Ion Channel Activity of the BH3 Only Bcl-2 Family Member, BID*

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BID is a member of the BH3-only subgroup of Bcl-2 family proteins that displays pro-apoptotic activity. The NH₂-terminal region of BID contains a caspase-8 (Casp-8) cleavage site and the cleaved form of BID translocates to mitochondrial membranes where it is a potent inducer of cytochrome c release. Secondary structure and fold predictions suggest that BID has a high degree of α-helical content and structural similarity to Bcl-X L, which itself is highly similar to bacterial pore-forming toxins. Moreover, circular dichroism analysis confirmed a high α-helical content of BID. Amino-terminal truncated BIDΔ1–55, mimicking the Casp-8-cleaved molecule, formed channels in planar bilayers at neutral pH and in liposomes at acidic pH. In contrast, full-length BID displayed channel activity only at nonphysiological pH 4.0 (but not at neutral pH) in planar bilayers and failed to form channels in liposomes even under acidic conditions. On a single channel level, BIDΔ1–55 channels were voltage-gated and exhibited multiconductance behavior at neutral pH. When full-length BID was cleaved by Casp-8, it too demonstrated channel activity similar to that seen with BIDΔ1–55. Thus, BID appears to share structural and functional similarity with other Bcl-2 family proteins known to have channel-forming activity, but its activity exhibits a novel form of activation: proteolytic cleavage.

Bcl-2 protein family members play an important role in governing a cell’s decision to heed or disregard signals to enter pathways for programmed cell death and apoptosis. The Bcl-2 protein family can be divided into two camps: pro- and anti-apoptotic, with proteins such as Bcl-2 and Bcl-X L acting to prevent cell death and proteins such as Bax and Bak encouraging cell death. The sequences of these proteins share pockets of similarity in regions designated BH1–4.¹

The three-dimensional structure of Bcl-X L shows the protein to be a bundle of seven α-helices with two central predominantly hydrophobic helices forming the core of the molecule. This structure is reminiscent of pore-forming bacterial toxins diptheria and colicins A, E1, and Ia, suggesting that Bcl-X L may have channel-forming potential (1). Indeed, in vitro channel-forming activity was demonstrated for Bcl-X L, Bcl-2, and Bax (2, 3, 4). Predicted structures for Bcl-2 and Bax can be modeled using the coordinates of Bcl-X L, suggesting that these proteins share similar structural features (5).

The amino acid sequences of one branch of the Bcl-2 family diverge substantially from Bcl-X L, Bcl-2, Bax, and many other Bcl-2 family proteins, sharing similarity only within the ~16 amino acid BH3 domain. This “BH3-only” subset includes BID, BAD, BIK, and HRK (reviewed in Ref. 6), which are all pro-apoptotic proteins. These proteins are presently thought to lack intrinsic activity, acting as trans-dominant inhibitors by use of their BH3 domains to interact with the BH3-binding pockets of anti-apoptotic proteins (7). In this view, BH3 proteins may play a passive role in apoptosis promotion by displacing anti-apoptotic proteins such as Bcl-X L from interactions with Bax, Apaf-1, or other pro-apoptotic proteins, freeing them to exert pro-apoptotic activities.

Mitochondria also play significant roles in apoptosis (reviewed in Ref. 8). Bcl-2 family members possessing a hydrophobic COOH-terminal membrane anchoring domain (e.g. Bcl-2, Bcl-X L) typically localize to mitochondrial membranes (reviewed in Refs. 6 and 8), although some members such as Bax can be transient mitochondrial residents that translocate from the cytosol to the mitochondria in response to several death signals (9, 10). Escape of cytochrome c (Cyt c) from mitochondria represents a critical event in initiating the caspase activation cascade, through its interaction with Apaf-1, which induces processing and activation of the cell death protease caspase-9 (Casp-9) (11, 12). The portal through which Cyt c passes into the cytoplasm is unknown, although Bax and the mitochondrial permeability pore complex appear to play important roles in Cyt c release (13, 14).

In response to Fas receptor ligation, pro-Casp-8 is recruited to the death-receptor complex where local aggregation allows Casp-8 processing (15). This activation is followed by Cyt c release and subsequent activation of downstream caspases such as Casp-3, -6, and -7 (16). Recently, the participation of BID in Cyt c release from mitochondria in Fas-stimulated cells was demonstrated (17, 18).

