Three-dimensional Structure of Transporter Associated with Antigen Processing (TAP) Obtained by Single Particle Image Analysis*

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The transporter associated with antigen processing (TAP) is an ATP binding cassette transporter responsible for peptide translocation into the lumen of the endoplasmic reticulum for assembly with major histocompatibility complex class I molecules. Immunoadfinity-purified TAP particles comprising TAP1 and TAP2 polypeptides, and TAP2 particles alone were characterized after detergent solubilization and studied by electron microscopy. Projection structures of TAP1+2 particles reveal a molecule ~10 nm across with a deeply staining central region, whereas TAP2 molecules are smaller in projection. A three-dimensional structure of TAP reveals it is isolated as a single heterodimeric complex, with the TAP1 and TAP2 subunits combining to create a central 3-nm-diameter pocket on the predicted endoplasmic reticulum-lumenal side. Its structural similarity to other ABC transporters demonstrates a common tertiary structure for this diverse family of membrane proteins.

ATP binding cassette (ABC) transports are ubiquitous and can form a significant component of an organism’s genome, for example 2% in Escherichia coli (1). They are defined by the ABC domain, a nucleotide hydrolysis domain whose activity powers substrate transport (2), and are classically composed of at least four domains, two transmembrane domains (TMDs) and two cytoplasmic and soluble nucleotide binding domains (NBDs). These domains can be expressed together as a single modular polypeptide or separately.

TAP is an ABC transporter expressed in the endoplasmic reticulum (ER), which supplies peptides from the cytosol for binding by MHC class I molecules (3–8). In the absence of TAP the assembly of MHC class I and, consequently, antigen presentation are both severely impaired (9–12). TAP is expressed as two polypeptide subunits, TAP1 and TAP2, each composed of one TMD and one NBD, which together form the functional transporter. Models for the predicted structure of TAP have relied on the sequence-based prediction of multiple transmembrane-spanning regions in the TMDs (13), the location of point mutations or naturally occurring polymorphisms affecting peptide selection and transport (14–17), or the cytosolic or ER luminal orientation of TAPs engineered to contain reporter molecules (18, 19). More recently, expression of truncated TAP polypeptides suggests interactions between both the TMDs and NBDs of TAP1 and TAP2 (20–22). Nevertheless, no direct structural imaging of this crucial molecule to the development of cellular immune responses has yet been obtained.

There is a need for structural studies of whole ABC transporters to permit insights into their overall structure and functions. Single particle image analysis has successfully been applied to molecules of comparable size to TAP (23–26). Furthermore, low resolution electron microscopic structures of the ABC transporters P-glycoprotein and MRP1 have been determined using a combination of single particle and crystallographic analysis (27, 28). Recently a structure for MsbA, a prokaryotic half-ABC transporter, has been published, allowing the packing of the six transmembrane helices in the TMD and the intracellular domains to be visualized for the first time (29). The structure of NBDs has been known for longer and at higher resolution. The tertiary fold of the NBD is highly conserved, although no consensus has emerged as to the extent of the quaternary interaction between the two NBDs (30–34). In the MsbA structure, the NBDs do not contact at all apart from crystal contacts, whereas in all the preceding NBD-only structures, the NBDs contact each other over extensive dimer interfaces. A recent structure for the TAP1 NBD provided the first exception to this, with no significant interface detected (35).

Biochemical evidence for the cooperativity of the NBDs would argue for significant contacts (36–38). In this report we have studied the structure of TAP by electron microscopy of detergent-solubilized immunopurified particles. Single particle analysis was used to compare TAP from cells expressing the TAP1-TAP2 heterodimer with particles from cells expressing TAP2 alone. Three-dimensional reconstruction of the TAP heterodimer was also carried out that allowed direct comparison with the structure of P-glycoprotein. The resulting assignment of features in the molecule to previously characterized domains provides the first direct structural evidence supporting models of TAP that have until now been predominantly predictive in nature.

