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NMR determination that an extended BH3 motif of pro-apoptotic BID is specifically bound to BCL-X_L[†]

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Abstract

The BH3 motif of the pro-survival family of proteins, BCL, is also present in pro-apoptotic proteins like BID and BAX. Homo- and hetero-oligomerization interactions of the BH3 motif are generally recognized as the critical component of their apoptotic activities. In full-length BID, the putative hydrophobic binding surface of its BH3 motif is substantially occluded by intramolecular contacts, many of which are removed on BID's transformation to tBID by cleavage with caspase 8, required for tBID's pro-apoptotic action on mitochondria, thereby releasing cytochrome *c*.

Keywords

NMR; ¹H; ¹³C; ¹⁵N; BID; BCL-X_L; apoptosis; BH3 motif; death agonist; helical propensity

As a step toward more complete characterization of the hetero-oligomeric complexes of the BCL interacting domain (BID) and B cell lymphoma associated gene (BCL) family of molecules, we report here the formation of a tight complex of the BH3 motif sequence of BID with BCL-X_L. In contrast to the previous report of BAK BH3 motif with BCL-X_L, the BID BH3 peptide (PDSESQEEIMHNIARKLAQIGDDI) forms an α -helix significantly extended to the *N*-terminus, as monitored by ¹⁵N{¹H} nOe determination and by ¹³C chemical shifts. Modeling of the BID BH3 motif/BCL-X_L based on the previous structure showed that the extended helix fitted well into an extended cavity in BCL-X_L. Mutagenesis of the peptide and BCL-X_L based on this model identified the key residues in the BH3 motif, but suggested that some conformational flexibility is likely in the BCL surface.

The extended recognition of BID's BH3 motif indicates that the range of specificity for binding partners to the BCL family is highly complex, that specificity may incorporate conformational changes from the free proteins, and that these may be of significance in the specific targeting of these complexes to membrane and other targets.

Programmed cell death is a highly evolutionarily conserved biological process critical for development and homeostasis in multicellular organisms.^{1,2} This process allows the removal of redundant or damaged cells, playing a vital role in normal cellular development, tissue homeostasis, and immunological defense.^{3,4} Deregulation of this pathway can contribute to

[†]Dedicated in memory of Stanley J. Korsmeyer.

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cancer, autoimmune disease, and neurodegenerative disorders.^{5,6} Developmental or environmental signals can regulate signals for either cell death or cell survival. The study of complex signaling pathways of programmed cell death (apoptosis) has led to the identification of a large number of molecules involved in regulating apoptotic death, or promoting cell survival.^{7,8} As part of a critical apoptotic pathway regulating a checkpoint in mitochondria, the BCL-2 family of proteins comprises both anti- and pro-apoptotic regulators.⁹ While the continuing research in apoptosis indicates that several mechanisms are in effect, or may yet be uncovered, nonetheless the BCL-related family of anti- and pro-apoptotic agents is a major group providing checkpoints and activators of apoptosis.

Membership in the BCL-2 family is defined on the basis of homology to at least one of four conserved sequence motifs known as BCL-2 homology motifs, BH1 to BH4 (sometimes referred to as *domains*). Most anti-apoptotic family members, such as BCL-2 and BCL-X_L, have BH1 and BH2 motifs and many have all four BH motifs. In contrast, many of the pro-apoptotic family members (BID, BAD, BIK, BIM, BLK, HRK) possess only the conserved BH3 motif.¹⁰ The ratio of anti- and pro-apoptotic molecules apparently determines whether a cell will respond to a proximal apoptotic stimulus. This competition is mediated, at least in part, by competitive dimerization between anti- and pro-apoptotic pairs.⁵ However, other roles including self association into ion transporters in membranes, and specific association with membrane-associated receptors (protein or lipid) in organelle membranes have also been considered.¹¹

The BCL-2 family member BID belongs to the BH3-only class of pro-apoptotic proteins. Like other members of this class of molecules, BID shows no recognizable sequence homology to BH1, BH2, or BH4 domains, and requires a functional BH3 domain for its dimerization and pro-apoptotic activity.^{12–14} Caspase-8 mediates the cleavage of an inactive 23 kDa cytosolic BID to produce a truncated 15 kDa fragment (referred to as *tBID*) that translocates to the mitochondria causing cytochrome *c* release, activation of caspases, and the final commitment to cell death.^{15–17}