BID is a 195-residue, 22-kDa protein that lacks the hydrophobic COOH-terminal domain often found in Bcl-2 family proteins and which has a predominantly cytosolic localization (19). BID interacts with Bcl-2, Bcl-X L, and Bax via its BH3 domain and can counteract the cytoprotective effects of Bcl-2 and Bcl-X L (19). The murine BID amino acid sequence contains cytochrome c; POPE, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidyl-ethanolamine; POPG, 1-palmitoyl-2-oleoyl-sn-glycero-3-rac-glycerol.

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¹ The abbreviations used are: BH, Bcl-2 homology domain; DOPC, 1,2-dioleoylphosphatidylcholine; DOPG, 1,2-dioleoylphosphatidylglycerol; GST, glutathione S-transferase; S, siemens; Casp, caspase; Cyt c, cytochrome c; POPE, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidyl-ethanolamine; POPG, 1-palmitoyl-2-oleoyl-sn-glycero-3-rac-glycerol.

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a putative Casp-8 cleavage site (57-t-Q-T-n-G-61) within its NH₂ terminus and BID is indeed cleaved between residues 59 and 60 by Casp-8 both in vivo and in vitro (17, 18). The intact BID protein is found in the cytoplasm, but upon cleavage, truncated BID translocates to mitochondria (17, 18). In vitro, truncated, but not intact BID, is a potent inducer of Cyt c release from isolated mitochondria, prompting release of more than 80% of total Cyt c after treatment with nanogram amounts of Casp-8-cleaved BID (18). Thus, BID serves as a linker between the Fas receptor at the plasma membrane and the mitochondrial cell death machinery. The conversion from the cytosolic, soluble intact BID to the mitochondrial membrane-associated NH₂-terminal truncated BID and subsequent Cyt c release implies that truncated BID may play a role in priming the mitochondrial membrane to become more permeable, permitting Cyt c to escape. To explore this possibility, the membrane activity of both wild-type and truncated recombinant murine BID was examined using liposome and planar bilayer assays.

MATERIALS AND METHODS

Plasmid Preparation—A plasmid encoding a GST-BID fusion protein was constructed by liberating an EcoRI-XhoI DNA coding murine BID (1–195) and subcloning into pGEX4Ti (Amersham Pharmacia Biotech). BIDΔ1–55 was constructed after introduction of an EcoRI site between the codons for residues 55 and 66 using forward (5'-CTCGAG-AGCAATTCCAGACAGAC-3') and reverse (5'-CTCGAAGCTTCAGATGTCAGAGG-3') primers in a polymerase chain reaction reaction to amplify a ~0.5-kilobase pair fragment flanked by EcoRI-XhoI sites. Residue 55, rather than 59, was chosen to minimize the number of base pair changes necessary to introduce the EcoRI site. The fragment was then inserted into pGEX4Ti following EcoRI and XhoI digestion. The presence of the deletion was confirmed by DNA sequencing.

Protein Purification—Wild-type BID and BIDΔ1–55 were produced as GST fusion proteins from pGEX vectors using Escherichia coli BL21 (DE3) as the host strain. The purification method is identical for each protein. An overnight culture (5 ml) was used to inoculate 1 liter of LB (DE3) as the host strain. The purification method is identical for each E. coli strain. The intact BID protein were loaded, the low intensity of the BIDΔ1–55 may be due to its low aromatic amino acid content.

Fig. 1. Analysis of recombinant BID and BIDΔ1–55 proteins. Wild-type BID (left) and truncated BIDΔ1–55 (right) were purified using GST-Sepharose affinity chromatography. Molecular mass markers (kilodaltons) are indicated. Although similar amounts of each protein were loaded, the low intensity of the BIDΔ1–55 may be due to its low aromatic amino acid content.

