. 6B, third and fourth bars). Strikingly, these analyses indicated that there was almost no change in TAP1 levels upon incubation at 34 °C and that there was only a 25% reduction in TAP2 levels (after normalizing for the small reduction in immunoprecipitable tapasin) (Fig. 6B, bars 3 and 4, respectively). Thus, the tapasin-associated populations of each TAP subunit appear to have significantly higher thermostability compared with the corresponding total populations. Because TAP1 is expected to be largely in complex with TAP2 in the analyses shown in Fig. 6, it is possible that the more pronounced effect of tapasin on TAP1 thermostability compared with TAP2 thermostability (Fig. 6B) is a consequence of a more stable interaction of tapasin with TAP1-TAP2 complexes compared with the free subunits. Tapasin interaction with the TAP1-TAP2 complex could reduce TAP complex dissociation and the consequent more rapid inactivation. However, tapasin also appears to be capable of stabilizing free TAP2 to some extent as 75% of tapasin-associated TAP2 is stable upon 34 °C incubation (Fig. 6B, last bar).

**DISCUSSION**

Earlier studies in which rat TAP1 and TAP2 were transfected into the TAP-deficient human T2 cell line showed that calreticulin, tapasin, and MHC class I molecules associated with TAP1 when expressed in the absence of TAP2 or with TAP2 expressed in the absence of TAP1 (although the efficiency of association was lower with the TAP1 transfectants compared with the TAP2 transfectants) (26, 27). Although these studies suggested that tapasin could interact with both TAP subunits, the presence of other co-precipitating proteins (calreticulin, MHC class I) made it difficult to previously unambiguously attribute the observed associations to the occurrence of tapasin interactions with both subunits of the TAP transporter. Indeed other experiments indicate the possibility of direct TAP-class I interaction (28). Because insect cells lack all the specialized components of the MHC class I antigen presentation pathway, we could define interactions between specific components of the TAP complex by infecting cells with baculoviruses encoding the appropriate constructs. Metabolic labeling and co-immunoprecipitation analyses revealed that both TAP1 and TAP2 independently co-precipitated with tapasin (Fig. 1). Likewise, the NBD-exchanged TAP chimeras T1MT2C and T2MT1C also interacted with tapasin (Fig. 1). Constructs encoding just the MSRAs of TAP1 and TAP2 associated with each other as well as with tapasin. Taken together, the interaction analyses suggest that tapasin interacts with the membrane-spanning domains of both TAP1 and TAP2.

What are possible functional consequences of tapasin interaction with the membrane-spanning regions of both TAP1 and TAP2? The primary TAP1-TAP2 interaction interface appears to be located within the membrane-spanning regions of TAP1 and TAP2 (Fig. 2). By interacting with both TAP1 and TAP2 (Fig. 1), tapasin appears to stabilize a functional TAP1-TAP2 complex (Fig. 5). In Drosophila SC-2 cells transfected with different components of the MHC class I antigen presentation pathway, the presence of murine tapasin increased the amount of murine TAP2 that co-precipitated with TAP1 (29). These observations taken together with our present results (Fig. 6B) suggest that tapasin stabilizes both TAP subunits but may interact more effectively with the TAP1-TAP2 complex compared with the isolated subunits. It will be of interest to establish whether tapasin has independent binding sites in its struc-
ture for both TAP1 and TAP2.

A putative function assigned to tapasin is that of editing the MHC class I peptide repertoire and promoting the binding of high affinity peptides that results in MHC class I complexes of higher stability in the presence of tapasin (for review, see Ref. 15). It was thus also of interest to ask whether tapasin might have an effect on altering the repertoire of peptides that could associate with TAP1-TAP2 complexes. If tapasin promoted the binding of low affinity peptides to TAP complexes, tapasin could enhance the range of peptides that are translocated and, thus, available for assembly with MHC class I molecules. We studied the binding of two different class I-specific peptides to TAP complexes, one of which was a high affinity peptide and the second, a moderate affinity peptide. We observed only 2–4-fold differences in the derived \( K_d \) values for peptide binding to TAP1-TAP2 complexes compared with TAP1-TAP2-tapasin complexes (Fig. 4). Although it is possible that other peptide sequences will be identified that are more profoundly influenced in their binding to TAP complexes by the presence of tapasin, our present analyses suggest that tapasin is unlikely to alter the repertoire of TAP-translocatable peptides.

The peptide binding site of TAP1-TAP2 complexes is thought to reside in cytoplasmic loops that connect the membrane-spanning domains of TAP1 and TAP2 (for review, see Ref. 3). Residues implicated in peptide binding are contained within the T1M/T2M constructs, which associate with tapasin. It is likely that tapasin interactions with the TAP subunits introduce small conformational alterations into the peptide binding site of TAP1-TAP2 complexes that result in the observed reduction in the peptide binding affinity in the presence of tapasin (Fig. 4). The peptide binding affinity measurements reported here correspond to the “resting state” of TAP1-TAP2 complexes. We cannot presently assess whether these tapasin-induced changes in peptide binding affinity by TAP1-TAP2 complexes in the resting state will translate to more profound differences in “transition state” conformations of TAP complexes during a transport cycle. A better understanding of different conformational states of TAP complexes will be required to address that question.