EXPERIMENTAL PROCEDURES

Cell Lines—T2, T2-TAP2 (expressing rat TAP2 only), T2-TAP1 (expressing rat TAP1 only), and T2 TAP1+2 (expressing rat TAP1 and TAP2) cells (39, 40) were maintained in RPMI 1640 medium with 5% fetal calf serum and 1.0 mg/ml G418 (Life Technologies, Inc.).
Flow Cytometry—Cells were washed into PFN medium (phosphate-buffered saline, 2% fetal calf serum, 0.1% azide) and incubated with monoclonal antibody MEI (anti-HLA-B) tissue culture supernatant (a gift from J. Taurog, University of Texas) followed by fluorescein isothiocyanate conjugated anti-mouse IgG (Sigma). Analysis was performed on a Becton Dickinson FacsSort using CellQuest software.

Immunosolation of TAP—Immunosolation utilized sheep anti-TAP2 antisera raised against the C-terminal 14 residues of rat TAP2 (KYYAHVLQQSIMEA) (40). 2 x 10⁶ cells were lysed in detergent buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris, pH 7.6, 5 mM MgCl₂, 1 mM phenylmethylsulfonyl fluoride). Immunoaffinity beads were prepared by equilibrating 5 μg of purified anti-TAP2 antisera with 50 μl of protein G-Sepharose. Pre-cleared lysates (50 μl of protein G-Sepharose, 1 h) were incubated sequentially in 1.5-ml aliquots with the same immunoaffinity beads for 30 min each. Beads were washed extensively before eluting with 50 μl above C-terminal TAP2 peptide. Eluted samples were cleared of any residual immunoglobulin by two further clearings with protein G-Sepharose with 20,000 x g-centrifuge spins in between. Samples were adjusted to 20% glycerol as cryoprotectant and stored at -80 °C. Western blotting of detergent-solubilized cells was carried out as previously described (40).

ATP and Peptide Labeling of TAP—Antibody immobilized using sheep anti-TAP1 or -TAP2 antisera (40) or peptide-eluted TAP polypeptides was incubated in a volume of 30 μl of lysis buffer with 1 μCi of [α-32P]triphosphate (Affinity Labeling Technology, Lexington, KY) for 15 min on ice, then exposed to UV irradiation at 365 nm for 10 min on ice. In some experiments unlabeled ATP was added at a concentration of 1 mM. For antibody-immobilized TAP polypeptides, free 8N₃ATP was removed by washes in lysis buffer. Particles in solution were diluted in 500 μl of lysis buffer and re-immunoprecipitated with monoclonal antibody MAC394 (41), recognizing rat TAP2 (at an epitope different from the C-terminal sheep sera), which was a gift from M. Knittler and J. Howard (University of Cologne). For SDS denaturation, samples were adjusted to contain 1% w/v SDS, heated to 80 °C for 5 min, cooled on ice, diluted as above in lysis buffer, and immunoprecipitated with sheep anti-rat TAP1 antisera or monoclonal antibody MAC394. Samples were heated in reducing sample buffer and analyzed by 6% SDS-PAGE.

Ethylene glycol bis(succinimidyldimaleate) (EGS, Sigma) labeling was performed on cell lysates prepared at a concentration of 20 x 10⁶ in phosphate-buffered saline containing 1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride. Peptides were added to a final concentration of 20 μM for 30 min on ice followed by EGS at a concentration of 1 mM for a further 30 min. Samples were heated in reducing sample buffer and analyzed by 6% SDS-PAGE and Western blotting with rabbit anti-TAP2 sera 116 (41).

Immunosolubilized TAP polypeptides were washed in lysis buffer. Eluted TAP particles were re-immunoprecipitated with MAC394 and analyzed by SDS-PAGE as described above. Densitometry was performed using NIH Image software.

