Reconstitution of peptide-binding activity by TAP in proteoliposomes

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Abstract The purification and functional reconstitution of the transporter associated with antigen processing (TAP) is crucial for a complete molecular understanding of its action. Here, we report the conditions for the successful solubilization of human TAP from cellular membranes while maintaining TAP peptide-binding activity. In addition, solubilized TAP was incorporated into proteoliposomes and shown to possess specific peptide-binding activity. These studies provide the foundation for future attempts to achieve the complete functional reconstitution of TAP, which includes peptide transport.

Key words: TAP; Reconstitution; Antigen processing; Proteoliposome

1. Introduction

The transporter associated with antigen processing (TAP) plays a critical role in the major histocompatibility complex (MHC) class I antigen presentation pathway. TAP transports antigenic peptides from the compartment in which they are made, the cytosol, into the lumen of the ER, where they can associate with newly synthesized class I molecules and β_2-microglobulin. TAP is essential because without a source of peptides the class I molecules remain unstable and will not exit the ER [1]. This results in a dramatic decrease in the cell surface expression of class I molecules, as has been revealed by cells which harbor TAP mutations [2,3], or in cells where TAP expression has been down-regulated [4]. Without MHC class I expression viral-infected cells or tumor cells cannot be recognized and destroyed by $CD8^+$ cytotoxic T cells, leading to unchecked viral infection and tumor growth.

TAP is a member of the ATP-binding cassette (ABC) family of transporters, which include the multidrug resistance P-glycoprotein (MDR) [5], the cystic fibrosis transmembrane conductance regulator (CFTR) [6], and the mating factor transporter in yeast (STE6) [7]. Consistent with the other transporters in this family, the TAP complex possesses 2 ATP-binding domains and is predicted to possess 12 membrane spanning regions. TAP is expressed as a heterodimer of 2 subunits called TAP.1 and TAP.2, which must associate in order to form a functional transporter [8]. Until now the study of TAP structure and function has been limited to TAP in intact cellular membranes, i.e. in permeabilized cells [9,10] and microsomes [11,12]. Although these studies have produced valuable information on how TAP works, in order to understand TAP function in greater molecular detail, the functional reconstitution of TAP into artificial membranes will be necessary. This will facilitate both the structural analysis of TAP and studies on the mechanism of TAP action.

The purification and reconstitution of TAP is necessary to determine how TAP behaves in isolation, away from the plethora of proteins which interact with TAP, such as MHC class I molecules, tapasin, calnexin, and calreticulin [13,14]. Current data suggest that TAP can act alone, as peptide transport readily takes place in microsomes derived from TAP-expressing insect cells, which do not express the associated proteins [15]. However, it needs to be determined unequivocally whether TAP alone can transport peptides, and this can only be done through purification and functional reconstitution.

Toward this end we have attempted to functionally reconstitute TAP into proteoliposomes. We have been able to successfully solubilize and reconstitute TAP into artificial lipid membranes and maintain peptide-binding activity. These are important steps toward the complete functional reconstitution of TAP which would include the reconstitution of peptide transport across the liposome membrane. These studies therefore provide valuable information toward the reconstitution of TAP and a more complete understanding of TAP biology.

2. Materials and methods

2.1. Cell culture

Raji cells, a B-lymphoblastoid line derived from a patient with Burkitt's lymphoma [16], were maintained at 37°C in RPMI (GIBCO-BRL, Grand Island, NY) supplemented with 10% bovine calf serum, 1 mM pyruvate, 2 mM glutamine, and 100 units/ml each of penicillin and streptomycin. The TAP-deficient .174 cells [3] were grown in IMDM (GIBCO-BRL) supplemented with 10% bovine calf serum, 2 mM glutamine, and 100 units/ml each of penicillin and streptomycin.

