Leakage and aggregation of phospholipid vesicles induced by the BH3-only Bcl-2 family member, BID

Dayong Zhai, Qi Miao, Xiaofeng Xin and Fuyu Yang

National Laboratory of Biomacromolecules, Chinese Academy of Sciences, Beijing, China

BID is a BH3 domain-only member of the Bcl-2 family that acts as an apoptotic agonist in programmed cell death. After cleavage by caspase-8, the N-terminal of BID (N-BID) stays in the cytosol while the C-terminal of BID (C-BID) translocates to mitochondria, leading to cytochrome c release in vivo and in vitro. We have previously reported that BID or truncated BID (tBID) can induce the release of entrapped trypsin and cytochrome c from large unilamellar vesicles (LUVs). Further studies have been performed and are presented here; the results demonstrate that C-BID, like BID and tBID, induces vesicle leakage, whereas N-BID or the BID mutants BID (D59A) and BID (G94E) fail to have any significant effects. The affinity of the above-mentioned proteins for soybean phospholipid LUVs (SLUVs) decreased in an order similar to their leakage-inducing capability: tBID > BID > BID (D59A), while N-BID and BID (G94E) were unable to bind to the vesicles at all. BID-induced leakage was dependent on the lipid composition of vesicles. Acidic phospholipid (e.g. phosphatidic acid or phosphatidylglycerol) was necessary for BID-induced leakage while the presence of phosphatidylethanolamine or cholesterol reduced the leakage. It was also found C-BID is better able to penetrate the soybean phospholipid monolayer than BID or tBID. A further finding was that tBID, but not full-length BID, could stimulate the aggregation of SLUVs. Finally, Bcl-xL, an apoptotic antagonist in programmed cell death, can prevent the aggregation of LUVs induced by tBID, but not the release of entrapped trypsin. It is postulated that two separate domains of tBID are responsible for inducing leakage and aggregation of phospholipid vesicles.

Keywords: aggregation; apoptosis; BID; large unilamellar vesicles; leakage.

Apoptosis is an evolutionarily conserved process critical in various biological events, such as embryonic development, maintenance of tissue homeostasis, removal of noninstructed, misinstructed and damaged cells, and immunological defense [1]. Various stimuli, including developmental and environmental ones, deliver complex signals to promote apoptosis or survival. A large number of pro-apoptotic and anti-apoptotic molecules have been identified as playing principal roles in apoptosis, in which members of the Bcl-2 family act as regulators [2].

Mitochondria, which were once thought simply to generate energy for a cell, have been implicated as important sensors and regulators in apoptosis [2]. NMR analysis of Bcl-xL±BAK BH3 peptide complex has revealed both hydrophobic and electrostatic interactions between the Bcl-xL pocket and a BH3 amphipathic a-helical peptide from BAK [7]. Deletions in BAK and an extensive mutation analysis of BAK suggest that the BH3 domain serves as a minimal ‘death domain’ critical for both dimerization and killing [8].

The three-dimensional structure of Bcl-xL contains a bundle of seven α helices with two central, predominantly hydrophobic, helices forming the core of the molecule while BID also has two central hydrophobic helices surrounded by six amphipathic helices [9–11]. Both of these resemble pore-forming bacterial toxins although their homologous sequence is limited, suggesting they may have channel-forming potential. In fact, BAX, Bcl-xL and Bcl-2 have already been shown to have channel activity in artificial lipid membranes [12–14], and this has also been recently reported for BID [15]. The ability of these proteins to form ion channels has fostered the idea that they may open pores or produce breaks in the mitochondrial outer membranes, allowing exit of cytochrome c [16]. Moreover, BAX and BAK have been shown to stimulate the opening of the voltage-dependent anion channel, a mitochondrial channel through which cytochrome c permeates [17].

BID is a BH3 domain-only protein that lacks transmembrane domains and is predominantly localized in the cytosol [18]. It demonstrates unique and important properties after cleavage by...
caspase-8; the 15-kDa BH3-containing C-terminal translocates from cytosol to the mitochondrial membrane and leads to cytochrome c release, while N-BID remains localized in the cytosol in vivo [19,20]. The data from the structure of BID and the yeast two hybrid experiments suggest that N-BID has an inhibitory action associated with the C-terminal BH3 domain [10,11,21], which may represent an important means of regulating the activity of the whole BID molecule.

