Bax Is Present as a High Molecular Weight Oligomer/Complex in the Mitochondrial Membrane of Apoptotic Cells*

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Mitochondrial Membrane of Apoptotic Cells*

Apoptosis is mediated through two major pathways, the death receptor pathway and the mitochondrial pathway (1). The mitochondrial pathway is controlled and regulated by the Bcl-2 family of proteins (2–4). This protein family can be divided into anti-apoptotic (Bcl-2, Bcl-XL, Bcl-w, Mcl-1, A1) and proapoptotic (Bax, Bak, Bok/Mtd, Bcl-Xs, Bid, Bad, Bik/Nbk, Bim, Blk) members (5–7). Although the overall amino acid sequence homology between the family members is relatively low, they contain highly conserved domains, referred to as Bcl-2 homology domains (BH1 to -4). The activity of these proteins appears to be regulated, at least partly, by formation of homo- and heterocomplexes (8–13). The conserved BH domains are involved in these interactions. In the proapoptotic proteins, Bax and Bak, the BH3 domain is essential for complex formation as well as for their “killing” effect (4, 14–17). The hydrophobic C-terminal domain present in some of the proteins has been implicated in targeting the proteins to the mitochondrial membrane. Bcl-2 prevents Bax oligomerization and insertion into the mitochondrial membrane. The outer mitochondrial membrane protein voltage-dependent anion channel and the inner mitochondrial membrane protein adenosine nucleotide translocator do not coexist with the large molecular weight Bax oligomers/complexes on gel filtration. Bax oligomerization appears to be required for its proapoptotic activity, and the Bax oligomer/complex might constitute the structural entirety of the cytochrome c-conducting channel in the outer mitochondrial membrane.

Bax is a Bcl-2 family protein with proapoptotic activity, which has been shown to trigger cytochrome c release from mitochondria both in vitro and in vivo. In control HeLa cells, Bax is present in the cytosol and weakly associated with mitochondria as a monomer with an apparent molecular mass of 20,000 Da. After treatment of the HeLa cells with the apoptosis inducer staurosporine or UV irradiation, Bax associated with mitochondria is present as two large molecular weight oligomers/complexes of 96,000 and 260,000 Da, which are integrated into the mitochondrial membrane. Bcl-2 prevents Bax oligomerization and insertion into the mitochondrial membrane. The outer mitochondrial membrane protein voltage-dependent anion channel and the inner mitochondrial membrane protein adenosine nucleotide translocator do not coexist with the large molecular weight Bax oligomers/complexes on gel filtration. Bax oligomerization appears to be required for its proapoptotic activity, and the Bax oligomer/complex might constitute the structural entirety of the cytochrome c-conducting channel in the outer mitochondrial membrane.

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The three-dimensional solution structure of the full-length Bax protein has recently been solved by NMR (33). The Bax structure shows a high similarity to the overall conformation of the two other Bcl-2 family proteins for which structural information is available, the antiapoptotic protein Bcl-Xl and the BH3 domain-only protein Bid (34–36). The proteins contain central hydrophobic helices (a5 and a6) surrounded by amphipathic helices. Of the three proteins, the Bax structure is the only one that contains the C-terminal hydrophobic domain. This domain forms a helix (a9) that protects the BH3-containing hydrophobic cleft on the protein. The structures of the Bcl-2 proteins are reminiscent of diphtheria toxin and the colicins A and E1. These toxins are pore-forming proteins that function as membrane channels that allow passage of ions or small polypeptides. Bax and other Bcl-2 family proteins have been shown to possess channel-forming activity in artificial membranes (37–40).

We have shown that in contrast to oligomeric Bax, monomeric recombinant Bax cannot form channels in liposomes nor trigger cytochrome c release from isolated mitochondria (41). Recently, Bax tetramers were shown to form a channel large enough to allow the release of cytochrome c from liposomes (42). Here we show that, in cultured cells exposed to the apoptosis inducer staurosporine or UV irradiation, Bax forms oligomers, which possibly form complexes with yet unidentified mitochondrial membrane proteins. The Bax oligomers are found inserted into the mitochondrial membrane. Moreover, we show that in the presence of Bcl-2, Bax oligomer formation and insertion into the mitochondrial membrane is inhibited.