2.2. Peptides

B27#3 (RRYQKSTEL) [17] and the photoreactive KB11 (AKVPLRPMTYKA, with a biotinylated lysine at position 11) were synthesized and purified at the Keck Foundation Biotechnology Resource Laboratory at Yale University School of Medicine and were generously provided by Dr. Peter Cresswell. The peptides were iodinated as previously described [9]. Briefly, 20 nmoles of peptide was iodinated using the method of chloramine T, and the unbound iodine was removed by gel filtration on a Sephadex G-10 column. The specific activity of the peptides was 15–50 cpm/fmol.

2.3. Phospholipids

Phospholipids were obtained from Avanti Polar Lipids (Alabaster, AL). All the phospholipids were supplied in chloroform, except sphingomyelin, which was supplied as a powder. The lipid mixture was composed of phosphatidylcholine (1000 mg), phosphatidylethanolamine (500 mg), phosphatidylserine (200 mg), and sphingomyelin

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Abbreviations: ABC, ATP-binding cassette; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate; HSAB, *N*-hydroxysuccinimidyl-4-azidobenzoate; MHC, major histocompatibility complex; NOG, *n*-octyl glucoside; TAP, transporter associated with antigen processing; TX-100, Triton X-100

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(200 mg). The lipids were combined and the chloroform was evaporated off under nitrogen gas. The dried phospholipids were subsequently washed twice with 20 ml diethyl ether. Finally, the phospholipid mixture was lyophilized to dryness for 6 h, and then resuspended in 2 mM 2-mercaptoethanol for a final concentration of 50 mg lipid/ml. The resuspended lipids were stored in aliquots at −80°C.

2.4. Membrane preparation

Raji cell pellets were stored at −80°C. The cell pellets were thawed and resuspended in a volume of 2 ml/10^8 cells on ice in Tris–buffered saline (TBS) (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.02% NaN_3), containing the following mixture of protease inhibitors: 500 μM phenylmethylsulfonyl fluoride (PMSF), 2 μg/ml aprotinin, 100 mM benzamidine, and 0.7 μg/ml pepstatin. The suspension was spun for 5 min at 1000×*g* to pellet the cells which remained intact. The supernatant was removed and saved while the pellet was resuspended in a volume of 1 ml/10^8 cells in 10 mM Tris-HCl, pH 7.4, 0.02% NaN_3. The suspension was clarified for 5 min at 1000×*g* as above. The supernatant from the second extraction was combined with the supernatant from the first extraction, and the pooled supernatant fraction was spun at high speed (100 000×*g*) for 45 min at 4°C to isolate the membranes. The membranes were prepared fresh the day of the experiment.

2.5. Membrane solubilization

Membrane pellets from 10^8 Raji cells each (4.1 mg total protein) were resuspended in 1 ml of phosphate-buffered saline (PBS) (10 mM sodium phosphate, pH 7.4, 150 mM NaCl) containing 20% glycerol, 10% phospholipid mixture (5 mg/ml final concentration), 1 mM dithiothreitol (DTT), protease inhibitors as before, and one of the following detergents: 1% Triton X-100, 2% *N*-octylglucoside (NOG), 2% digitonin, 1.6% CHAPS, 1.6% BigCHAP, or 1.6% cholic acid. The mixtures were incubated for 1 h on ice, followed by ultra-centrifugation at 100 000×*g* for 1 h to remove any unsolubilized material. The supernatants, containing the solubilized membrane proteins, were used immediately for peptide-binding assays or for incorporation into proteoliposomes. Protein determinations were performed using the Bio-Rad DC Protein Assay (Hercules, CA).

2.6. Proteoliposome formation

One ml of the phospholipid mixture (50 mg/ml) was sonicated to translucency using an ultrasonicator (Laboratory Supplies Co., Hicksville, NY). 100 μl of the sonicated phospholipids were added to 1 ml of the high speed supernatants (2.0 mg total protein) from above, containing solubilized TAP, and the mixture was incubated on ice for 30 min. Proteoliposomes were formed by diluting the samples 25-fold in PBS/1 mM DTT and incubating for 30 min at room temperature as previously described [18]. The proteoliposomes were harvested by centrifugation at 100 000×*g* for 1 h at 4°C.