Our present studies with tapasin and previous studies with nucleotides (6) indicate that neither tapasin nor nucleotides per se are required for high affinity peptide binding to TAP complexes (Fig. 5). However, nucleotides are critical for maintaining TAP complex stability at physiological temperatures (Refs. 7 and 8 and Fig. 5). Using peptide binding assays to quantify structurally intact TAP complexes, we compared the effects of both tapasin and nucleotides in maintaining TAP complex stability (Fig. 5). TAP complexes containing tapasin were reproducibly more resistant to inactivation upon 34 °C incubation compared with TAP complexes that lacked tapasin (Fig. 5B). Nucleotides markedly enhanced the stability of both TAP1-TAP2 complexes as well as of TAP1-TAP2-tapasin complexes. The presence of tapasin conferred enhanced stability to TAP complexes in the presence of nucleotides, but the stabilizing effect of tapasin was most apparent at low nucleotide concentrations (no exogenous nucleotide) (Fig. 5). We suggest that the observed effect of tapasin on TAP1-TAP2 structural stability (Figs. 5 and 6) might account for the observations of enhanced TAP expression levels in the presence of tapasin in human and mouse cells (14, 25). In the insect cell system, the effects of tapasin on TAP protein expression levels were more difficult to assess under conditions of competing infections with multiple viruses. In 721.220 cells, the human tapasin-deficient cell line, TAP1 protein expression levels were increased by 2.5–3-fold upon transfection with tapasin (14). Consistent with the increase in TAP expression, there was an ~3-fold increase in peptide binding as well as translocation in tapasin-expressing cells compared with tapasin-deficient cells. Similar func-

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**Fig. 6. Thermostability of TAP1-TAP2 and TAP1-TAP2-tapasin interactions.** A, metabolically labeled cells infected with viruses encoding either TAP1-eGFP-TAP2 or TAP1-eGFP-TAP2-tapasin were incubated at 4 or 34 °C in peptide binding assay buffer as indicated. Cells were subsequently lysed, and proteins were immunoprecipitated (IP) with antibodies against anti-GFP (top panel), anti-TAP2 antiserum (middle panel), and anti-tapasin (bottom panel). Proteins were visualized by SDS-PAGE and phosphorimaging analysis. B, intensity ratios (34/4) of immunoprecipitated TAP1 (first and third bars) or TAP2 (second and fourth bars) were quantified in the anti-GFP (first bar), anti-TAP2 (second bar), or anti-tapasin (third and fourth bars)-based immunoprecipitations of cells expressing TAP1 and TAP2 alone or TAP1, TAP2, and tapasin as indicated. The data shown here are from a single immunoprecipitation analysis but are representative of two independent experiments.
tional effects of tapasin were observed in cells derived from tapasin-deficient mice, with the extent of the effect showing a cell-type dependence. Murine tapasin may have a much larger effect on murine TAP expression levels in some cell types compared with the relatively small (2.5–3-fold) effects of human tapasin on human TAP expression levels in the Epstein-Barr virus-transformed 721.220 cells (25, 30). In the absence of tapasin, the higher levels of inactive TAP complexes that we observe in the in vitro assays at 34 °C (Fig. 5) as well as the lower levels of thermostable TAP subunits (Fig. 6) might translate in vivo to a greater proportion of TAP complexes that are targeted for degradation. The extent of this effect might be cell type-specific and TAP species-specific and likely to be dependent on the inherent stability of particular TAP subunits.

In summary, our studies demonstrate the binding of tapasin to both TAP subunits, most likely to residues contained within the membrane-spanning regions of both TAP1 and TAP2. Complex formation with tapasin appears to induce conformational alterations in the peptide binding site of TAP1-TAP2 complexes. Nucleotide binding to the TAP NBD has a marked influence on TAP complex stability at physiological temperatures as previously described (8), an important consideration in interpreting the effects of TAP NBD mutants with reduced nucleotide binding affinities. Tapasin enhances the structural stability of the peptide binding site of TAP1-TAP2 complexes both in the presence and absence of nucleotides. Finally, tapasin interactions with the TAP subunits persist upon incubation of the complexes at near-physiological temperatures, and tapasin enhances the thermostability of both TAP subunits.