MATERIALS AND METHODS

The Superdex 200 (16/60) column was from Amersham Pharmacia Biotech, and 14% polyacrylamide gels and 10% NuPage gels were from

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Novex (San Diego, CA). CHAPS\(^1\) was from Roche Biochemicals, and Triton X-100 was from Fluka. Polyclonal anti-Bax antibodies were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY), monoclonal anti-Bax antibodies were from R & D Systems (Minneapolis, MN), monoclonal anti-Bcl-2 antibodies were from Genosys (Cambridge, England), polyclonal anti-Haemagglutinin; Mops, 4-morpholinepropanesulfonic acid; rBax, recombinant monomeric Bax.

Cytosolic samples were centrifuged at 100,000 \(g\) and resuspended in 1% Triton X-100. Protein concentration was measured with the BioRad protein assay kit.

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RESULTS

Bax in Extracts from Control and Apoptotic HeLa Cells—Cultured HeLa cells have been used to study the quaternary structure of Bax in apoptotic cells. Apoptosis was induced by culturing the cells in the presence of 1 μM staurosporine for 16 h or by UV irradiation. Consistent with published results, in untreated HeLa cells, Bax was found both in the cytosol and associated with the mitochondria (Fig. 1). In apoptotic cells, Bax almost completely disappeared from the cytosol. Although a small increase in Bax associated with the mitochondria was seen in the apoptotic cells, this does not fully account for the decreased cytosolic Bax concentration (Fig. 1), suggesting that cytosolic Bax that is not translocated to the mitochondria is eliminated in apoptotic cells, possibly through proteolysis (47).

Bax Quaternary Structure in Triton X-100 Mitochondrial Extracts from Control and Apoptotic HeLa Cells—To study the quaternary structure of Bax associated with mitochondria from control and apoptotic HeLa cells, mitochondrial proteins were extracted with 2% Triton X-100 as described under “Materials and Methods.” The soluble mitochondrial extracts were analyzed on gel filtration, and the fractions from the column were analyzed by Western blot with anti-Bax antibody. Gel filtration separates molecules according to the Stokes radius and gives an estimate of their molecular weights and thus of their quaternary structure. Extracts from control or apoptotic HeLa cell mitochondria eluted as a single peak at fractions 20–24, corresponding to a molecular mass of 260,000 Da (Fig. 2). Monomeric Bax was found neither in control nor apoptotic mitochondrial extract. Furthermore, analysis of other mitochondrial membrane proteins that have been suggested to interact with Bax, including Bak, Bel-XL, and VDAC, all eluted with Bax as high molecular weight complexes in fractions 20–24. Again, no difference was observed between mitochondrial extracts from control and apoptotic cells (Fig. 2). However, the mitochondrial membrane protein Cox IV eluted with a main peak in fractions 28–30 and a minor peak in fractions 22–24. The void volume of the column was at fraction 12.

Triton X-100 has been reported to induce dimerization of Bax and other Bel-2 family members (48). In a previous study, we reported that Triton X-100 was able to trigger oligomerization of soluble monomeric Bax (41). Therefore, Triton might have induced the oligomerization of Bax and possibly other proteins extracted from the mitochondrial membrane, masking any differences in protein quaternary structure between control and apoptotic cells. Previous studies on the ability of various detergents to trigger Bax oligomerization showed that in contrast to Triton X-100 and octyl glucoside, CHAPS had no effect on Bax quaternary structure (41). Therefore, we examined whether CHAPS could extract Bax from mitochondria. As shown in Fig. 3, 2% CHAPS was as efficient as 2% Triton X-100 in extracting Bax from the mitochondrial membrane of both control and apoptotic cells. This is an important control to ensure that we were not extracting and analyzing only a subpopulation of Bax from the mitochondria.

Bax Quaternary Structure in CHAPS Mitochondrial Extracts from Control and Apoptotic HeLa Cells—Mitochondria were isolated by differential centrifugation from control and apoptotic HeLa cells, proteins were extracted with 2% CHAPS as described under “Materials and Methods,” and the soluble mitochondrial extracts were analyzed on gel filtration. In the mitochondrial extract from control cells, Bax eluted in fractions 36–40. This corresponds to a molecular mass of 20,000 Da, which is close to the theoretical calculated molecular mass of monomeric Bax, 22,000 Da (Fig. 4A). In contrast, in the mitochondrial extract from cells where apoptosis had been induced by staurosporine treatment, Bax eluted mainly as large oligomers in fractions 20–28. Two peaks with estimated molecular masses of 260,000 and 96,000 Da were consistently detected (n = 9) (Fig. 4A). The amount of monomeric Bax eluting in fractions 36–38 varied between preparations and could depend on the percentage of cells that had entered apoptosis at the time of harvest. Identical results were obtained with a monoclonal anti-Bax antibody, confirming that the protein detected was indeed Bax (results not shown).