Fig. 2. Far-UV circular dichroism spectra of BID and BIDΔ1–55. BID (○), pH 7.0; +, pH 4.0) and truncated BIDΔ1–55 (×) were dialyzed into 20 mM potassium phosphate, pH 7.0, or 20 mM sodium acetate, pH 4.0, and the spectra scanned from 250 to 190 nm. Protein concentrations were 14 and 10 μM for wild-type and truncated BID, respectively. Spectra represent the average of three scans (5 s averaging time) corrected for background intensity by subtraction of the appropriate buffer blank.

Liposome Preparation and Cl⁻ Efflux Measurements—Large unilamellar vesicles composed of 70% dioleoylphosphatidylcholine (DOPC) and 30% dioleoylphosphatidylglycerol (DOPG) were produced as described previously (4). The liposomes were diluted to a final concentration of 10 mg/ml in 10 mM dimethyl sulfoxide, 100 mM choline nitrate, 2 mM Ca(NO₃)₂, at either pH 4.0 or pH 6.0. Valinomycin was added to a final concentration of 15 mM to generate an inside-negative potential. BID wild-type or BIDΔ1–55 was added at the indicated concentrations, and residual Cl⁻ was released following addition of Triton X-100 (0.1% v/v). The total amount of Cl⁻ released was compared against a calibration curve produced by successive additions of KCl. Electrode assembly is as described previously (4).

Molecular Modeling—Structure prediction for BID was attempted using a threading approach (21). The algorithm produced several possible predictions with similar significance scores. Out of the first four predictions, two were disqualified, because their sequence alignments failed to produce a three-dimensional model. The remaining predictions out of the first four were: apolipoporphrin III (PDB code 1aep, a four-helical bundle) and Bcl-XL (1maz). The 1aep prediction was discarded because removal of the NH₂-terminal fragment up to Asp-59 (which is cleaved by caspase to produce an active protein) does not result in a change of BH3 domain accessible surface. Two models of BID, either the full-length or Δ1–59 based on the Bcl-XL structure were then built using the MODELLER program (22).

Planar Bilayer Preparation and Single Channel Recording—Phospholipid bilayer membranes were formed as described previously (23). Solvent containing membranes were formed by placing a bubble of lipid onto the end of the Teflon tube approximately 300 microns in diameter.
The design of the chamber allowed rapid introduction of solution into immediate proximity with the membrane in a volume of only 50 microliters. Agar salt bridges were used to connect the electrodes to the solutions and voltage clamp conditions were employed in all experiments. Lipids were purchased from Avanti Polar Lipids (Birmingham, AL) and stored at −70 °C. In some cases, azolectin was used for membranes to increase sensitivity but this was unnecessary for BID (Δ1–55). Current was recorded with an Axopatch amplifier (Axon Instruments, Sunnyvale, CA) and stored on videotape for later playback and analysis. Membrane capacitance and resistance were monitored in order to assure the formation of reproducible membranes.

RESULTS AND DISCUSSION

To explore the possibility that BID interacts with membranes, recombinant BID and BIDΔ1–55 were expressed as GST fusion proteins in E. coli. BIDΔ1–55 represents a recombinant mimic of the ~15-kDa Casp-8 cleavage product of BID and was used to avoid any ambiguities associated with incomplete digestion of full-length BID or the possible confounding presence of Casp-8. The BID proteins were liberated from the GST moiety by thrombin digestion and determined to be >90% purity, as determined by SDS-polyacrylamide gel electrophoresis analysis with Coomassie staining (Fig. 1). The faintness of the BIDΔ1–55 band may be attributable to its lack of aromatic amino acids (one tyrosine and no tryptophans) required for Coomassie Blue binding (24).

To assess whether the truncated form of BID possesses a significantly different structure, circular dichroism (CD) spectra were obtained for each protein. The far UV-CD spectrum (185–250 nm) is dominated by the amide bond absorption and is highly sensitive to the presence of ordered secondary structure. The far-UV spectrum for both wild-type and truncated forms of BID display minima at ~202 and 222 nm and are characteristic of proteins having at least 50–60% α-helical secondary structure (25) (Fig. 2). Little change was seen in the shape or amplitude of the far-UV spectrum between the wild-type and BIDΔ1–55, indicating that the α-helical nature of these two proteins remains intact despite the loss of the first 55 residues (Fig. 2). These CD data also demonstrate that BID and BIDΔ1–55 possess a high α-helical content. The high α-helical content of BID and BIDΔ1–55 is reminiscent of the channel-forming Bcl-X, Bel-2, and Bax proteins as well as structurally related bacterial toxins such as the pore-forming colicins and diphtheria toxin.