It is still unclear how BID leads to cytochrome c release from mitochondria. Some authors speculate that BID may cause cytochrome c release via formation of ion channels or pores [15]. Others, however, argue that BID may directly perturb the membrane integrity by interacting with membrane lipids [20]. Another putative function of BID is that tBID can trigger the clustering of mitochondria in cells during the process of apoptosis [20]. Li et al. suggest that two independent pathways for the BID-mediated destruction of mitochondria in cell death may operate, based on analysis of the structure of BID [10].

The relative simplicity of the model system based on pure lipid bilayers invites its use in the study of the molecular mechanisms of membrane–protein interactions. We have previously reported that BID can induce trypsin as well as cytochrome c release from the internal medium of LUVs [22]. Here, we used this model system to study the molecular mechanism underlying the actions of BID and other Bcl-2 proteins. Data show that in contrast to C-BID, the mutants of BID, BID (D59A), BID (G94E), and N-BID have almost no leakage-inducing ability. Moreover, tBID rather than BID can lead to the aggregation of LUVs. The leakage of vesicles by BID and the aggregation-inducing effect of tBID may be due to the different nature of the two hydrophobic domains, mimicking the functions of BID in vivo.

**MATERIALS AND METHODS**

**Materials**

Human BID, BID (D59A), BID (G94E) and caspase-8 expression plasmids were kindly provided by X. Wang (Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX, USA). The Bcl-2–GST expression plasmid was provided by J. Yuan (Department of Cell Biology, Harvard Medical School, Boston, MA, USA). The BAK-GST expression plasmid was obtained from Y. Tsujimoto (Biomedical Research Center, Osaka University, Japan). Trypsin, proteinase K, cholesterol, dioleoylglycerophosphatidic acid (DOPA), dioleoylglycerophosphoglycerol (DOPG), dioleoylglycerophosphoethanolamine (DOPE) and dioleoylglycerophosphocholine (DOPC) were obtained from Sigma. Ph-CO-Arg-OEt was from the Shanghai Institute of Biochemistry (Chinese Academy of Sciences, Shanghai, China).

**Expression and purification of recombinant proteins**

Human BID, BID (D59A), BID (G94E), BAK, Bcl-2, and caspase-8 were expressed recombinantly as has been described previously [19,20]. The plasmids for expression of the proteins were transformed into bacteria BL21 (DE3) cells; proteins were purified from the cell lysate using nickel affinity (Qiagen) or GST affinity chromatography. A Bio-scale Q5 column (Bio-Rad) was used for further purification. Truncated BID was obtained by adding 1 : 50 (vol/vol) caspase-8, and incubating overnight at 4 °C. After dilution, the sample was loaded on to a Bio-scale S5 column (Bio-Rad). Elution was performed with a linear gradient of 0 to 100 mM NaCl in 20 mM Hepes, pH 7.4, containing 10 mM KCl, 2 mM MgCl₂, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylene sulfonyl fluoride (buffer A). After re-equilibration with buffer A, the pH was raised to 12 to obtain C-BID.

**Liposome preparation and detection of liposome leakage**

LUVs were prepared as in Rietveld et al. [23]. Briefly, 2.5 mL of dioleoylglycerolphosphatidic acid (DOPC) were added to a dry lipid film of 5 μm phospholipid. After sonication for 20 min at 4 °C with a bath sonicator, and evaporation of ether under reduced pressure, the vesicles were dialyzed overnight at 4 °C in Pipes buffer. The vesicles formed were then fractionated by centrifugation in a Beckman Optima TLX table-top ultracentrifuge (TLA-100.3 rotor) at low speed (15 min, 5564 g, 4 °C) to discard the multilamellar vesicles, and high speed (30 min, 42 000 g, 4 °C) to collect the LUVs. The LUVs were washed at least three times to remove the nonenclosed trypsin. The phospholipid concentration was determined by perchloric acid destruction. Various concentrations of the test proteins were added to 100 μL volumes of the LUV suspension, and pre-incubated at 30 °C for 15 min. Ph-CO-Arg-OEt was introduced as substrate to measure the degradation rate by the released trypsin at 253 nm by using a Shimadzu UV-2101PC spectrophotometer.