Transmission Electron Microscopy—Samples were added to freshly glow-dried (rendered hydrophilic) copper/carbon mesh grids for 30 s, and the sides were blotted with Whatman grade 50 filter paper and negatively stained in 4% uranyl acetate. Micrographs of the grids were taken with a Philips CM10 (University of Leeds) and a Philips Technai 10 (UMIST) electron microscope operating at 100 kV. The micrographs were scanned using a Leaf Microdensitometer (University of Leeds) and a Zeiss scanner (University of Sheffield).

Single Particle Analysis—Image files were initially contrast transfer function (CTF)-corrected using the CRISP image processing package (Calidris Software, Stockholm). Particles were then selected using SPIDER (42), and the densities were normalized between several micrographs. Reference-free alignment was carried out, and the results were

![Image](image-url)
statistically analyzed. Particles were sorted into groups for averaging using hierarchical ascendant classification with complete linkage. For each average, Fourier ring correlation values dropped below 0.5 was chosen and was determined by fitting the data with a four-parameter Boltzman function. A threshold for each class that gave the best averages in terms of resolution was thus determined.

Three-dimensional Reconstruction—The random conical reconstruction methods we used were part of a suite of procedures written for SPIDER (www.wadsworth.org/spider_doc/spider/docs/spider.html), adapted to include CTF correction in SPIDER. The change in the CTF across the micrographs of the tilted specimen was determined using the program ctfind2.com (43). Particles from untitled micrographs were selected with their equivalent tilted partners (40°) from the underfocused side of the tilted one. An individual CTF correction was applied to each of the tilted particles selected based on its positional coordinates. CTF correction was not needed to align the untitled data set, but a Fermi low pass filter was used to improve the fidelity of the alignment. Reference-free alignment was carried out on the untitled data set (1013 particles), and hierarchical clustering was used to sort these into the major groups. Three classes (215, 215, and 251 particles) were chosen as before for reconstruction of volumes by back projection from the tilted particles (44). Each volume was put through an angular refinement step (see Pawel’s random conical reconstruction methods on the above-mentioned SPIDER web site) by generating projections from the initial three-dimensional structure and translationally realigning the original tilted particles to these newly generated projections. The realigned particles were then used to recreate a new volume. This refinement step was carried out six times, and the final volume was used for merging the data. The refined volumes from the three separate groups were then compared and rotationally aligned using a three-dimensional correlation search before combining to give a merged reconstruction. This reconstruction was also subject to a similar refinement procedure. This volume represented 67% (683 particles) of the initial data set (1013 particles). Resolution was assessed by splitting its tilted projection data set into two, constructing two “sub-volumes,” and calculating the Fourier shell correlation between them using a cut-off of 2/πN (where N is the number of pixels in each Fourier shell).

RESULTS

Purification and Characterization of TAP—We utilized the TAP-deficient T2 human lymphoblastoid cell line, which had been transfected with both rat TAP1 and TAP2 or with rat TAP2 alone. Peptide specificity and transport activity, and MHC class I-chaperone interactions with TAP polypeptides have been well documented in these transfectant lines (39, 40, 45–47). Western blot analysis of detergent-solubilized cell lysates confirmed the expression of both TAP polypeptides in the T2-TAP1 + 2 transfected and of TAP2 only in T2-TAP2 cells (Fig. 1b). Flow cytometric analysis of the cell surface levels of MHC class I confirmed no increase in MHC class I on T2-TAP2 cells compared with T2 cells, whereas T2-TAP1 + 2 displayed at least 10-fold more MHC class I (Fig. 1a). This confirmed that before immunoprecipitation, the TAP heterodimer is functional.
in peptide transport, whereas TAP2 on its own is not.