Western blot analysis with marker antibodies for various organelles showed that the mitochondria isolated by differential centrifugation was contaminated mainly by ER (Fig. 5). To ensure that the Bax oligomers were associated with the mitochondria, the mitochondrial fraction was further purified on a sucrose gradient; this purification step improved the purity of the preparation. The sucrose gradient-purified mitochondria confirmed the result; oligomeric Bax (fractions 20–28) was found in the mitochondrial extract from apoptotic cells, whereas monomeric Bax (fractions 36–40) was present in both apoptotic and control mitochondrial extracts (Fig. 4B).

In addition, inducing apoptosis by exposing HeLa cells to UV irradiation also induced oligomerization of Bax associated with
the mitochondria. Similar to mitochondrial extract from staurosporine-treated cells, two peaks of Bax oligomers at fractions 20–22 (Mr 260,000) and fractions 26–28 (Mr 96,000) were detected on gel filtration (Fig. 6). Furthermore, we treated isolated HeLa mitochondria with 50 nM caspase 8-cleaved Bid for 30 min. The extract from the Bid-treated mitochondria contained the same Bax oligomers as mitochondria from staurosporine- or UV-treated HeLa cells (Fig. 6). Thus, inducing apoptosis by three different stimuli all resulted in Bax oligomerization.

Composition of the Bax Oligomers—In addition to its interactions with Bcl-2 family members, Bax has also been reported to interact with other mitochondrial membrane proteins. Interactions with the outer mitochondrial membrane protein VDAC and the inner membrane protein ANT, both part of the permeability transition pore, have been reported (49, 50). To investigate whether the Bax oligomers contained these proteins, the fractions from the gel filtration column were analyzed for Bak, Bcl-XL, VDAC, and ANT (Fig. 4A).
Two major peaks of Bak were detected, eluting in fractions 24–26 and 30–32. A low amount was also present over a wide molecular weight range (fractions 14–32), which suggests various forms of oligomers and/or complexes. However, no difference in the distribution between control and apoptotic cells was detected. Thus, since the Bax oligomers are not present in the mitochondrial membrane of nonapoptotic cells, interactions between Bak and the Bax oligomers are unlikely. Bcl-XL was similarly detected over a large range (fractions 16–32) with a major peak in fractions 26–32. As for Bak, no difference between control and apoptotic cells was detected.

VDAC eluted after the Bax oligomers (fractions 20–28) as a sharp peak at fractions 30–32 (48,000 Da) in the mitochondrial extract from both control and apoptotic cells. ANT eluted before the Bax oligomers at fractions 14–16 (650,000 Da). No difference was detected between the control and apoptotic mitochondrial extracts. These results show that neither VDAC nor ANT is part of the Bax oligomers extracted from mitochondria of apoptotic cells.

The Bax Oligomers—The elution profile of oligomeric recombinant Bax from the gel filtration column showed two peaks
eluting at fractions 22–24 ($M_r$ 160,000) and 27–29 ($M_r$ 80,000) (Fig. 6). This indicates that recombinant Bax forms two distinct oligomers containing different numbers of monomeric units. It appears that the larger oligomer (160,000 Da) could be a dimer of the smaller oligomer (80,000 Da). In the mitochondrial extract from apoptotic HeLa cells, we also detected two Bax peaks, the first peak eluting at fractions 20–22 ($M_r$ 260,000) and the second peak at fractions 26–28 ($M_r$ 96,000) (Figs. 4 and 6). The Bax oligomers from apoptotic mitochondrial extracts eluted at higher molecular weights compared with the recombinant protein. This difference in apparent molecular weight might indicate that in the mitochondrial membrane the Bax oligomers form stable complexes with as yet unidentified proteins.