**BID binding to LUVs**

LUVs were prepared as above without entrapped trypsin. BID, BID (D59A), BID (G94E), or N-BID (0.1 mg·mL⁻¹) was incubated at different lipid:protein molar ratios in 100 μL Pipes buffer for 1 h at 30 °C. The proteins binding to the LUVs were separated from the free parts by centrifugation at 96 000 g in a Beckman Optima TLX table-top ultracentrifuge (TLA-100.3 rotor) for 30 min at 30 °C. The protein concentration in the supernatant was determined by the Bradford method [24].

**Penetrating of the phospholipid monolayer by BID**

A film balance, type Han-2000, designed and made in our laboratory was used to study the membrane penetrating ability of proteins. Briefly, 3 mL of Pipes buffer as a subphase was added into the mini-trough, which has been described previously [25]. The monomolecular lipid layer was spread to give the desired initial surface pressure by dropping aliquots of lipid dissolved in chloroform on the aqueous surface. The surface pressure of the monolayer was measured by the Wilhelmy plate method using plates cut from filter paper and rinsed with methanol prior to use. After the initial surface pressure had stabilized to a plateau value, the sample was then rapidly mixed with bulk solution, and radically diffused to the upper monolayer-spreading disk. All measurements were performed at room temperature. Usually, an increase of the surface pressure Δp is measured as a function of the initial surface pressure p. A plot of Δp versus p yields a straight line with negative slope that intersects the abscissa at the value named as the limiting surface pressure.

**CD measurements**

CD measurements were carried out on a JASCO J-720 instrument. Measurements were taken using a 1-mm path
length. All spectra were recorded in 1.0-nm wavelength increments with a 1-s time constant and a full-scale sensitivity of 20 millidegrees. Each spectrum was the average of six scans corrected for background solvent effects by subtraction of the appropriate buffer blank. BID, BID (D59A), BID (G94E), and N-BID were diluted to a concentration of 20 m in NaCl/Pi buffer; C-BID was diluted in 10 m Tris buffer, pH 12. The lipid concentration was 1 m. Spectra were scanned in the far UV from 250 to 190 nm.

**Turbidity measurement**

The aggregation of SLUVs after the proteins had been introduced was monitored spectrophotometrically at 600 nm in a cuvette with 1-cm path length using a Shimadzu UV-2101 PC spectrophotometer. Aliquots of BID, tBID or tBID (G94E) were added successively to the suspension of SLUVs (1 m lipid concentration) in Pipes buffer. Truncated BID or tBID (G94E) pre-incubated with Bcl-xL at 4°C for 10 min (1:1, mol:mol) was introduced and aggregation detected as above. The molar ratios of lipid to protein ranged from 2000:1 to 100:1.

**RESULTS**

**Purification of BID, and its fragments and mutants**

Recombinant BID, BID (D59A), BID (G94E), BAK, Bcl-xL, and caspase-8 were purified in accordance with previously published methods [19,20]. Truncated BID was prepared as the product of BID cleaved by caspase-8 following overnight incubation at 4°C; N-BID was still tightly associated with C-BID in the buffer. After dilution, the tBID sample was loaded to the Bio-scale S5 column. The N-BID was easily washed out with 0.1 m NaCl, while C-BID could only be eluted when the pH value was above 12. The purity of the proteins was above 95% as determined by SDS/PAGE analysis with Coomassie blue staining (Fig. 1.).