Acknowledgments—We thank Alero Fregene for generating the tapasin-encoding baculovirus and other technical assistance early in the project. We thank Dr. Ping Wang for the tapasin cDNA and Dr. Robert Trowsdale for the human TAP1 and TAP2 cDNAs. We thank Dr. Robert Tampé for the baculoviruses encoding TAP1 and TAP2 and the 148.3 antibody. Dr. Peter Cresswell for the anti-tapasin antiserum, and Dr. M. J. Andrelewicz for the anti-TAP1 and anti-TAP2 antisera. We are grateful to Dr. R. Neubig for use of the PTI fluorimeter. We thank the University of Michigan Biomedical Research Core facilities for peptide synthesis and purification, the University of Michigan hybridoma core for 43S.3 ascites fluids, the Cell Biology laboratories for use of computer resources, the National Cell Culture Center for tapasin amplification, and the University of Michigan Reproductive Sciences Program for peptide iodination.

REFERENCES
1. Pamer, E., and Cresswell, P. (1998) Annu. Rev. Immunol. 16, 323–358
2. Abele, R., and Tampe, R. (1999) Biochim. Biophys. Acta 1461, 405–419
3. Momburg, F., and Hammerling, G. J. (1998) Adv. Immunol. 68, 191–256
4. Cresswell, P., Bangia, N., Dick, T., and Diedrich, G. (1999) Immunol. Rev. 172, 21–28
5. Andrelewicz, M. J., and Cresswell, P. (1994) Immunity 1, 7–14
6. Lapinski, P. E., Neubig, R. R., and Raghavan, M. (2001) J. Biol. Chem. 276, 7526–7533
7. Saveanu, L., Daniel, S., and van Endert, P. M. (2001) J. Biol. Chem. 276, 22107–22113
8. van Endert, P. M. (1999) J. Biol. Chem. 274, 14632–14638
9. Neefjes, J. J., Momburg, F., and Hammerling, G. J. (1995) Science 261, 769–771
10. Li, S., Sjogren, H. O., Hellman, U., Pettersson, R. F., and Wang, P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8708–8713
11. Ortmann, B., Copeman, J., Lehner, P. J., Sadasivan, B., Herberg, J. A., Grandeaa, A. G., Riddell, S. R., Tampé, R., Spies, T., Trowsdale, J., and Cresswell, P. (1997) Science 277, 1306–1309
12. Sadasivan, B., Lehner, P. J., Ortmann, B., Spies, T., and Cresswell, P. (1996) Immunity 5, 103–114
13. Bangia, N., Lehner, P. J., Hughes, E. A., Surman, M., and Cresswell, P. (1999) Eur. J. Immunol. 29, 1858–1870
14. Lehner, P. J., Surman, M. J., and Cresswell, P. (1998) Immunity 8, 221–231
15. Grandeaa, A. G., III, and Van Kael, L. (2001) Trends Immunol. 22, 194–199
16. Meyer, T. H., van Endert, P. M., Uebel, S., Ehring, B., and Tampe, R. (1994) FEBS Lett. 351, 445–447
17. Arora, S., Lapinski, P. E., and Raghavan, M. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 7241–7246
18. Lapinski, P. E., Miller, G. G., Tampe, R., and Raghavan, M. (2000) J. Biol. Chem. 275, 6831–6840
19. Andrelewicz, M. J., Anderson, K. S., and Cresswell, P. (1999) Proc. Natl. Acad. Sci. U. S. A. 90, 9130–9134
20. van Endert, P. M., Tampe, R., Meyer, T. H., Tisch, R., Bach, J. P., and McDevitt, H. O. (1994) Immunity 1, 491–500
21. Neumann, L., and Tampe, R. (1999) J. Mol. Biol. 294, 1203–1213
22. Vos, J. C., Spee, P., Momburg, F., and Neefjes, J. J. (1999) J. Immunol. 163, 6679–6685
23. Li, S., Paulsson, K. M., Chen, S., Sjogren, H. O., and Wang, P. (2000) J. Biol. Chem. 275, 1581–1586
24. Cheng, Y., and Prusoff, W. H. (1973) Biochem. Pharmacol. 22, 3099–3108
25. Grandeaa, A. G., III, Golovina, T. N., Hamilton, S. E., Siriram, V., Spies, T., Brutkiewicz, R. R., Hartly, J. T., Eisenlohr, L. C., and Van Kael, L. (2000) Immunity 13, 213–222
26. Antoniou, A. N., Ford, S., Pilley, E. S., Blake, N., and Powis, S. J. (2002) Immunology 106, 182–189
27. Powis, S. J. (1997) Eur. J. Immunol. 27, 2744–2747
28. Lauvau, G., Gubler, B., Cohen, H., Daniel, S, Caillat-Zuken, S., and van Endert, P. M. (1999) J. Biol. Chem. 274, 31349–31358
29. Schoenhals, G. J., Krishna, R. M., Grandeaa, A. G., III, Spies, T., Peterson, P. A., Yang, Y., and Fruh, K. (1999) EMBO J. 18, 743–753
30. Momburg, F., and Tan, P. (2002) Mol. Immunol. 39, 217–233