Alternatively, 1 ml of the phospholipid mixture was solubilized in 2% NOG in PBS for 1 h at room temperature. The solubilized lipids were clarified by centrifugation at 100 000×*g* for 30 min at room temperature. Liposomes were then formed by diluting the solubilized lipid 25-fold in PBS followed by centrifugation at 100 000×*g* for 45 min at room temperature. The pellet was resuspended in 2 ml PBS and used immediately for the formation of proteoliposomes. Proteoliposomes were formed by mixing 2 ml of the liposome solution with 4 ml of solubilized Raji membrane preparation in 1.6% CHAPS (16.5 mg total protein), followed by dialysis versus 50 ml PBS with the addition of 0.5 g detergent-binding beads (SM-2 Biobeads, Bio-Rad, Hercules, CA) for overnight at 4°C (with one exchange of fresh buffer and Biobeads). The proteoliposomes were harvested by centrifugation of the dialysate at 100 000×*g* for 30 min at 4°C.

2.7. Peptide-binding assays

2.7.1. Peptide binding to TAP bound to Sepharose beads. Raji membranes (from 1×10^8 cells, 4.1 mg total protein) were solubilized in various detergents as described above. After the high speed spin, the supernatants, containing the soluble TAP proteins, were used for the peptide-binding assay. Sepharose-CL4B beads (25 μl) conjugated to the anti-TAP.1 monoclonal antibody 148.3 [15] were added to the supernatants and allowed to incubate with rotation for 45 min at 4°C. The beads were then washed once in 1 ml wash buffer (PBS, 0.5% detergent, and 20% glycerol). The beads were resuspended in 1 ml binding buffer (PBS, 0.5% detergent, 20% glycerol, and 1 mM DTT) and ^{125}I-B27#3 (100 nM) was added and the mixtures were allowed to incubate with rotation for 30 min at 4°C. The Sepharose beads, containing bound TAP and radiolabeled peptide, were washed 3 times in wash buffer and counted in the γ-counter (1470 Wizard, Wallac, Gaithersburg, MD).

2.7.2. Peptide binding to TAP in proteoliposomes. Proteoliposome pellets, derived from 4.1 mg total membrane protein, were resuspended in 500 μl intracellular transport (ICT) buffer (50 mM HEPES, pH 7.0, 78 mM KCl, 4 mM $MgCl_2$, 8.4 mM $CaCl_2$, 10 mM EGTA, 1 mM DTT, and 4 mg/ml bovine serum albumin, see [9]). ^{125}I-B27#3 (100 nM) was added to the proteoliposome suspension and allowed to incubate for 20 min on ice. 1 ml of ICT buffer was added to each suspension followed by centrifugation at 100 000×*g* for 15 min at 4°C. The proteoliposomes were washed one more time in 1 ml ICT buffer followed by centrifugation for 15 min as above. The washed proteoliposome pellets were then counted in the γ-counter.

2.8. TAP photolabeling in membranes and proteoliposomes

Membrane pellets (4.1 mg total protein) or proteoliposome pellets (derived from 4.1 mg total membrane protein) were resuspended in 500 μl ICT buffer and transferred to 3.5 cm plastic dishes. The photo-