**Comparison of trypsin release from LUVs induced by BID, C-BID, N-BID and its mutants**

Trypsin-containing LUVs have previously been used to monitor protein translocation across the model membranes [26]. Here this system was used to assay the BID-induced leakage of contents from the internal medium of the liposomes. Leakage is studied as a function of a constant lipid:BID ratio, BID concentration (lipid concentration kept constant), and lipid concentration (BID concentration kept constant). At a constant lipid:BID ratio of 2000:1, the extent of leakage after 15 min reduced as the lipid concentrations decreased (Fig. 2A). This is expected on the basis of the partitioning of the proteins between the bilayer and the aqueous phase; as the lipid concentration is...
lowered, the fraction of the peptide that becomes vesicle-associated decreases. When BID concentration was held constant, increasing the lipid concentration led to a decrease in the extent of leakage (Fig. 2B). As the number of vesicles in suspension grows with increasing lipid concentration, the results are in agreement with the pore-forming hypothesis. In other words, a defined number of peptides is required for the formation of a channel or pore in the bilayer. These data also indicate that once in bilayer, BID does not rapidly redistribute between different vesicles. Finally, at constant lipid, increasing BID concentration induced a greater extent of leakage until a maximum value was reached (Fig. 2C). This result is consistent with a monomer–multimer assembly pore-forming process being involved in the mechanism of leakage. In all cases, the lipid:BID ratios were determined from the amount of vesicles and BID initially added to the cuvette. The effective ratio depends on the lipid:water partition coefficient of BID.

When tBID is added to trypsin-encapsulated LUVs, it triggered release more efficiently than did the full-length BID. To investigate how BID destabilizes the LUVs, BID mutants and its fragments were examined. Both BID (D59A), a mutant that cannot be cleaved by caspase-8, and BID (G94E), which contains a point mutation in the BH3 domain, were unable to induce cytochrome c release from mitochondria. Strikingly, BID (D59A) and BID (G94E) were shown to lose the ability to induce trypsin release from LUVs at the same concentrations as BID did. A partial leakage of trypsin could only be observed when excessive amounts of these mutants were added (Fig. 3A).

After cleavage by caspase-8, C-BID translocates to mitochondria to induce the release of cytochrome c, whereas N-BID remains in the cytosol [19,20]. It is difficult to separate C-BID from N-BID in the experiments carried out in vitro, but a portion of C-BID could be precipitated by adjusting the pH of the medium to 7.0. The partially aggregated C-BID could trigger the release of entrapped trypsin from the liposomes. In contrast, N-BID was incapable of inducing leakage even at higher concentrations (Fig. 3B).

BAK, caspase-8 alone or BID proteolyzed with proteinase K were used as controls. None of these showed leakage-inducing activity. To investigate further whether BID is able to induce the release of other molecules, fluorescein sulfonate (FS) or cytochrome c were entrapped in LUVs. A similar releasing effect could also be observed following BID treatment (data not shown).

**BID-induced leakage of vesicles with different lipid compositions**

To test whether the charge of the membrane constitutes an important factor in destabilizing liposomes, the effect of BID or tBID on leakage of LUVs composed of DOPA and 1,2-dioleoylphosphatidylcholine, or DOPG and 1,2-dioleoylphosphatidylcholine, at ratios of 2 : 8, 3 : 7, 4 : 6, and 5 : 5 (mol/mol), were studied. The results indicated that none of the lipid matrices tested changed the ability of the proteins to induce leakage (data not shown). This confirms that membranes containing varying amounts of negatively charged phospholipids allow the proteins to act similarly. Regarding 1,2-dioleoylphosphatidylcholine-only LUVs entrapped with trypsin, the efficiency of induction decreased drastically (Fig. 4). It may deduced that a negative surface potential of a lipid bilayer is necessary for BID or tBID to bind to membranes during the initial step of the interaction, albeit that the motif.
directing leakage may be insensitive to the charge density on the membrane surface.

DOPE, the second most abundant phospholipid of mitochondria, or cholesterol was introduced into vesicles to investigate whether lipid composition would alter the interaction of BID with the vesicles. When the percentage of DOPE was about 10%, the efficiency of BID induction was not obvious. Adding 30% DOPE to bilayers containing 20% DOPG and 50% DOPC resulted in a steep decrease in the leakage efficiency (Fig. 4), indicated by an increase in the protein:lipid ratio needed to get the same final leakage (about ten-fold more protein was required). Similar results were obtained in the case of SLUVS (data not shown). Vesicles comprising 30% cholesterol, 20% DOPG and 50% DOPC also caused a clear drop in the induction efficiency of BID (Fig. 4). These vesicle leakage results resemble those obtained using the pore-forming peptide synthetic GALA, which is similarly affected by DOPE and cholesterol [27,28].