To further investigate the composition of the Bax oligomers, we overexpressed Bax fused to three different epitope tags (HA, Myc, and His) in HEK cells. Since overexpression of Bax induces apoptosis, the transfected cells were cultured in the presence of the caspase inhibitor ZVAD to prevent transfected cells from dying and thus selecting for nontransfected cells. We verified by Western blot that the tagged Bax was localized, like endogenous Bax, both in the cytosol and the mitochondria (Fig. 7A). Mitochondria were then isolated from transfected and untransfected HEK cells, membrane proteins were extracted with 2% CHAPS, and the extracts were analyzed on gel filtration. To the cytosol from the transfected cells, 2% CHAPS was added, and the samples were analyzed on gel filtration. In the mitochondrial extract from untransfected cells and in the cytosol from transfected cells, only Bax monomers were detected (Fig. 8). However, in the mitochondrial extract from transfected cells (apoptotic cells), Bax oligomers similar to those in staurosporine or UV-irradiated HeLa cells were detected (Fig. 8). The majority of Bax in the oligomers was endogenous untagged Bax; thus, with the anti-Bax antibodies we did not detect the upper band corresponding to HA- and Myc-tagged Bax. To ensure that the tagged proteins were part of the oligomers, we...
further analyzed the fractions of the mitochondria extract from the transfected cells for the presence of tagged Bax. Although the concentration of the tagged proteins was low, we detected all three tags in the fractions containing Bax oligomers, fractions 18–30 (Fig. 8). To further investigate the composition of the Bax oligomers, we performed cross-linking experiments on isolated mitochondria from the Bax-transfected cells as described under “Materials and Methods.” Bax was subsequently immunoprecipitated with anti-His antibodies, and the precipitated sample was analyzed on Western blot with anti-Bax, anti-HA, and anti-Myc antibodies. As seen in Fig. 7B, Bax oligomers precipitated with anti-His antibodies were reactive to Bax, HA, and Myc antibodies. On the contrary, no immunoreactivity was detected with anti-VDAC antibodies. These results show that the Bax oligomers from mitochondria of apoptotic cells are composed of multiple Bax monomers and are not only Bax monomers in complex with other proteins. Bax oligomers were not detected in the cytosol (Fig. 7B). It has been shown that in unactivated monomeric Bax the N terminus is not exposed and not immunoreactive (46); this presumably explains why we did not precipitate the monomeric N-terminal His-tagged Bax in the cytosol. The molecular weights of the Bax-reactive bands in the mitochondrial extract were estimated, and the corresponding number of Bax molecules in the complexes were calculated (Fig. 7C). Bands corresponding to Bax mono-, di-, tri-, and tetramers were identified. Although higher molecular weight bands can be detected, the molecular weights cannot be estimated in this region of the SDS-PAGE separation. One band (Fig. 7C, band d) between a Bax trimer and tetramer could correspond to a complex with an unidentified protein of ~15 kDa.

**Bax Localization in the Mitochondria**—We have recently shown that Bid treatment of isolated HeLa mitochondria results in the insertion of Bax into the outer mitochondrial membrane (51). Here we investigated whether the change in Bax quaternary structure was required for integration into the mitochondrial membrane during apoptosis. We treated mitochondria from control and apoptotic cells with 0.1 M Na$_2$CO$_3$, pH 12, which solubilizes proteins attached to the membrane, whereas proteins integrated into the lipid membrane remain associated with the membrane fraction. After this treatment, the soluble (attached proteins) and the membrane fraction (integrated proteins) were analyzed by Western blot. We confirmed the findings by Eskes et al. (51) that in control cells Bax was only attached to the mitochondrial membrane, whereas in the apoptotic cells most of the protein was inserted into the membrane (Fig. 9A). Bak and Bcl-X$_L$ were integrated into the membrane in both control and apoptotic cells. As expected, the membrane protein VDAC was found to be integrated into the membrane in both samples. As shown in Fig. 4, mitochondria from apoptotic cells contained both monomeric and oligomeric Bax. We then investigated the nature of the quaternary structure of membrane-integrated Bax. As shown in Fig. 9B, membrane-integrated Bax was present as the oligomer. A low amount of monomeric Bax was detected in the sodium carbonate-washed mitochondrial pellet. However, this was also detected in the control cells and is presumably a contamination of attached protein that was not completely removed.