We chose to purify TAP by a rapid immunoaffinity-based methodology to avoid lengthy chromatography-based isolation, thus limiting the time-dependent inactivation of TAP after detergent solubilization (48). Triton X-100 was chosen to isolate the core subunits of TAP1 and -2 in the absence of associated class I MHC-chaperone complexes. Immunopurification was performed utilizing anti-rat TAP2 antisera immobilized on protein G-Sepharose. After extensive washing of the immune complexes, TAP was specifically eluted by the addition of the peptide, against which the antisera was generated. The purity of the TAP preparations was determined by analyzing a portion of the eluate by SDS-PAGE and silver staining (Fig. 1c). Similar attempts to isolate TAP1 using the relevant peptide resulted in very inefficient recovery of TAP1 and was not pursued further in this study. On average purifications using TAP2 reagents yielded between 50 and 200 ng of TAP polypeptides from \( \sim 2 \times 10^8 \) cells. T2-TAP2 cells produced a predominantly single species of approximate molecular mass 75,000 Da, characteristic of TAP2, whereas a doublet of similar mass representing TAP2 and TAP1 was isolated from T2-TAP1+2 cells. Western blotting verified the identity of the components (not shown). The co-isolation of TAP1 using anti-TAP2 antisera confirmed the functional interaction of these polypeptides, suggesting also that they have been purified in an unaltered conformation. Control purifications from untransfected T2 cell lysates produced no detectable TAP species (Fig. 1c). Some higher molecular mass contaminants were visible in all preparations including the control T2 lysate; however, the relatively high purity of the TAP preparations was confirmed.

Once solubilized in detergent, it is difficult to determine the peptide transport activity of TAP; we therefore studied the integrity of the ATP binding domains of TAP, which are known to intimately control the ability of TAP to bind and transport peptides (36, 37, 49, 50). The presence of an ATP-binding site was probed by incubation of immunosolated TAPs with \( 8N_3{\text{ATP}} \), which can be cross-linked once bound by exposure to UV irradiation. We first tested the ability of the TAP heterodimer, TAP2 alone, and also TAP1 alone (in T2-TAP1 cells) to be labeled by \( 8N_3{\text{ATP}} \) while still attached to antibody in immune complexes. As shown in Fig. 2a, both TAP2 and TAP1 labeled strongly when expressed singly. In the case of the heterodimer, whether immunoisolated by anti-TAP2 or -TAP1 reagents, a band was obtained that in comparison to single TAP chains, suggested that both ATP binding sites were equally accessible to \( 8N_3{\text{ATP}} \). A strikingly different pattern of labeling was obtained when we examined TAP particles free in solution after peptide-specific elution. In this instance poor labeling was observed with TAP2 alone (Fig. 2b), whereas in the TAP heterodimer, the majority of the labeling appeared to be on material of lower molecular mass than TAP2, suggesting almost exclusive labeling of TAP1. In both cases the presence of nonradioactive ATP inhibited the labeling. To confirm this observation we performed the same experiment but denatured a portion of the TAP heterodimer sample in SDS after labeling with \( 8N_3{\text{ATP}} \) and re-immunoprecipitated with reagents specific for TAP1 or TAP2. The majority of the labeling was cross-linked to TAP1 (Fig. 2c). Thus the ATP binding sites in the TAP heterodimer appear to differ in their ability to bind ATP when immobilized with antibody. Possibly the presence of antibody directed at the C terminus of either TAP1 or TAP2 may alter the conformation of the NBDs. However, the preferential labeling of TAP1 of TAP particles free in solution correlates with recent data that indicates that the ATP-binding site on TAP1 is more readily labeled than TAP2 (36, 51).