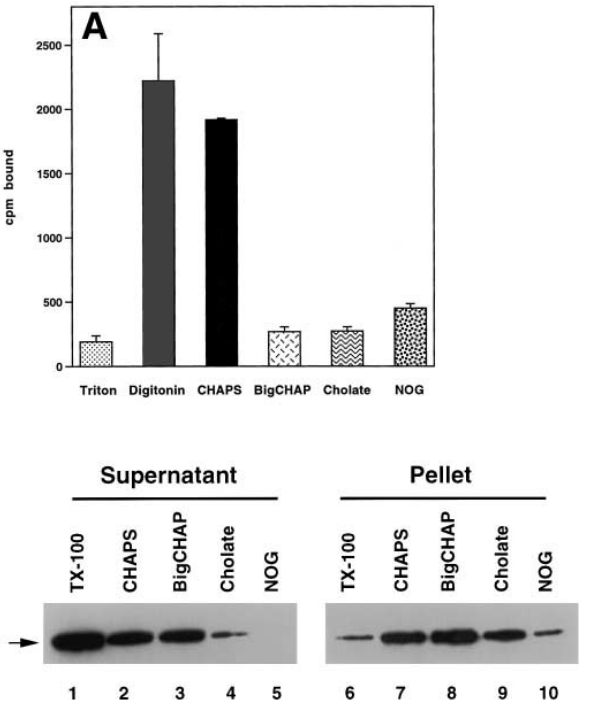

Fig. 1. A: Peptide binding to solubilized TAP bound to Sepharose-CL4B. Raji membranes (from 1×10^8 cells, 4.1 mg total protein) were solubilized in the detergents indicated. The extracts were clarified by centrifugation at 100 000×*g*, and the supernatants were used for the peptide binding assay as described in Section 2. Briefly, the samples were incubated with 25 μl of Sepharose-CL4B beads conjugated to the anti-TAP.1 antibody 148.3. The beads were washed to remove unbound TAP, and then ^{125}I-B27#3 was added to the beads. After washing a second time, bound ^{125}I-B27#3 was quantitated by counting in a γ-counter. The y-axis (cpm) represents the level of ^{125}I-B27#3 bound to the 148.3-conjugated Sepharose beads. The histograms represent the mean of duplicate points. B: Western blot analysis of the supernatant and pellet fractions from the initial solubilizations. Identical fractions of Raji membranes (4.0 mg total protein) were solubilized by the various detergents as described above (digitonin excluded). The extracts were spun at 100 000×*g* for 45 min, and the supernatant and pellet fractions were analyzed for TAP protein by Western blot. Briefly, supernatant fractions were immunoprecipitated with the anti-TAP.2 antibody 435.3 prior to 10% SDS-PAGE analysis, while the pellet fractions were analyzed directly by SDS-PAGE. Proteins were transferred to Immobilon-P membranes, blotted with the 435.3 antibody, and detected by enhanced chemiluminescence (ECL) and autoradiography.

reactive peptide KB11-HSAB was used to photolabel TAP as previously described [8]. ^{125}I-KB11-HSAB (200 nM) was added to the dishes, and incubation was carried out in the dark for 30 min on ice. The dishes, with tops removed, were exposed to UV light (254 nm wavelength) for 3 min. The photolabeled membranes or proteoliposomes were washed once by addition of 2.5 ml ICT buffer followed by centrifugation at 100 000 × *g* for 45 min. Photolabeled membranes or proteoliposomes were solubilized in PBS, 1% Triton X-100, 0.5 mM PMSF, and 5 mM iodoacetamide for 1 h on ice. The samples were clarified by centrifugation at 15 000 × *g* for 5 min. The solubilized TAP molecules were immunoprecipitated using 5 μg of the anti-TAP.2 monoclonal antibody 435.3 [19] and 25 μl of protein G-Sepharose beads (Sigma, St. Louis, MO). The beads were washed three times with wash buffer (TBS, pH 7.4, 0.5% Triton X-100), counted in the γ-counter, and analyzed by SDS-PAGE and autoradiography.

3. Results

3.1. Solubilization of TAP with retention of peptide-binding activity

To determine the optimum detergent for use in TAP reconstitution studies a series of detergents was tested for their capacity to solubilize TAP and still retain TAP peptide-binding activity. Membrane preparations from the human B-lymphoblastoid cell line Raji were solubilized in Triton X-100, digitonin, CHAPS, BigCHAP, cholate, and NOG as described in Section 2. The solubilized TAP molecules were then immobilized onto anti-TAP.1 antibody (148.3) conjugated Sepharose beads and assayed for the binding of ^{125}I-B27#3 peptide to the immobilized TAP molecules. The results, shown in Fig. 1A, revealed that two detergents, namely digitonin and CHAPS, retained a significant degree of peptide-binding activity compared to the other detergents tested. Of these two detergents, CHAPS appeared more promising for use in TAP reconstitution studies due to the inherent difficulties in working with digitonin.