The structure of BID sheds light on its role as a death agonist, and the requirements for pro-apoptotic action in the BCL-2 family. What makes a BCL-2 family member a death agonist? It appears that two criteria must be met for pro-apoptotic function. The first requirement is cellular localization. There is strong evidence for several BCL-2 family members that mitochondrial translocation is a key determinant for death-inducing activity. BID^{15–17} and BAX¹⁸ are inactive as cytosolic components but induce death upon their targeting to mitochondria. The activity of BAD, on the other hand, is downregulated by phosphorylation, which sequesters the protein in the cytosol away from mitochondria targets.¹⁴ The second, and perhaps more critical, criteria for death agonism is the exposure of the BH3 surface. The cleavage of BID by caspase-8 is likely to expose a BH3 motif that is buried in the uncleaved molecule. On the basis of our structure predictions for the BCL-2 family, some members of the BH3-only class appear to have their BH3 motif exposed, or at least not under the same conformational constraints as other members of the BCL-2 family.¹⁰ Molecules possessing an exposed BH3 motif (Egl-1, Hrk, Bik, Bim, tBID) might be expected to be constitutively active death agonists, while other members of the family possessing a ‘hidden’ BH3 motif are regulated by post-translation modification events (i.e. cleavage, phosphorylation) that expose the BH3 epitope^{19–21} which induce their pro-apoptotic function. The structure of BID and related molecules then strongly supported the original hypothesis that BH3 recognition, and potentially exposure of the hydrophobic face of the BH3, was a key event in pro-apoptotic action. Recent structural studies of BAX, a pro-apoptotic ‘multidomain’ member of BCL-2 family, showed that BH3 domain interaction surface of full-length BAX is occupied by C-terminal hydrophobic helix of BAX suggesting possible control over its mitochondrial

targeting and dimer formation. A similar control mechanism of BH3 domain-induced oligomerization was proposed for other 'multidomain' BCL-2 family members.²²

The conservation of sequential motifs in the BCL family led to detailed investigations of their interactions, and the identification of the BH3 motif as an interaction site in BAK's binding to BCL-2 and BCL-X_L.²³ In elegant structural studies,²⁴ the Fesik laboratory showed that this was the result of the BH3 forming a helix analogous to its fold in full-length analogs, producing complexes with a BH1 and BH2 face of BCL-X_L through hydrophobic and electrostatic contacts. Various screening methods including chemical-shift perturbation/NMR were used to identify small molecule mimics of the BAK BH3 motif²⁵ that are able to bind to BCL-X_L. The general surface area of BCL-X_L perturbed by the small molecules was similar to that perturbed by the BAK BH3 peptide. More recently, Walensky *et al.*²⁶ elegantly demonstrated that a hydrocarbon-stapled BH3 analog of the BID BH3 could provide activation of apoptosis *in vivo*.

In this paper we investigate structural determinants of the BID BH3 interaction with anti-apoptotic BCL-X_L. We developed selective binding assays that allowed us to quantitatively compare various alternate peptidic mimics of BID BH3 motif binding to BCL-X_L. Amino-terminal extension of BID BH3 domain led to an increase in BID BH3: BCL-X_L binding affinity by 1 order of magnitude. We describe the lengthened and altered specificity of the BID BH3 binding to BCL-X_L using site-directed mutagenesis and heteronuclear NMR.

RESULTS AND DISCUSSIONS

To understand how BID interacts with BCL-X_L and inhibits the ability of BCL-X_L to promote cell survival, we measured the binding affinity of BCL-X_L to a peptide corresponding to the BH3 region of BID protein (BID11s) by using a fluorescence polarization (FP) binding assay (Table 1). The BCL-X_L used for the binding assay lacks the putative C-terminal transmembrane region. A BID 16-mer peptide was synthesized in which Glu97 and Met97 were changed to those found in the BAK peptide—Glu-Met → Asp-Leu. His89, which is not conserved in BCL-2 family members and is located on the surface of the BCL-X_L/BAK peptide complex,²⁴ was mutated to the unnatural amino acid Dap (X in Table 1) for the synthesis of the cyclic peptide. A decrease in binding affinity by a factor of 10 compared with the BAK peptide was observed for the peptide derived from the BH3 region of BID, even though it contains most of the key residues in BAK that interact with BCL-X_L. Each peptide was fluorescein labeled (in table, X = 2,3 diamino propionic acid, stereoisomer-equivalent to L-amino acid). We concluded that the longer BID-based peptide either formed a more effective helix for binding or had additional contacts.