We also studied the ability of purified TAP particles to bind peptides. An iodinated model peptide TNKTVARYV (termed pep1 in Fig. 2) coupled to the photoreactive cross-linker HSAB (52) labeled the immunoinmobilized TAP heterodimer but not...
FIG. 4. Reference-free alignment and statistical analysis. A, hierarchical ascendant classification was performed to sort aligned particles into classes before averaging the members of each class together (top half, TAP1 + 2; bottom half, TAP2). Scale bar = 10 nm. B, resolution of each class (from A) was determined using Fourier ring correlation with a correlation of 0.5 taken as a threshold. Histogram bars are located under each corresponding class. C, five major classes for TAP1 + 2 (upper row) and TAP2 alone (lower row) at higher magnifications with evenly spaced contours used to delineate protein density, with the first contour at 0.75σ above the mean density. Scale bar, 10 nm.
TAP2 alone (Fig. 2d). However, initial attempts to label TAP particles in solution with pep1 were unsuccessful. We reasoned that the presence of the C-terminal TAP2 peptide KVYAHL-VQQRLEA (termed pep2) used to competitively elute TAP particles at 50 μM may inhibit the binding of the photoreactive reporter peptide. To test whether pep2 was capable of binding TAP, we utilized the ability of the chemical cross-linker EGS to cross-link TAP polypeptides into a large molecular mass complex, the formation of which is enhanced by TAP-binding peptides (50, 53). Detergent lysates of T2 TAP1+2 cells were incubated with pep1 or pep2, and cross-linking was induced by the addition of 1 mM EGS. Western blot analysis of the lysates indicated that both peptides increased the formation of a cross-linked TAP product of relative molecular mass 220 kDa (Fig. 2e). Thus the C-terminal TAP2 peptide binds to TAP and is likely to compete with the photoreactive reporter peptide. We therefore eluted TAP particles with a 10-fold reduced concentration of pep2 and retested the binding ability of iodinated HSAB-pep1. Under these conditions iodinated HSAB-pep1 labeled TAP particles, and the addition of 10 μM unlabeled pep1 or pep2 resulted in inhibition of 78 and 43% binding, respectively (Fig. 2f). Thus eluted TAP particles in solution retain the ability to bind peptides. Taken together with the ATP binding data, this suggests that the TAP particles in solution maintain good overall structural integrity.

**Structural Analysis of TAP Heterodimer and TAP2 Particles**—Transmission electron micrographs of the TAP heterodimer, TAP2, and untransfected T2 control preparations are shown in Fig. 3. Untransfected T2 cell preparations contained only very small debris, whereas the other two samples contained much larger particles. Thus the immunoisolation reaction per se does not appear to contribute particulate matter of similar size to the TAP immunosolubles. Particles in the TAP2 micrographs were on average 7–9 nm in diameter, whereas the TAP heterodimer particles were 9–12 nm in diameter. This information assisted in the TAP heterodimer and TAP2 particle selection process to help improve the data set.

Images of 686 TAP2 and 1436 TAP1+2 particles were selected (Fig. 3). Each image represents a projection (a two-dimensional view) of the protein structure. After reference-free alignment, particles were sorted into groups, and averages of different orientations of each molecule were generated (Fig. 4A). The choice of threshold to apply in the clustering was determined by Fourier ring correlation (Fig. 4B). There is pseudo-2-fold symmetry in the orientations. Averages B and C are examples of projections that show a central 3-nm-diameter-stain-accumulating region. Five of the most significant averages of TAP2 are also shown at the same scale as the TAP1+2 averages (Fig. 4C, bottom row). These averages have a spatial resolution of 26–35 Å, determined by Fourier ring correlation (Fig. 4B). Some orientations have a diamond shape (Fig. 4C, bottom row, d and e), and others have a trapezoid shape (Fig. 4C, bottom row, a–c). Along one axis, the length of the molecule changes from one side, 6–7 nm, to the other side, 7–8 nm. Along the other axis the length is constant in most averages, at 8–9 nm. This molecule has a deep semicircular groove in the side of some of the projections, such as projections d and e, which have a diameter of about 2–3 nm, giving a kinked “L-shaped” molecule with a total length along the arms of the L of about 11 nm and a width of 3–4 nm.