To determine whether the reduced level of peptide binding observed for several of the detergents above, i.e. Triton X-100, BigCHAP, cholate, and NOG, was due to poor solubilization of TAP by the respective detergents rather than decreased TAP peptide-binding capacity, Western blot analysis for TAP protein was performed on the supernatant and pellet fractions obtained from the initial solubilizations. The results, shown in Fig. 1B, revealed that indeed cholate and NOG had a significantly reduced ability to solubilize TAP (lanes 4 and 5), whereas Triton X-100, CHAPS and BigCHAP solubilized TAP to a much greater degree (lanes 1, 2, and 3). This indicates that the reduced levels of peptide binding by TAP in the presence of Triton X-100 and BigCHAP, is most likely due to a reduced capacity to bind peptide by the solubilized TAP molecules. However, the reduced levels of peptide binding by TAP in the presence of cholate and NOG may be in part due to poor solubilization of TAP. Nevertheless, the data support the choice of CHAPS for further use in TAP reconstitution studies. It is not clear why the levels of TAP in the NOG-solubilized supernatant and pellet fractions were so low, however, it should be noted that TAP could be detected in NOG-solubilized photolabeled membranes (see Fig. 2A).

3.2. TAP in proteoliposomes retains photoreactive peptide-binding activity

To illustrate further the capacity of CHAPS to solubilize TAP and maintain peptide-binding activity, TAP, in both Raji crude membranes and in proteoliposomes, was photolabeled using the ^{125}I-KB11-HSAB photoreactive peptide. In one set of experimental points Raji membranes were photolabeled