Comparison of the LUV binding of BID, and its fragments and mutants

To study whether the discrepancy in the competency of these proteins to induce leakage was attributed to the difference in their binding activity, the affinities of the BID proteins to the same LUVs were determined and compared. The SLUVs were incubated with BID, tBID, BID (D59A), BID (G94E) and N-BID. After centrifugation, free proteins in the supernatant were analyzed. BID proteins associated with SLUVs were plotted as a function of the lipid concentration (Fig. 5A); it can be observed that tBID has the highest affinity for SLUVs, BID (D59A) has a lower affinity for the membrane than does the wild type BID, and BID (G94E) and N-BID hardly bind to SLUVs.

Binding of BID to vesicles comprising: 20% DOPG and 80% 1,2-dioleoylphosphatidylcholine; 20% DOPG, 30% DOPE and 50% 1,2-dioleoylphosphatidylcholine; and DOPC only, was also compared. As shown in Fig. 5B, BID had a higher affinity for DOPG/DOPC vesicles than for DOPG/DOPE/DOPC vesicles, which was consistent with the disparity in the leakage-inducing activities with the two vesicle types. However, the discrepancy in binding affinity of tBID to these
vesicles was negligible. Compared with BID, tBID always showed higher affinity to the vesicles. Almost all of the BID proteins tested showed very weak binding affinity for the DOPC vesicles (data not shown). These results showed that the affinity of individual BID proteins to a particular membrane parallels their capacity to induce leakage.

Penetrance of the phospholipid monolayer

The experiments on vesicles were complemented by studying BID protein binding to a phospholipid monolayer. An increase in the surface pressure will be observed where the proteins have penetrated the phospholipid monolayer. As shown in Fig. 6A, at an initial surface pressure of about 20 mN·m⁻¹, C-BID had the highest penetrating ability among these proteins. The order was then tBID, BID and BID (D59A); BID (G94E) and N-BID had almost no penetrating ability. The surface pressure did not increase when BID was pretreated with proteinase K, indicating that the rise in surface pressure reflected the association of BID with the monolayer.

When using the BID protein-induced increase in surface pressure as a function of the initial surface pressure, an inverse linear relationship appears (Fig. 6B). The lipid-packing density of the liposomes used was equivalent to a monolayer surface pressure of 32–35 mN·m⁻¹ [25]. Our results indicate that BID could, conceivably, penetrate monolayers of soybean phospholipid. That the limiting surface pressure for C-BID, 44 mN·m⁻¹, was much higher than that for BID, 36 mN·m⁻¹, or tBID, 41 mN·m⁻¹, (Fig. 6B) implies that BID exposes more hydrophobic residues after cleavage with caspase-8.

Secondary structural changes in BID on interaction with phospholipid

The secondary structures of BID, BID (D59A), BID (G94E), tBID, N-BID and C-BID were determined and compared using CD spectra. The only minor difference was found in the shape or amplitude of the far-UV spectrum among BID, BID (D59A), BID (G94E), tBID and C-BID, while N-BID showed little α helix but an abundance of β structure (data not shown). No obvious differences occurred in any of the above proteins after lipid was introduced (data not shown). The high α-helix content of BID is reminiscent of the channel-forming Bcl-xL, Bcl-2 and BAX proteins, and the structurally related bacterial toxins such as the pore-forming colicins and diphtheria toxin [15].

Aggregation of SLUVs induced by tBID

In addition to inducing leakage from liposomes, it was interesting to note that tBID, but not full-length BID, could stimulate the aggregation of SLUVs, and that such aggregation was dependent on the protein concentration. Significant aggregation was observed only at BID concentrations greater than 1 μM (tBID : lipid of approximately 1 : 1000, mol/mol). The line in Fig. 7A shows the time course of the tBID-induced aggregation. Neither proteinase K-treated tBID, caspase-8 nor BAK caused aggregation. The turbidity was unaffected even when these proteins were tested at ratios up to 1 : 25 (protein : lipid, mol/mol).