When BID was added to KCl-loaded liposomes composed of 70% neutral (DOPC) and 30% acidic (DOPG), no chloride efflux could be observed, either at pH 4.0 or 7.0 (Fig. 3, curve c, and data not shown). In contrast, when BIDΔ1–55 was added in nanogram amounts to similar vesicles, the protein induced >50% release of encapsulated chloride at pH 4.0 (Fig. 3, curve a), but no ion release was detected at pH 7.0 (Fig. 3, curve d). The ion release induced by truncated BID displayed a partial

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**Fig. 3. BIDΔ1–55 induced Cl− efflux from KCl-loaded vesicles.** Either wild-type BID (curve d) or BIDΔ1–55 (curves a, b, and c) was added at t = 0 to 70% DOPC/30% DOPG vesicles suspended in 10 mM DMG buffer at pH 4.0 (curves a, b, and d) or pH 7.0 (curve c) in the presence (curves a, c, and d) or absence (curve b) of 15 mM valinomycin (added prior to protein addition). BID and BIDΔ1–55 were added to final concentrations of 1 μg/ml and 0.2 μg/ml, respectively. Arrows indicate the addition of 0.1% Triton X-100 to release residual chloride.

**Fig. 4. BID induces current only at acidic pH, while Casp-8-cleaved BID and BIDΔ1–55 form channels at neutral pH.** A, current is shown as a function of time through a membrane voltage-clamped at +50 mV. Wild-type BID (50 μg/ml) was added to the aqueous solution (100 mM KCl, 50 mM HEPES-Tris, pH 7.3). The flat current trace (t = 0) indicates that no channel activity was seen. At the first arrow, the chamber was perfused with BID-free solution, pH 7.3. A slight deflection was seen due to perfusion artifact. At the second arrow, the chamber was perfused with acidic BID-free solution (100 mM KCl, 50 mM citrate-Tris, pH 4.0). Membranes never showed activity in the absence of BID. The inset shows that individual “jumps” of current can be discerned at early times, perhaps representing single channel events. Membrane lipid was POPE:POPG (1:1). B, channel activity of Casp-8-activated BID. Current is shown as a function of time with voltage clamped at +50 mV. BID (60 μg/ml) was preincubated with Casp-8 (0.35 μM) in 100 mM KCl, 50 mM HEPES-Tris at 25 °C for 1 h prior to addition (arrow) to azolectin membrane. The increase in current indicates formation of channels by Casp-8-activated BID and was not observed either in the presence of uncleaved BID or Casp-8 alone at pH 7.3. Multiple simultaneous or overlapping channel openings are observed. C, current is shown as a function of time through a membrane to which BIDΔ1–55 had been added to a final concentration of 60 μg/ml. In the first half of the record, membrane voltage was held at +50 mV, and the BIDΔ1–55 channels were seen to be frequently opening and closing (at least two to three channels appear to be present, see inset). When the voltage is changed to −50 mV, the channels close and only rarely reopen. When the membrane is returned to +50 mV, the channel opening resumed at somewhat higher frequency. This reflects the stochastic nature of single channel openings and the fact that new channels occasionally appear in the bilayers. Aqueous solution was 100 mM KCl, 50 HEPES-Tris, pH 7.3. Lipid was POPE:POPG (1:1). Aqueous solutions and lipid were the same as in A.
lipids (100% DOPC) (not shown). The behavior of BID release was detected at pH 4.0 when the vesicles lacked acidic curve b cin was reduced (Fig. 3, the presence of a information on pH, voltage dependence, and ion conductance. To characterize the behavior of wild-type and truncated BID subgroup of molecules are responsible for ion release. In channel formation, excluding the possibility that a small activity was observed (Fig. 4B). Similar to Casp-8-cleaved BID, BIDΔ1–55 also demonstrated channel activity at neutral pH (Fig. 4C). Our ability to detect channels formed by BID Δ1–55 at neutral pH in planar bilayers but not in liposome-based ion efflux assays presumably reflects the greater sensitivity of the former method. Similar results were also obtained for Bcl-2 and Bcl-XL (2, 3).