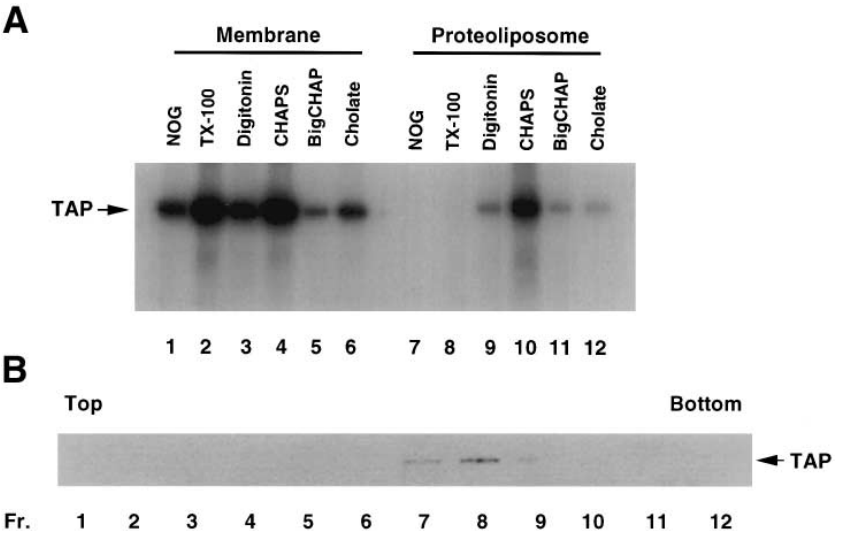

Fig. 2. A: TAP photolabeling in membranes and proteoliposomes. Raji membranes or proteoliposomes generated from 1 × 10^8 cells each (4.1 mg total protein) were photolabeled with ^{125}I-KB11-HSAB as described in Section 2. After photolabeling, the membrane pellets were solubilized in the series of detergents indicated, i.e. NOG, Triton X-100, digitonin, CHAPS, BigCHAP, and cholate, while the proteoliposome pellets were all solubilized in 1% Triton X-100. The solubilized TAP molecules were immunoprecipitated with the anti-TAP.2 antibody 435.3 and protein G-Sepharose. The precipitated, photolabeled TAP molecules were analyzed by SDS-PAGE (10%) and autoradiography. B: Analysis of TAP-containing proteoliposomes by sucrose gradient centrifugation. Proteoliposomes were prepared from Raji membranes (8.2 mg total protein) by the detergent dilution method. The proteoliposomes were resuspended in 3 ml 30% sucrose (gradient buffer, 50 mM Tris-HCl pH 7.4, 150 mM KOAc, 5 mM MgOAc) and overlayed successively with 3 ml 20%, 10%, and 5% sucrose. The gradient was spun at 150 000 × *g* for 4 h at 4°C. Fractions (1 ml) were collected from the top of the gradient, and were analyzed for TAP by immunoprecipitation with the anti-TAP.2 antibody 435.3, followed by Western blot analysis of the immunoprecipitated samples as described in the legend to Fig. 1.

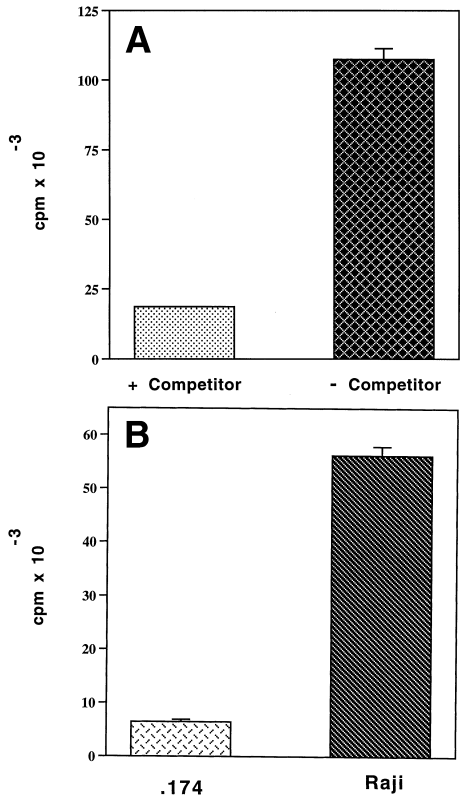

Fig. 3. Peptide binding to proteoliposomes containing TAP. ^{125}I-B27#3 binding to proteoliposomes made from Raji or TAP-negative .174 cell membranes (4.2 mg total protein each) was carried out as described in Section 2. A: ^{125}I-B27#3 binding to proteoliposomes derived from Raji was carried out in the presence and absence of unlabeled B27#3 competitor peptide (50 μM). B: ^{125}I-B27#3 binding to proteoliposomes derived from Raji and .174 membranes was performed in the absence of unlabeled B27#3. The y-axis represents the level of ^{125}I-B27#3 (cpm$\times 10^{-3}$) bound to the proteoliposomes. The histograms represent the mean of duplicate points.

prior to solubilization in detergent. The same series of detergents as above was used for the solubilization, i.e. NOG, Triton X-100, digitonin, CHAPS, BigCHAP, and cholate. In another set of points, Raji membranes were first solubilized in the detergent series and proteoliposomes generated from the solubilized TAP molecules for each detergent. The proteoliposomes were in turn photolabeled using the KB11 photopeptide. Photolabeled TAP molecules from all samples were immunoprecipitated using the anti-TAP.2 antibody 435.3, and analyzed by SDS-PAGE and autoradiography. The results of this analysis are shown in Fig. 2A. The detergents in the series were more or less successful in solubilizing TAP from photolabeled Raji membranes (left panel), especially Triton X-100 (lane 2) and CHAPS (lane 4). In contrast, only CHAPS generated proteoliposomes which could still bind the peptide and photolabel TAP (right panel, lane 10). Clearly, the other detergents generated proteoliposomes which had very low or no peptide-binding capacity. Surprisingly, digitonin produced proteoliposomes with very little peptide-binding capacity (lane 9) in spite of showing a high level of peptide binding in the prior assay (see Fig. 1A). This is most likely due to its poor ability to form proteoliposomes. The results unambiguously show that CHAPS is the detergent of choice for the generation of proteoliposomes containing TAP with retention of peptide-binding activity, and CHAPS is utilized for the remainder of this study.