It has been reported that Bcl-xL can counteract the function of BID, particularly tBID, due to the interaction of the hydrophobic cleft of the former with the BH3 domain of the latter [19]. This is supported by the results described in Fig. 7B; Bcl-xL could prevent the aggregation of LUVs induced by tBID, but had no obvious influence on tBID (G94E), in which the BH3 domain is changed by point mutation. Li et al. have described similar phenomena where overexpression of tBID has led to the clustering together of mitochondria in vivo [20].

**DISCUSSION**

BH3 domain-only proteins have been previously viewed as transdominant inhibitors that rely exclusively on dimerization with other Bcl-2 family proteins to exert effects on cell life and death [15]. In this work, we have further demonstrated that BID can directly induce the release of entrapped trypsin and cytochrome c release from LUVs, as has been previously reported [22]. The experiments described here were designed to understand the intrinsic activity of BID as a membrane-integrating or pore-forming entity in the model membrane system.

Firstly, we tested the hypothesis that BID causes leakage by a detergent-like mechanism. In our experiments with BID...
concentration kept constant, increasing the lipid concentration resulted in a decrease in the extent of leakage, while decreasing the concentration of the lipid led to a reduction in leakage. This finding is in disagreement with the detergent-like hypothesis, but is consistent with a monomer–multimer assembly pore-forming process [29]. Decreasing the lipid concentration lowers the extent of leakage at a constant lipid:BID ratio, which would confirm this.

It has been reported that lipid composition may affect the efficiency of leakage induction by a pore-forming peptide. For example, cholesterol significantly reduces the efficiency of leakage by the synthetic peptide GALA [27]. Our results also showed that cholesterol can lead to a clear drop in the efficiency of BID-induced leakage. It can be envisaged that penetration of BID into more rigid membranes is less favorable for pore forming. 1,2-Dioleoylphosphatidylethanolamine also has an obvious effect on BID-induced leakage. Such a reduction is partially due to the decrease in the binding affinity of BID protein for the liposomes, though it seems that for the same number of membrane-bound BIDs per vesicle, the amount of leakage from DOPE-containing liposomes is still several-fold less than that from the DOPA/DOPC or DOPG/DOPC vesicles. During the preparation of the present paper, Szoka et al. [28] reported that GALA-induced 1-aminoanaphthalene-3,6,8-trisulfonic acid/p-xylene-bis-pyridinium bromide (ANTS/DPX) leakage is also decreased when the vesicles contain DOPE. They attribute the reduction to the lowering of bilayer deformation energy caused by GALA aggregations that are adsorbed on the membrane surface in the presence of DOPE. Our results also show the influence of DOPE on the efficiency of leakage induced by BID. We postulate that the presence of DOPE, which is well known as a nonbilayer-forming phospholipid, may be unfavorable for the formation of pore structures, as in the case of GALA. It was reported previously that proteins such as Bcl-2 and Bcl-xL are almost unable to form ion channels in neutral vesicles [12–15]. In our case, a small amount of BID was found to bind with DOPC vesicles; hence only a small amount of entrapped molecules could be released. The results obtained with DOPE, cholesterol and 1,2-dioleoylphosphatidylethanolamine-containing vesicles further indicate that the pore-forming rather than the micellization mechanism may account for the BID-induced leakage.

The results of binding experiments with the monolayer may indicate that part of the differences between leakage-induction efficiencies of the different BID proteins is due to differences in binding affinities for liposomes, particularly in the case of BID mutants and fragments. Compared with wild-type BID, an excessive amount of BID (G94E) is required for the mutant to induce partial leakage. This result is in line with the lower affinity of BID (G94E) to vesicles. Hence, we can conclude that the BH3 domain of BID is necessary for its binding to membranes.

The difference in the leakage activity of BID (D59A) and wild-type BID is unexpected, because the mutation site of BID (D59A) is not in the BH3 domain or other helices. Although its binding activity with liposomes partially remains, it is no longer capable of inducing leakage of LUVs. As a consequence of the point mutation, a suitable conformation of BID for pore formation may not be attained.