We used the BIDΔ1–55 protein as a recombinant mimic of Casp8-cleaved BID to further characterize BID channel activity in planar bilayers (Fig. 5). BIDΔ1–55 displayed a membrane potential dependence that was similar to that observed for the pore-forming colicins (26) and to that observed for truncated BID in liposomes, in that the channel was open when a positive voltage was applied on the side to which BID was added but converted to a nonconductive state when the voltage was reversed to a negative voltage. Several BIDΔ1–55 single channel conductances with clear quantal sizes were discerned in these experiments (Fig. 5), including smaller conductances of 7.4 pS and larger conductances of 40 and 100 pS in 150 mM KCl (Fig. 5).

Although the sequence similarity between BID and Bcl-XL and other family members is confined to the BH3 domain, the high α-helical content of the protein and its ability to form ion channels in vitro prompted the consideration that BID may share structural similarity with Bcl-XL or other pore-forming proteins. Accordingly, structure prediction for BID was attempted using a threading approach (21), revealing extensive predicted structural similarity with Bcl-XL (Fig. 6). Using the MODELLER program, models for the full-length and truncated forms of BID were built using the Bcl-XL coordinates as a guide. The COOH-terminal portion of both BID and BIDΔ1–55 are predicted to correspond to the last six of the seven α-helices previously implicated in pore formation by Bcl-2 and Bax (3, 14) and an α-helix corre-

**FIG. 6. Predicted structures for BID and BIDΔ1–55.** A, Ribbon diagram for Bcl-XL. Red helices represent a5 and a6 of the central hydrophobic hairpin, and the blue helix contains the BH3 domain. B, predicted structure for wild-type BID modeled on Bcl-XL coordinates. Red helices correspond to helices a5 and a6 of Bcl-XL and the blue helix to the BH3 domain. C, predicted structure for BIDΔ1–55. Removal of the first helix (yellow, compare with B) results in increased exposure of the hydrophobic central helices and the BH3 domain also has increased exposed surface area. Both predicted accessible surface changes are only lower estimates because for much of the BID NH2-terminal region a model could not be built because of lack of similarity in this region to Bcl-XL.

BID at pH 4.0 is indistinguishable from that at pH 7.0 (Fig. 2). However, the lack of a clear quantal size for these channels may reflect rapid transitions among multiple conformations of molecules undergoing tertiary structure changes as they interact with the membrane.

Since BID is a Casp-8 target in vivo, we explored whether Casp-8-cleaved BID also displays channel activity similar to the mutant mimic BIDΔ1–55. Incubation of BID with Casp-8 at a 10:1 (mole:mole) ratio produced a cleaved form of BID (not shown) that exhibited channel activity within 1 min after addition of the cleavage mixture to planar bilayers at pH 7 (Fig. 4B). The activity detected in this assay is pH and voltage-dependent, excluding the possibility that a small subset of molecules are responsible for ion release.

To characterize the behavior of wild-type and truncated BID at a microscopic level, experiments were performed using planar bilayer membranes. This technique allows channels to be monitored at the single channel level and yields more specific information on pH, voltage dependence, and ion conductance. When wild-type BID was added to planar bilayers at pH 7.3 in the presence of a +50 mV membrane potential, no channel activity was observed (Fig. 4A). Yet when the chamber was flushed out and a pH 4.0 buffer solution was added, channel activity began immediately (Fig. 4A, expanded region), suggesting that the wild-type BID is able to associate with the membrane, but an acidic pH is necessary to achieve membrane insertion and channel activity. This channel activity does not appear to require that the protein undergo significant changes in secondary structure as the far-UV CD spectrum of wild-type BID was unaffected at pH 4.0 when the vesicles lacked acidic lipids (100% DOPC) (not shown). The behavior of BID release was detected at pH 4.0 when the vesicles lacked acidic lipids (100% DOPC) (not shown). The behavior of BIDΔ1–55 closely resembles that observed previously for Bcl-2 and Bcl-XL in that the activity detected in this assay is pH and voltage-dependent and requires acidic lipids (2, 3). The chloride efflux assay detects channel activity on a macroscopic level and requires that the bulk of the protein molecules are participating in channel formation, excluding the possibility that a small subgroup of molecules are responsible for ion release.