BH3 mimics

We designed and tested a conformationally constrained α -helix mimicking the full BH3 motif of BID and other pro-apoptotic members of the BCL-2 family. We implemented the strategy of using $i, i + 7$ side-chain links for the sequence NIAKHQAQIGDEMD, with the K ... D linkage being obtained by intermediate protection with allyl-K and Alloc-E (see Fig. 1). While α -helicity is apparent in the CD spectrum (data not shown), the magnitude of the 222 nm minimum is less than that expected from the formation of helix concordant with the BID solution structure helical content (~68%) (see Fig. 3 of Ref. 10) and may reflect time averaging of the structure between an α -helix and more random structures. Cyclization by exo-peptidic linkages produced only a modest apparent affinity increment in comparison to the optimized hydrocarbon-linked sequences of others.²⁶

CD spectra were acquired for the peptides in 30% TFE. From the mean residue ellipticity at 222 nm, the percentage of α -helix was derived and compared to the affinity of each peptide

for BCL-X_L (Table 1). As hypothesized, the cyclic peptide possesses a greater helix propensity compared to the linear equivalents but did not show dramatically higher affinities. We conclude that the general surface interaction of all these rigidized peptides is similar to the original packing observed in the BCL-X_L/BAK peptide complex²⁴.

To explain the tight binding of the extended BID BH3 peptide to BCL-X_L, we considered that the BID peptide might form additional interactions with BCL-X_L. Therefore, the longer peptide with a 10-residue extension of the BID 16-mer at the *N*-terminus was synthesized (BID11, Table 1). This longer peptide containing 26 amino acids was found to bind tightly to BCL-X_L with a *K*_d of 250 nM, and has a higher helix propensity than the 16-mer peptide.

Binding affinity measurements using fluorescence polarization

To obtain binding affinities for various BID peptides to BCL-X_L, the fluorescein derivative was chosen to provide a fluorophore for FP binding assay. The fluorophore was formed by an *N*-terminally or both *N*-terminally and Lys side-chain linked 5-carbamide fluorescein moiety. The labeled peptides were first tested in a saturation binding experiment against BCL-X_L (Fig. 2, Table 1).

The results show that, despite the addition of an *N*-terminal fluorescein, the affinity measured for the BAK peptide is in good agreement with previously published results.²⁴ A second FP experiment for measuring binding affinity was designed as a competition assay, which requires careful selection of the probe characteristics. The results in Table 1 show that BID11 has higher polarization change upon binding to BCL-X_L, which is of importance primarily because it delimits the signal-to-noise ratio of the assay. On the basis of *K*_d and polarization values, we selected the probe Fluor-BID11 for measuring the binding affinities for other peptides.

NMR assignments of the BID peptide complexed to BCL-X_L

To test whether the increase in binding affinity observed for BID 26-mer compared to the BID 16-mer results from higher helix propensity, or from the additional interactions between BCL-X_L and *N*-terminal residues of the BID peptide, we used heteronuclear NMR to characterize the BCL/BID peptide interaction. The deletion mutant of BCL-X_L used in the NMR studies is the same as that used for binding assay, which lacks the putative *C*-terminal transmembrane region and residues 45 to 84 which constitute a flexible loop previously shown to be dispensable for the anti-apoptotic activity.²⁷ The BID 26-mer peptide corresponds to the BH3 region of BID protein with ten residue extension at the *N*-terminus (BID11e), in which Met97 was mutated to Leu to avoid a cyanogen cleavage site in the middle of the peptide. We determined a *K*_d of 250 nM for BID11e/BCL-X_L binding from the FP competition assay. Furthermore, the *K*_d of the 40-amino acid peptide including the BH3 region and *N*-terminal residues extended to Asp59, the caspase cleavage site in the full-length BID protein, is the same as for the 26-residue peptide. In the ¹H{¹⁵N} HSQC spectra of free and complexed [U-¹⁵N, ¹³C] BID11e (Fig. 3), all the amide positions undergo significant chemical-shift changes upon binding, which is in accord with the change to a new structure on high-affinity binding, consistent with the fluorescence studies and with the expectation that the free peptide was either wholly or predominantly unstructured.