**FIG. 5. Three-dimensional structure of TAP, displayed as stereoviews.** Stereoview gallery of the three-dimensional reconstruction of TAP generated from different angles at a threshold of 0.1 (3.69σ above the mean). All angles refer to a rotation in either the y plane or the x plane in relation to the first view (top, marked 0°). The structure is rotated 45° per view along the y axis four times and 90° along the x axis in the final view. Bar, 10 nm.
To reconstruct a volume from projection data, micrographs of a set of particles imaged-tilted and -untilted were used. Rotations and shifts from alignment data of the untitled particles are applied to the tilted ones taking into account the angle of tilt and back projection used to reconstruct a volume. Three-dimensional reconstruction of TAP1+2 was carried out from a data set of 1013 particles originating from two pairs of tilted and untilted micrographs. Reconstruction of TAP2 particles was not performed because of their smaller size. The untilted particles were first aligned and clustered into major groups. The averages produced were very similar to those produced initially and had similar resolution (data not shown). The three best groups were chosen (comprising 215, 215, and 251 particles) and back projections were reconstructed, and these were merged as described under "Experimental Procedures" to give the volume shown in Fig. 5. There was a significant increase in signal:noise proceeding from the initial structures (with a resolution estimate 42–45 Å) to the final merged and refined structure (with a resolution estimate of 30–40 Å, see Fig. 6).

Several orientations of the three-dimensional structure (Fig. 5) resemble quite closely different projections of TAP1+2 and are also comparable in size and shape to that of the three-dimensional-structure of P-glycoprotein (27). The central, pocket-like, darkly staining region of TAP1+2 is clearly visible on the surface (Fig. 5, 0° rotation). The inner structure of the transporter is shown in Fig. 7A with cross-sections taken through the complex along various directions. Structural similarity is observed between some of the sections and the TAP2 data. There is a significant level of pseudo-2-fold symmetry in the structure of TAP1+2, with a division into two apparent halves in several views that not only is visible from the outside but is also apparent from the sectioned views.

**DISCUSSION**

Expression of TAP1 and TAP2 as a heterodimer is essential for the proper supply of peptides into the ER and subsequent assembly of MHC class I molecules (9–11, 54). The flow cytometry performed in Fig. 1 demonstrates that MHC class I is restored to the cell surface only in the presence of the TAP heterodimer and not by TAP2 alone. For the purposes of our purification, this behavior indicates that TAP is functional before solubilization. Detergent solubilized, immunopurified TAP, after specific-peptide elution, could be labeled with the photoreactive ATP analogue 8N₃ATP (Fig. 2). Both the TAP heterodimer and TAP2 particles were labeled, although TAP2 labeled less efficiently. This poor labeling of TAP2 also occurred in the TAP heterodimer, with TAP1 the principal ATP acceptor. This is in agreement with other experiments performed on detergent-solubilized TAPs (36, 51) and suggests that our particle preparations retain structural integrity. TAP particles, in the presence of reduced levels of the eluting peptide, were also capable of binding and cross-linking to an iodinated reporter peptide, further suggesting good integrity. Our demonstration that the peptide used to elute TAP particles also binds to TAP suggests that the structural model presented here may represent peptide-bound TAP. We are currently investigating other purification protocols that do not rely on peptide-based elution to determine differences between peptide-bound and peptide-free structures of TAP.

The behavior of the TAP heterodimer and TAP2 in the presence of immobilizing antibody is intriguing. Expressed separately, both NBDs appear to bind ATP (Fig. 2a), and this is replicated in the TAP heterodimer. This suggests that we may be able to use antibodies (or purified Fab’s) to probe various states of the NBDs of TAP in further single particle experiments. The data indicate that our novel rapid immunoaffinity purification of TAP produces relatively high purity material that can be utilized in both functional and structural studies.