Fragments of BID were used to identify which part may be responsible for inducing LUV leakage. The results showed that C-BID keeps the leakage-inducing activity, while N-BID loses both the ability to bind to liposomes and leakage-inducing activity. We can propose that C-BID is responsible for inducing leakage of vesicles. Moreover, the results from monolayer experiments also show that C-BID has the highest potential ability to penetrate into membranes compared with BID or tBID.

The N-terminal segment itself has no leakage-inducing ability, but may serve as an inhibitor of pro-apoptotic activity. The N-terminal domain remains intact at approximately the same position and orientation after caspase-8 cleavage [10,11], but even in the uncleaved BID, the N-terminal helices may temporarily detach from the protein core, leading to some activity of the uncleaved protein [10]. Our results showed that full-length BID contains much of its intrinsic function leading to leakage of liposomes, which may be due to the temporary detachment of N-terminal segment.

In addition to inducing the passage of cytochrome c, tBID can trigger the clustering of mitochondria around the nucleus to form a ring in the early stage of apoptosis after transient transfection of tBID-GFP into an ec dysone-inducible system [20]. In the present paper, it is interesting to note that tBID, but not full-length BID, shows the ability to aggregate SLUVs. The extent of aggregation depends on the amount of tBID added. These results mimic the function of tBID in vivo [20]. Full-length BID can induce the leakage but is unable to aggregate LUVs.

Recently, the three-dimensional structures of both BID and tBID have been solved [10,11]. The surface electrostatic potential of BID does not reveal any unusually charged regions. However, two hydrophobic patches appear on the surface. One is the BH3 domain, where four partially conserved hydrophobic residues are exposed (I82, I86, L90, M97), the other is a large hydrophobic cleft formed by L105, Y140, V150, L151 and L154. The biological implication of the BID hydrophobic cleft remains to be investigated. Inasmuch as cleavage by caspase-8 can greatly enhance the pro-apoptotic activity of BID, it has been hypothesized that such a dramatic activation is accompanied by a conformational change after cleavage. However, the overall structural integrity of BID is preserved on caspase-8 cleavage. Minor conformational change predominantly occurred in the loop region near the cleavage site [10], but the results from McDonnell et al. [11] suggest that a higher extent of the BH3 domain may be exposed following cleavage. Considering the difference between BID and tBID in inducing the aggregation of LUVs, we postulate that different domains of BID molecule may be responsible for the aggregation and leakage of LUVs. In other words, the BH3 domain of tBID plays its main role in the clustering of liposomes, the BH3-independent hydrophobic cleft is more important for inducing leakage. Insertion of the hydrophobic H6 and H7 helical hairpin perpendicularly into the lipid bilayer may form putative pores, structurally similar to the pore-forming domain of bacterial toxins [30]. This is further supported by the observation that Bcl-xL can counteract tBID-induced aggregation as the BH3 amphipathic helix of tBID binds to the hydrophobic cleft of Bcl-xL [20]. Adding Bcl-xL to tBID (G94E), which has a point mutation in the BH3 domain, fails to prevent LUV aggregation. Furthermore, it is interesting to note that, in contrary to the results obtained with mitochondria in vivo, Bcl-xL cannot inhibit the activity of BID or tBID-induced leakage of LUVs. Possibly, in the former case, a BH3-dependent pathway for inducing leakage of mitochondrial membrane may co-exist with a BH3-independent pathway [20].

The in vivo assays using model membrane systems have facilitated the study of the mechanism by which BID damages mitochondria during apoptosis. Results from different approaches showed that the pore-forming rather than the micellization mechanism may account for the BID-induced
leakage of liposomes. Based on the results obtained, we tentatively suggest that the BH3 domain and the hydrophobic cleft of tBID may be responsible for the aggregation and leakage of phospholipid vesicles, respectively.

ACKNOWLEDGEMENTS

This work was financially supported by the National Natural Science Foundation of China (39730130) and the Chinese Academy of Sciences. We thank X. Wang, J. Yuan and Y. Tsujimoto for their generous gifts. We are also grateful to X. Han and X. Hang for their interesting discussions.

REFERENCES