Backbone resonance assignments using a [U-¹⁵N, ¹³C] BID11e/BCL-X_L sample

Sequential assignments were achieved using the HNCACB/CBCA (CO) NH²⁸ pair of experiments so that connectivities could be traced through two independent, through-bond pathways. A ¹H{¹⁵N} NOESY-HSQC experiment allowed tracing of some amide–amide connectivities through the α -helical structures. Overall, all amide backbone resonances except Ile 83 were sequentially assigned by using this sample. The amide backbone resonance of Ile 83 was assigned using a specific [¹⁵N, ¹³C-Ile] BID11e/BCL-X_L sample.

The secondary structure of the BID11E complexed with BCL-X_L was identified through the analysis of $^{13}\text{C}^\alpha$ and $^{13}\text{C}^\beta$ shifts.²⁹ Segments of the protein experience a downfield shift with C^α resonances and upfield shift with C^β carbons when located in helices, and C^β carbons experience an upfield and C^α , a downfield shift when located in the β -strands. The BH3 region derived from the BID protein shows a condensed group of positive C^α and C^β differences compared with the empirical values, which indicates that it adopts an α -helix when complexed to BCL-X_L (Fig. 4). Interestingly, the α -helix is *N*-terminally extended to the residue Gln79 located beyond the BH3 region, which suggests that the peptide might form additional interactions with BCL-X_L.

To further identify the boundaries of the folded peptide structure, we performed a steady-state $^{15}\text{N}\{^1\text{H}\}$ nOe experiment. The protein segments that do not participate in the folded structure have negative nOe values because of their high degree of local flexibility and motions in the subnanosecond to nanosecond timescale. As seen from Fig. 5, *N*-terminal residues 1–4 have negative $^{15}\text{N}\{^1\text{H}\}$ nOe values and experience essentially minimal restrictions from the rest of the complex. This boundary of well-defined tertiary structure of the BID11e/BCL-X_L complex is consistent with the secondary structural part determined from Chemical-Shift Index.

Mutagenesis of the BID peptide and BCL-X_L protein

To determine what additional interactions between BCL-X_L and the *N*-terminal residues of the BID peptide are responsible for the increase in binding affinity observed for the BID 26-mer compared the BID 16-mer, the *N*-terminal residues 79–83 were added to the NMR structure of BCL-X_L/BAK complex, modeled, and energy-minimized using Insight (MSI) (Figs 6 and 7). An additional helical segment of BID BH3 fits well in the hydrophobic groove of BCL-X_L introducing no steric clashes (Fig. 6) and presenting hydrophobic Ile83 and polar Gln79 for direct interaction with the groove.

In the model proposed, the residues Thr109 and Val17 located in the helix 4 of BCL-X_L are involved in the hydrophobic interactions with *N*-terminal residues Gln79 and Ile83 of the BID peptide. We performed site-directed mutagenesis of the protein and the peptide on the basis of this mapping result to explore the extra binding sites. Individual mutations of two residues located at the *N*-termini of BID peptide, Q79A and I83A, resulted in 2.8-fold and 2.3-fold decrease of binding affinity to BCL-X_L ($\Delta 45$ –84), respectively. Mutation T109A does not affect the binding affinity, and V117L located in the helix 4 of BCL-X_L results in a 2.3-fold decrease of the binding affinity to BID peptide. However, the binding affinity of BID11e I83A peptide to mutant protein BCL-X_L ($\Delta 45$ –84) V117L has a 4.6-fold decrease compared to BID11e to BCL-X_L ($\Delta 45$ –84), which is additive of the titrations of two complementary mutants. The result indicates that Gln79 and Ile 83 of the BID peptide do significantly contribute to an overall 10 times increase in binding affinity of extended BID BH3 towards BCL-X_L. Val17 of BCL-X_L, which lies outside of the BAK peptide: BCL-X_L interaction surface, also makes a contact with BID BH3 but may not interact directly with Ile83. This result highlights the necessity of further high-resolution experimental work on the extended BID BH3: BCL-X_L complex. Mutation T109A may not be dramatic enough to introduce significant change in BID BH3:BCL-X_L binding affinity (Fig. 7).