Regarding the domain organization of TAP, Fig. 7B presents a comparison of the TAP1+2 heterodimer with structures of P-glycoprotein and dimeric NBDs. The high degree of similarity between the extracellular view of P-glycoprotein and the proposed lumenal side of TAP1+2 is clear. This is the first confirmation that ABC transporters share similar three-dimensional motifs at low resolution. A ring of protein, which is divided into two similarly sized domains, surrounds the deeply stained region. This ring could correspond to the two TMDs and their lumenal loops on the basis of the P-glycoprotein structure. The footprint of each domain in the ring (22 × 56 Å) would correspond well with the space required for 7–8 transmembrane helices and short connecting loops (18, 19, 22) and also matches the (slightly smaller) footprint of the six TMD helices in the half-transporter MsB (29). This area is most likely to contain the domains in closest proximity to the MHC class I binding grooves awaiting suitable peptides (40, 55, 56) and is also the most likely target site for antibodies.
area for the interaction of US6, the viral inhibitor of TAP expressed by human cytomegalovirus (51, 57). The three-dimensional structures of TAP and P-glycoprotein appear less similar from the cytoplasmic side/intracellular views (Fig. 7: TAP1+2 184°, P-glycoprotein intracellular). Rather than being due to real structural differences, this could be due to different thresholds or differential staining. In support of this conclusion, the three-dimensional structure of P-glycoprotein from two-dimensional crystals shows a very strong similarity to the TAP1+2 184° view with two similar, rotationally symmetrical, 40-Å diameter domains (data not shown).

The molecule is clearly dimeric. TAP1 transmembrane helices 2–6 and TAP2 helices 1–5 have been shown to be essential for co-association, and TAP2 has been shown to stabilize the
topology of transmembrane helix 6 of TAP1, indicating a close functional association (19, 22). TAP also migrates with an estimated molecular mass of 200 ± 50 kDa in size exclusion chromatography (54) and can be chemically cross-linked into a product of 220 kDa (53), further suggesting that TAP is a heterodimer with a stoichiometry of 1:1. These experimental observations are reflected in the three-dimensional-structure of TAP1–2, which has a strong division into two halves in many views (Fig. 5).

A model has been proposed for a head-head, tail-tail association of the TAP subunits, which would suggest that the TMDs should have a pseudo-mirror symmetry (22). This would imply that the angle at which the helices in TAP1 would penetrate the membrane is likely to be a mirror of the angle of the TAP2 helices, i.e. an altered packing of the helices in the two subunits. Where pseudo-2-fold symmetry is apparent in the three-dimensional views, the 2 halves seem to be rotated by a 180° rotation rather than a mirror plane (e.g. see the two lobes outlined in Fig. 7B, TAP1–2 184° and the footprints outlined in the 0°). This could imply a head-tail, head-tail organization that is observed for other heterodimeric membrane proteins (58–61). At the current resolution it is impossible to resolve individual helices in the potential TMDs, and thus, their orientation remains to be determined.

The published x-ray crystallographic structures of the NBD dimer HisP (32) and of the NBD homologue Rad50 dimer (31) have been filtered to 25 Å resolution for comparison with TAP (Fig. 7B). It is interesting to note the predicted cytoplasmic side of TAP has comparable dimensions to the NBDs and also shows two rotationally symmetrical 40-Å-diameter lobes (outlined in Fig. 7B, TAP1–2 184°). This two-lobed structure forms a cup shape or groove toward the top as seen in the 138° view. It is of similar dimensions and shape to the one formed by Rad50. Comparing the shape of the two lobes of TAP to the HisP dimer appears more difficult. Thus the organization of TAP NBDs may more closely resemble the Rad50 crystal structure packing rather than HisP. In the Rad50 dimer, each ATP is actually sandwiched between the signature motif of one monomer and the p-loop of the other, suggesting a high degree of cooperativity between monomers. Evidence for cooperativity of the NBDs is available for P-glycoprotein and also for TAP (36–38). Fluorescence studies with P-glycoprotein demonstrate fluorophore binding to its two Walker A motifs at only ∼22 Å apart in detergent solution and ∼16 Å in the membrane (62), which may support the Rad50 model. In the Rad50 dimer structure, the highly conserved signature sequence is involved in ATP binding, whereas in the HisP dimer structure, the signature motif is distant from the ATP binding site. This together with the dimer interface being more extensive in Rad50 than in HisP lends weight to the idea that the Rad50 packing may be more relevant to the situation in vivo. The extra mass provided by the cytoplasmic loops (16, 63, 64), which may be ∼30–40% of the total mass, complicates the resolution of the NBDs in our three-dimensional-structure. In the boomerang-shaped MshA structure, the intracellular domain loops connecting the TMD to the NBD are almost colinear with the TMD helices, but the NBD is rotated relative to this axis by a 45° kink. In the TAP and P-glycoprotein structures, there is no evidence for extensively protruding NBDs, but rather there is a relatively compact structure on the cytoplasmic face. A possible explanation for this may be that the two halves of the latter ABC transporters are more extensively kinked, allowing the NBDs to fold back to associate with each other and the TMDs.