dependence of an outside-positive voltage, as the amount of ion released in the absence of the K+-specific ionophore valinomycin was reduced (Fig. 3, curve b). No BIDΔ1–55-induced ion release was detected at pH 4.0 when the vesicles lacked acidic lipids (100% DOPC) (not shown). The behavior of BIDΔ1–55 closely resembles that observed previously for Bcl-2 and Bcl-XL in that the activity detected in this assay is pH and voltage-dependent and requires acidic lipids (2, 3). The chloride efflux assay detects channel activity on a macroscopic level and requires that the bulk of the protein molecules are participating in channel formation, excluding the possibility that a small subgroup of molecules are responsible for ion release.

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sponding to the BH3 dimerization domain (Fig. 6). The NH₂-
terminal region of full-length BID proximal to the caspase
 cleavage site does not model well on the Bcl-XL structure,
which corresponds to the BH4-containing first α-helix in Bcl-
X₉. In contrast to BID, attempts to model other BH3-only
proteins on the Bcl-XL coordinates were unsuccessful, includ-
ing BAD, Bik, Bik, Hrk, and Egl (not shown), thus suggesting
that these members of the Bcl family do not possess struc-
tures similar to Bcl-XL and related pore-forming proteins.

Two observations related to the effects of cleavage of BID are
predicted by comparisons of the models for full-length and
truncated BID. First, removal of the NH₂-terminal segment
(1–55) results in a 60 Å² increase of exposed hydrophobic sur-
face area of the central pair of helices (α4–α5 in this model),
which are candidate pore-forming regions of the protein. Con-
sequently, removal of the NH₂-terminal region by proteolysis
may promote the association of the cleaved BID protein with
membranes. Second, excising the NH₂-terminal segment is pre-
dicted to result in increased accessibility of the BH3 dimeriza-
tion domain by 90 Å². Since the hydrophobic surface of BH3
domains is known to be involved in dimerization among Bcl-2
family proteins (27), this suggests that cleavage of BID may
also promote its heterodimerization with Bcl-2 family members
(Fig. 6).

Taken together, the findings presented here argue that while
BID shares very limited amino acid sequence similarity with
Bcl-XL and other documented pore-forming members of the
Bcl-2 protein family, BID may nevertheless be a structurally
similar protein that also shares pore-forming capability. Re-
cent determination of the three-dimensional structure of BID,
which was reported while this work was under review (28, 29),
supports this view. The pore-forming activity of BID is unique
in that it occurs primarily following proteolytic cleavage. Al-
though full-length BID did display some channel activity on
planar bilayers at acidic pH, this may reflect that the NH₂-
terminus has become “unwrapped” from the protein at low pH
in the same manner as previously documented for colicins (26).
Thus, low pH may promote BID conformations that allow some
of the uncleaved molecules to insert into membranes under
conditions of nonphysiological pH. However, in vivo, where pH
ever essentially drops below pH 6.0, the NH₂-terminal do-
main of BID presumably requires physical removal by Casp-8
cleavage, although other mechanisms involving interactions
with other proteins cannot be excluded.

Previously, BH3-only proteins have been viewed as trans-
dominant inhibitors that relied exclusively on dimerization
with other Bcl-2 family proteins to exert effects on cell life
and death. The results presented here suggest that some BH3-only
proteins may have intrinsic activities as membrane-integrating
or channel proteins, necessitating re-evaluation of the role of
this sub-branch of the Bcl-2 family.