To confirm that the proteoliposomes generated in this study were in fact proteoliposomes and not aggregated/high density protein/lipid complexes, a sample of the proteoliposomes generated from Raji membranes was run on a sucrose density gradient. TAP-containing liposomes were subsequently immunoprecipitated from the fractions and analyzed by Western blot analysis. As revealed in Fig. 2B, the TAP-containing fractions (predominantly fractions 7 and 8) were found approximately two-thirds of the way into the gradient of 5% to 30% sucrose, and did not appear to migrate as high density protein/lipid aggregates. This indicates that liposomes containing TAP were formed during the TAP reconstitution procedure.

3.3. TAP in proteoliposomes specifically binds ^{125}I-labeled peptides

The ultimate goal of these studies is to reconstitute peptide transport, and in this sense the use of photoreactive peptides to measure peptide binding was prohibitive. Therefore we tested the proteoliposomes for their capacity to bind ^{125}I-B27#3. Shown in Fig. 3 are the results of peptide binding to reconstituted TAP in liposomes in the absence and presence of excess unlabeled B27#3 (Fig. 3A), and ^{125}I-B27#3 binding to proteoliposomes generated from Raji and the TAP-negative cell line .174 (Fig. 3B). The results indicate that ^{125}I-B27#3 binding to proteoliposomes is specific. The binding was competed with excess unlabeled peptide, and peptide binding to TAP-negative proteoliposomes (.174) was much lower in comparison to TAP-positive proteoliposomes (Raji). The high level of background binding is most likely due to the sticky nature of the proteoliposomes, and measures to reduce the level of background binding are being explored. Saturation peptide-binding analysis was also performed on TAP reconstituted into proteoliposomes. The results, shown in Fig. 4, reveal that ^{125}I-B27#3 binding to proteoliposomes is satura-

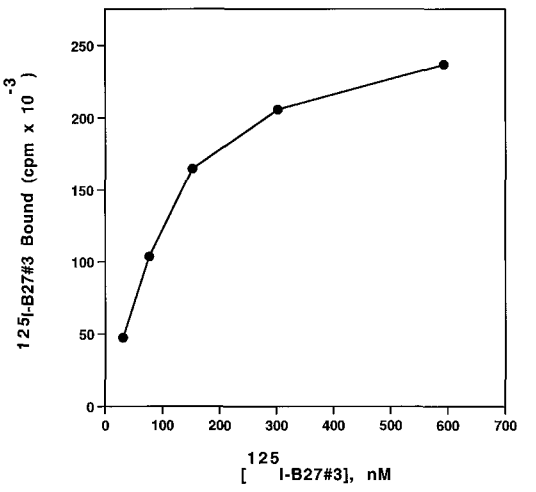

Fig. 4. Saturation binding of ^{125}I-B27#3 to proteoliposomes. ^{125}I-B27#3 binding to proteoliposomes derived from Raji membranes (2.0 mg total membrane protein per point) was performed at different concentrations of the labeled peptide (x-axis). Each point represents the mean of peptide binding to duplicate samples with the background binding (amount of ^{125}I-B27#3 bound in the presence of 100 μM unlabeled B27#3) subtracted.

ble. The estimated maximum bound peptide (B_{max}) was approximately 5 pmols which directly relates to the amount of active TAP. In addition, the K_d of the binding reaction was ~150 nM, which is consistent with previous data on peptide binding to TAP in microsomes [20].