Our observation of the lengthening and altered specificity of the BID BH3 motif towards anti-apoptotic BCL-X_L highlights the uniqueness of homo- and hetero-dimerization processes of different BCL-2 family members and may be important for the development of highly specific and selective mimics of BH3 motif to control apoptotic signals, possibly using the hydrocarbon stapling strategy²⁶ or similar peptidomimetic approaches.

MATERIALS AND METHODS

Expression and purification of BCL-X_L and mutants

The deletion mutants of BCL-X_L and mutants containing single-residue mutations were constructed from the expression vector for BCL-X_L (residues 1 to 209) by PCR using primers containing a BamHI upstream restriction site and a downstream EcoRI restriction site. The amplified genes were cloned in frame into the expression vector PGEX-2T (Pharmacia). The protein was expressed in *Escherichia coli* BL21 (DE3) using the T7 expression system. Cells, freshly transformed with plasmid, were grown to late log phase. Protein expression was induced by addition of 0.5 mM isopropyl thio- β -D-galactoside (IPTG). After growth for another 3 h at 37 °C, cells were harvested by centrifugation. The cell pellet was re-suspended in lysis solution (1% Triton X-100, 5 mM EDTA, PBS (140 mM NaCl, 5 mM phosphate, pH 7.3), 1 mM DTT, and protease inhibitors (Aprotinin, PMSF) and sonicated for 10 min periods on ice. The lysate was clarified by centrifugation at 4800 g for 20 min at 4 °C, and the supernatant was then incubated with glutathione-agarose beads (5 ml beads/1 l culture) for 2 h at 4 °C with Nutator mixing. The suspension was centrifuged at 2000 rpm for 5 min and the pellet was re-suspended and washed twice with 10 ml of high-salt buffer (sonication buffer with additional 100 mM NaCl) at 4 °C. Glutathione S-transferase (GST) was cleaved from the fusion protein with human thrombin (150 units) in 50 mM Tris-HCl pH 7.5, 100 mM NaCl, 2.5 mM CaCl₂, 2 mM DTT for 12 hours at 4 °C. The digestion was quenched with 1 mM AEBSF. The protein solution was then dialyzed against NMR buffer (20 mM phosphate buffer pH 7.2; 50 mM NaCl; 5 mM EDTA; 1 mM DTT).

BID peptide expression and purification

BID peptide was constructed from the expression vector for full-length BID protein by PCR. The amplified gene was subsequently cloned into the HindIII-BamHI sites of a phagemid-T7 expression vector, PTMHa.³⁰ Residue Met 97 was mutated to Leu using the megaprimer method.³¹ The DNA sequence was verified by DNA sequencing. In the PTMHa vector, the desired sequences are expressed as chimeric proteins containing a modified form of the TrpLE leader sequence, in which a *N*-terminal (His)₉ tag has been added, which can be removed upon cleavage by cyanogen bromide (CNBr). The peptide was expressed in *E. coli* BL21(DE3) using the T7 expression system. Peptide expression was induced by addition of 0.5 mM IPTG. After growth for another 5 h at 37 °C, the bacteria were harvested by centrifugation and the pellets were stored frozen. Inclusion bodies of the cell lysate were isolated by sonicating in sucrose (50 mM Tris, 25% sucrose, 1 mM EDTA, pH 8) and Triton X buffer (20 mM Tris, 1% Triton X-100, 1 mM EDTA, pH 8) separately.³² The resulting pellet was treated with CNBr (0.03 g/ml, 70% formic acid, 2 h). After removal of CNBr, the protein was dialyzed against 5% acetic acid and lyophilized. The protein was solubilized in 6 M GdmCl, 0.1 M sodium phosphate, and 10 mM Tris pH 8.0). The solution was passed over a nickel-chelating column to remove the leader sequence containing the His tag and any uncleaved chimeric protein. The protein was purified to homogeneity by reverse-phase HPLC, using a Vydac C-18 preparative column and a linear gradient of acetonitrile containing 0.1% trifluoroacetic acid. The protein identity was confirmed by mass spectroscopy. Uniformly ¹⁵N-, ¹³C-labeled peptide was prepared by growing the *E. coli* strain BL21 (DE3) over-expressing BID1le on a minimal medium containing ¹⁵NH₄Cl and ¹³C-glucose.