**Bax Oligomerization Does Not Appear in Bcl-2-overexpressing Cells**—A HeLa cell line overexpressing Bcl-2 (HeLa-Bcl-2) is resistant to staurosporine-induced apoptosis (44). We treated these cells with staurosporine for 16 h and analyzed Bax associated with the mitochondria of treated and control cells. Both control and staurosporine-treated cells contained only Bax...
monomers. No Bax oligomers were detected in mitochondria isolated from staurosporine-treated cells (Fig. 10). Bcl-2 eluted in fractions 26–30 in both control and staurosporine-treated cells. No difference was found in the elution profiles of Bak, Bcl-XL, and VDAC was detected between control and apoptotic cells. To determine whether Bax had been inserted into the mitochondrial membrane in the staurosporine-treated cells, we treated the isolated mitochondria with sodium carbonate. As seen in Fig. 9C, the monomeric Bax was only found attached to the mitochondrial membrane; no Bax was inserted in the membrane of this staurosporine-treated cells. Bcl-2 was found to be inserted into the mitochondrial membranes from both control and staurosporine-treated cells (Fig. 9C).

**DISCUSSION**

The release of cytochrome c from mitochondria has been shown to play a crucial role in many apoptotic signaling cascades through the activation of the downstream effector caspases (28). Several studies point to Bax as a trigger of apoptosis through the activation of the downstream effector protein known to play a crucial role in many apoptotic signaling cascades.

Bax triggers cytochrome c release. (29–31). Based on the ability of this protein to form channels in synthetic lipid membranes, several models have been proposed that would allow Bax to form a cytochrome c-conducting channel. Bax oligomerization and insertion in the outer mitochondrial membrane resulting in a “Bax-only” channel or hybrid channels formed by Bax binding to VDAC or ANT represent alternative possible mechanisms (52, 53). Thus, at the molecular level, it remains unclear how Bax triggers cytochrome c release.

Here, using gel filtration analysis and cross-linking, we show the formation of high molecular weight Bax oligomers in mitochondrial membranes of apoptotic cells. Previous cross-linking experiments of Bax in mitochondrial membranes, followed by immunoprecipitation and Western blot analysis, have revealed the existence of several Bax-immunoreactive bands, suggesting the presence of Bax oligomers (51, 54). The use of Bax fused to various tags allowed us, for the first time, to demonstrate the formation of Bax oligomers during apoptosis. A direct relationship between formation of large Bax oligomers and apoptosis was further demonstrated by gel filtration analysis. Analysis of membrane proteins requires solubilization with detergents. The choice of detergent is critical, since the protein quaternary structure and complex interactions may be affected by the detergent (55, 56). Hsu and Youle (48) have reported that cytosolic Bax from thymic cells displayed different conformational states that depended on the detergent to which they were exposed. That certain detergents induce significant conformational changes in Bax was shown in a recent publication by Suzuki et al. (33). In the study by Suzuki et al. (33), results from NMR experiments support the formation of large Bax oligomers. In agreement with these data, we found that Triton X-100, but not CHAPS, was able to trigger oligomerization of pure recombinant monomeric Bax (rBax) (41). Importantly, unlike Bax, Bcl-2 and Bcl-XL monomers did not oligomerize in the presence of various detergents, including Triton X-100 (41, 57). Indeed, as expected, when we extracted mitochondrial membrane proteins from control and apoptotic cells with Triton X-100, Bax consistently eluted on gel filtration chromatography as a high molecular weight oligomer. In fact, several proteins analyzed, including Bak, Bcl-XL, and a major component of the permeability transition pore, VDAC, that has been reported to interact with Bax, coeluted with Bax in a discrete peak corresponding to a molecular mass of 260,000 Da. However, the mitochondrial membrane protein Cox IV did not coelute with the 260,000-Da complexes, indicating that the extraction procedure did not induce a general protein aggregation or only a fragmentation of the mitochondrial membrane.