4. Discussion

The reconstitution of TAP into proteoliposomes is important for further structural and functional analysis of TAP. A key step in this procedure is the solubilization of TAP in detergent with the retention of TAP activity. This type of analysis is purely empirical and can only be done by testing different detergents. We were able to show that CHAPS is the detergent of choice for TAP reconstitution experiments. Peptide binding to TAP solubilized in CHAPS (Fig. 1), as well as photoaffinity labeling of TAP solubilized in CHAPS and incorporated into proteoliposomes (Fig. 2), clearly shows that CHAPS retains the highest level of peptide-binding activity. Digitonin also retained a certain level of TAP activity, however, due to the inherent problems with working with digitonin, it was not chosen for further use. Although CHAPS may not be the most efficient detergent for solubilizing membranes, the fact that it retains TAP peptide-binding activity while the other detergents do not is a critical factor. In fact, based upon the Western analysis shown in Fig. 1, CHAPS is able to solubilize approximately 50% of the TAP molecules associated with the original membranes, and based upon the maximum level of peptide binding to proteoliposomes in Fig. 4, this represents a minimum of 750 ng TAP per 2.0 mg total membrane protein.

A prerequisite for the reconstitution of peptide transport via TAP incorporated into proteoliposomes is the successful reconstitution of peptide binding to TAP. We have been able to reconstitute peptide binding to TAP in proteoliposomes. As shown in Fig. 3 this peptide binding is specific. It is competed by unlabeled competitor peptide (Fig. 3A), and the labeled peptide binds very poorly to proteoliposomes derived from TAP-negative membranes (Fig. 3B). With the conditions for peptide binding worked out, peptide transport experiments were undertaken. These were done by adding ATP and labeled peptide to proteoliposomes followed by incubation at 37°C to allow for the incorporation of the peptide into the liposomes. Unfortunately, the level of peptide incorporation in the presence of ATP was only slightly above the level of incorporation in the absence of ATP (data not shown). Therefore, the state of TAP in the proteoliposomes, although good enough to allow for peptide binding, does not appear to support peptide transport, and further refinement of the reconstitution system will be required.

Another aspect of TAP reconstitution is the purity of TAP. We have chosen to pursue the functional reconstitution of TAP using proteoliposomes prepared from crude cellular membranes. B-lymphoblastoid cell lines such as Swei and Raji produce relatively large amounts of TAP, and provide a good source of material for reconstitution studies. In addition, membranes from the B-lymphoblastoid lines are easily prepared, and may contain the necessary TAP-associated proteins or cofactors to facilitate peptide transport. Our strategy is to first define the conditions for peptide transport in proteoliposomes derived from crude cellular membranes, and once these conditions have been worked out, attempt to reconstitute using purified TAP. In this way, the number of experimental variables will be kept to a minimum at each step. Ultimately, one will have to utilize a TAP overexpression system, i.e. baculovirus, to generate enough protein to purify TAP successfully.

The reconstitution of peptide transport by TAP remains a challenge for future studies. The related ABC transporters, multidrug resistance P-glycoprotein (MDR) and the cystic fibrosis transmembrane conductance regulator (CFTR) have been successfully purified and reconstituted [21–23]. Nevertheless, substrate transport in the reconstituted systems has proven difficult to show. For P-glycoprotein the vast majority of reports on functional reconstitution have involved the restoration of drug-induced ATPase activity and not drug transport [24]. The main problem is that the hydrophobic nature of MDR substrates results in high non-specific binding to proteoliposomes, and lack of accumulation in vesicles. Furthermore, CFTR is considered a regulated chloride channel and therefore the transport properties of CFTR are fundamentally different from those of P-glycoprotein and TAP. In the case of TAP, the peptide transport process is inherently more complex than peptide binding. For example, peptide transport requires the binding and hydrolysis of ATP, the translocation of the peptide across the lipid bilayer, and the accumulation of the peptide within the liposome. All these factors will need to be addressed to achieve successful peptide transport in a reconstituted system.

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