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REFERENCES
1. Muchmore, S. W., Sattler, M., Liang, H., Meadow, R. P., Harlan, J. E., Yoon,
H. S., Nettesheim, D., Chang, B. S., Thompson, C. B., Wong, S. L., Ng, S. L., and
Fesik, S. W. (1996) Nature 381, 335–341
2. Mina, A. J., Velez, P., Schendel, S. L., Liang, H., Muchmore, S. W., Fesik,
S. W., and Fill, M. (1997) Nature 385, 353–357
3. Schendel, S. L., Xie, Z., Montal, M. O., Matsuyama, S., Montal, M., and Reed,
J. C. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5113–5118
4. Antonsson, B., Contin, F., Ciavatta, A., Montesuis, S., Lewis, S., Martinou, I.,
Bernasconi, L., Bernard, A., Mermod, J. J., Mazzei, G., Maundrell, K.,
Gamble, F., Sadoul, R., and Martinou, J. C. (1997) Science 277, 370–372
5. Schendel, S. L., Montal, M., and Reed, J. C. (1998) Cell Death Differ. 5,
372–380
6. Adams, J. M., and Cory, S. (1998) Science 281, 1322–1325
7. Kelekci, A., and Thompson, C. B. (1998) Trends Cell Biol. 8, 324–330
8. Green, D. R., and Reed, J. C. (1998) Science 281, 1399–1312
9. Wolter, J. G., Hsu, Y. T., Smith, C. L., Nechushtan, A., Zi, X. G., and Youle,
R. J. (1997) J. Cell Biol. 139, 1251–1292
10. Gross, A., Jockel, J., Wei, M. C., and Korsmeyer, S. J. (1998) J. Cell Biol. 143,
207–215
11. Liu, X., Kim, C. N., Yang, J., Jemmerson, R., and Wang, X. (1996) Cell 86,
147–157
12. Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S. M., Ahmad, M., Alnemri,
E. S., and Wang, X. (1997) Cell 91, 479–489
13. Jurgensmeier, J. M., Xie, Z., Deveraux, Q., Ellerby, L., Breides, D., and Reed,
J. C. (1999) Proc. Natl. Acad. Sci. U. S. A. 95, 4997–5002
14. Marzo, I., Brenner, C., Zamzami, N., Jurgensmeier, J. M., Susin, S. A., Vieira,
H. L., Prevost, M. C., Xie, Z., Matsuyama, S., Reed, J. C., and Kroemer, G.
(1998) Science 281, 2027–2031
15. Green, D. R. (1998) Cell 94, 695–698
16. Srinivasula, S. M., Ahmad, M., Fredandes-Alnemri, T., Litwack, G., and
Alnemri, E. S. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14486–14491
17. Li, H., Zhu, H., Xu, C.-J., and Yuan, J. (1998) Cell 94, 491–501
18. Luo, X., Budihardjo, I., Zou, H., Slaughte, C., and Wang, X. (1998) Cell 94,
481–490
19. Wang, K., Yin, X.-M., Chao, D. T., Milliman, C. L., and Korsmeyer, S. J. (1996)
Genes Dev. 10, 2859–2869
20. Fling, S. P, and Gregerson, D. S. (1986) Anal. Biochem. 155, 83–88
21. Jaraszewski, L., Rychlewski, L., Zhang, B., and Godzik, A. (1998) Protein Sci.
7, 1431–1440
22. Sali, A., and Blundell, T. L. (1993) J. Mol. Biol. 234, 779–815
23. Mirzabekov, T. A., Silverstein, A. Y., and Kagan, B. L. (1999) Methods
Enzymol. 294, 661–674
24. Congdon, R. W., Muth, G. W., and Splittgerber, A. G. (1993) Annu. Rev. Biophys.
Biomol. Struct. 22, 611–641
25. Johnson, W. C. (1990) Proteins Struct. Funct. Genet. 7, 205–214
26. Cramer, W. A., Heymann, J. B., Schendel, S. L., Deiry, B. N., Cohen, F. S.,
Elkins, P. A., and Staufacher, C. V. (1995) Annu. Rev. Biophys. Biomol.
Struct. 24, 611–641
27. Sattler, M., Liang, H., Nettesheim, D., Meadow, R. P., Harlan, J. E., Eberstadt,
M., Yoon, M. S., Shuker, S. B., Chang, B. S., Minn, A. J., Thompson, C. B.,
and Fesik, S. W. (1997) Science 275, 983–986
28. Chou, J. J., Li, H., Salvesen, G. S., Yuan, J., and Wagner, G. (1999) Cell 96,
615–624
29. McDonnell, J. M., Fushman, D., Milliman, C. L., Korsmeyer, S. J., and
Cowburn, D. (1999) Cell 96, 625–643