The fact that CHAPS does not trigger oligomerization of recombinant and cytosolic monomeric Bax indicated that CHAPS was a more appropriate detergent to study Bax oligomerization in mitochondria during apoptosis. Using this detergent, we found that while mitochondrial Bax from control cells eluted as a monomer on gel filtration, the protein from mitochondria of apoptotic cells consistently eluted as large molecular weight oligomers, which were distributed in two major peaks corresponding to masses of 260,000 and 96,000 Da. This Bax elution profile was obtained in mitochondrial extracts from HeLa cells undergoing apoptosis induced by either staurosporine or UV irradiation. Moreover, we found that the addition of caspase 8-cut Bid, a Bax activator, to mitochondria isolated from HeLa cells was able to trigger the formation of similar large molecular weight Bax oligomers. These data suggest that oligomerization of Bax and its ability to form large oligomers may be a phenomenon common to several apoptotic pathways involving Bax. The Bax oligomers were always tightly inserted in the mitochondrial membranes, and they were never detected in the cytosol, suggesting that they may form at or within the outer mitochondrial membrane. However, we cannot rule out the possibility that these large Bax oligomers may form outside of mitochondria and, once formed, rapidly insert into the outer mitochondrial membrane, preventing their cytosolic detection. This latter hypothesis would be consistent with earlier data showing that Bax translocate from the cytosol to mitochondria during apoptosis (58–60) and that enforced dimerization of Bax is accompanied by its insertion in the mitochondrial membrane (54). However, in the latter study, the Bax-enforced dimers failed to trigger cytochrome c release from mitochondria, suggesting that Bax dimers are not the active quaternary structure. A recent study shows that Bax is able to trigger the release of cytochrome c from liposome. The quaternary structure of the cytochrome c-conducting Bax channels was estimated to be a tetramer (42).

Interestingly, we found that in Bcl-2-overexpressing cells that are resistant to staurosporine-induced apoptosis (44), Bax oligomerization did not occur. This result is consistent with our previous data showing that Bcl-2 prevents the conformational change of Bax, which precedes its activation and membrane insertion (46, 51). In the Bcl-2-overexpressing cells, Bcl-2 was found both in the cytosol and inserted in the mitochondrial membrane. It is unclear which of these two Bcl-2 pools prevents the conformational change that Bax undergoes during apoptosis.

Recently, we have shown that soluble monomers of rBax isolated from *E. coli* in the absence of detergent fail to display channel activity in liposomes and are unable to trigger cytochrome c release from liver mitochondria (41). However, when rBax is extracted from *E. coli* membranes with CHAPS, it is recovered as oligomers. Importantly, these oligomers display channel activity, indicating that CHAPS extraction is compatible with the preservation of Bax activity (data not shown). Strikingly, when we analyzed pure rBax extracted from *E. coli* membranes with CHAPS or Triton X-100 by gel filtration, two oligomer populations of 160,000 and 80,000 Da were detected. The significantly higher molecular mass of the Bax oligomers from mitochondrial membranes compared with the rBax oligomers suggests either a difference in the number of Bax subunits composing the oligomers or an association with other mitochondrial proteins.

In addition, we used gel filtration to examine the mitochondrial membrane extracts for the presence of other Bcl-2 family members (Bak, Bcl-XL, Bcl-2) as well as for VDAC and ANT. We observed that neither VDAC nor ANT copurified with Bax. Moreover, VDAC and ANT were eluted in different fractions. This result is consistent with previous data that showed that...
when ANT was extracted with CHAPS it was recovered bound to cyclophilin D alone, whereas in Triton X-100 extracts, the complex contained an equimolar amount of VDAC (61, 62). Since there is the possibility that, in the presence of CHAPS, interactions between Bax, ANT, and VDAC do not withstand the extraction procedure, we cannot exclude the possibility that these proteins interact within membranes. However, in contrast to previous results (49, 50), we have never been able to demonstrate such interactions by cross-linking experiments (51) or by immunoprecipitation (Fig. 7B and data not shown). These data suggest that even within membranes, interactions between Bax and ANT or Bax and VDAC may be weak or transient or, alternatively, that only minor amounts of these proteins interact with each other at any one time.

It is important to note that, in contrast to Bax, the quaternary structures of Bak, Bel2, Bel-2, VDAC, and ANT do not change significantly during apoptosis. All proteins appear to be part of large molecular weight complexes. Interactions between Bel-2 and VDAC or Bel-XL and Bak have previously been reported (49, 63, 64). The copurification of Bel-2 with VDAC and of Bel-XL with Bak is consistent with these data. However, copurification does not necessarily indicate direct interactions between the proteins.

In conclusion, we have demonstrated the formation of two high molecular weight Bax oligomers in mitochondrial membranes of cells undergoing apoptosis. The formation of these Bax oligomers involves a series of events, including a change in Bax conformation triggered by Bid or other “BH3-only” proteins (46) and insertion into the outer mitochondrial membrane (51). The exact protein composition of these oligomers now remains to be determined, although we already know that the core structure is composed of several Bax subunits. Moreover, it remains to be determined whether these large Bax oligomers constitute the cytochrome c-conducting channel in mitochondria.

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Bax Oligomerization