Slices through TAP generated in Fig. 7A show internal features of the heterodimer, with TAP2 projections included for comparison. Comparisons of the projections of TAP with TAP2 proved difficult (e.g. TAP2 appeared too large, Fig. 4C), suggesting that the dominant views of TAP2 we obtained were from a different angle. However, slices through the three-dimensional structure of TAP may resolve this problem by looking more similar in size and shape to TAP2. The three-dimensional structure thus provides a means of reconciling the TAP heterodimer and TAP2 projection data. The selected slices cross the three-dimensional structure across two different planes, both intersecting a putative axis of pseudo-2-fold rotational symmetry. In comparison with TAP2 projections, it is not immediately apparent which sliced view is representative of the half-TAP subunit. The more asymmetric view (Fig. 7A, bottom center) may represent the different subunits, as the monomeric components (either TAP1 or TAP2) have an asymmetric primary structure made up of one NBD and one TMD. The end-on view in Fig. 5, where TAP1–2 is rotated 90° around the x axis, supports this interpretation as it shares similar features to TAP2 projection classes shown in Fig. 4C (bottom row, a–c). That TAP2 expressed on its own should retain at least some similar conformation compared to when complexed with TAP1 can be inferred both from its ability to bind ATP (Fig. 2), albeit weakly, and its retained ability to interact with MHC class I molecules, tapasin, and chaperones, features shared with rat TAP1 when expressed singly (40, 65).

This work represents the first structural imaging of the detergent-solubilized TAP ABC transporter, a transporter crucial to antigen presentation by MHC class I molecules, and the development of CD8+ T lymphocytes (10, 12, 66). The projection structure of TAP at low resolution is broadly comparable with that of other ABC transporters, P-glycoprotein, and MRP1 (27, 28). The first structural data for a eukaryotic half-transporter, TAP2, comprising a single transmembrane domain associated with a single nucleotide binding domain is also shown. In comparison to the bacterial half-transporter, MshA, the data implies an extensive kinking of the TAP2 molecule. A three-dimensional structure of TAP is presented that illuminates the heterodimeric nature of TAP and allows visualization of how the two subunits come together. The polarity of the molecule can be inferred from comparison to P-glycoprotein, which enables the mapping of domains onto the three-dimensional-structure. It also enables mapping of these features back to the NBD dimers, which sheds some insight into how the TMDs and NBDs may interact in vivo. Together, it can be seen how two separate TAP subunits can dimerize to form the complete ABC transporter. The model of TAP presented here forms the basic core of the TAP assembly complex. Using the information gained in this study we will now be able to perform similar purifications in milder detergent conditions such as occur with digitonin, whereby the interactions between TAP and MHC class I-calreticulin-Erp57-tapasin complexes are preserved (56). With up to four MHC class I-chaperone complexes interacting per TAP molecule, the predicted size of such a complex containing MHC class I/B2m, tapasin, calreticulin, and Erp57 would be ∼1000 kDa. This may assist in the localization of the as yet unknown sites of interaction between TAP and MHC class I-chaperone complexes, and the use of Fabs directed at individual components of the complex may yield information on their relative organization and orientation. Imaging of TAP therefore presents a unique model for the understanding of interactions between ABC transporters and their substrate receptors.

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