Synthesis of peptide for cyclization

We used Fmoc (9-fluorenyl) methoxycarbonyl chemistry³³ to carry out solid-phase synthesis. Fmoc removal was achieved by piperidine-DMF (20%) cleavage, and standard BOP/HOBT/NMM activation. To synthesize cyclic peptide, we used *N*^α-Fmoc, *N*^ε-Dde Lys derivate and *N*^α-Fmoc, *O*^β-Alloc Asp derivate as building blocks; side chains were assembled after removal of Dde with hydrazine hydrate-DMF (2%) and removal of Alloc with Pd (Ph₃P)₄/CH₃Cl/

AcOH/NMM, respectively. A rigid linker was introduced using Fmoc-*p*-(aminoethyl) phenylacetic acid (Fig. 1) as a building block. Side-chain deprotection was obtained by hydrogenation with Pd, and cyclization by use of the procedure of Phelan.³⁴ The synthesized peptides were characterized by ESI mass spectroscopy.

Fluorescence titration

FP experiments were performed at 18 °C on an ISA Fluorolog III (JY Horiba) spectrofluorometer. Fluorescein-containing probes were read with standard cut-off filters for the fluorescein fluorophore ($\lambda_{\text{ex}} = 485 \text{ nm}$, $\lambda_{\text{em}} = 520 \text{ nm}$). Saturation experiments were performed using fixed concentrations of the probe (20 nM) titrated against increasing concentration of BCL-X_L proteins. Data were analyzed using Origin (Microsoft, Inc.) to fit the equation $(\text{probe bound})/(\text{total probe}) = (\text{free protein})/(\text{Kd} + [\text{free protein}])$ making the assumption that $(\text{free protein}) \cong (\text{total protein})$, which holds for our experimental conditions. Competitive binding experiments were performed using fixed concentrations of the probe (20 nM) complexed with proteins titrated against increasing concentration of the second unlabeled peptide. Standard buffer conditions were PBS buffer, pH 7.2; 2 mM DTT; 1 mM EDTA plus 0.01% bovine gamma-globulin (BGG).

NMR experiments

NMR spectra were acquired at 30 °C on a Bruker DMX500 or DMX600 NMR spectrometer. 700 μM [U-¹⁵N, ¹³C] BID11e sample and 1 mM [U-¹⁵N, ¹³C] BID11e/BCL-X_L were in NMR buffer (20 mM phosphate buffer pH 7.2; 50 mM NaCl; 5 mM EDTA; 1 mM DTT, 0.02% NaN₃). The HNCA, CBCA(CO)NH, HNHA, ¹⁵N-edited NOESY and ¹⁵N{¹H} steady-state experiments were collected using previously described protocols.^{35,36} All spectra were processed using NmrPipe³⁷ and assignments were made with in-house software.

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Figure 1.
Chemical structure of the rigid linker of BID1cls.

Figure 2.

Saturation binding assay for fluor-BID1le with BCL-X_L. Buffer: 20 mM phosphate, pH 7.2; 50 mM NaCl; 2 mM DTT; 1 mM EDTA 0.01% BGG.

Figure 3.
 $^1\text{H}\{^{15}\text{N}\}$ HSQC Map free (a) and bound (b) of $[\text{U } ^{15}\text{N}, ^{13}\text{C}]$ BID1le to BCL-X_L.

Figure 4. Apparent helicity (ordinate $\Delta^{13}\text{C}^\alpha - \Delta^{13}\text{C}^\beta$ chemical shifts in ppm) for the bound form of BID11e peptide. The horizontal line indicates the 'accepted' BH3 recognition in the BAK peptide interaction,²⁴ residues Gln77–Asp86.

Figure 5. $^{15}\text{N}\{^1\text{H}\}$ NOE for BID 11e binding to BCL-X_L. The peptide corresponds to the BID sequence Pro74–His99 (NP 031 570).

Figure 6. Depiction of the BCL-X_L/BAK interface from coordinates from the work of Sattler *et al.*²⁴ (a) Ribbon depiction, (b) Surface representation of the binding pocket of BCL-X_L with a ribbon of bound BAK peptide. This structure provides a model for anchoring the BID BH3 model in figure 7.

Figure 7.
Graphical representation of site-directed mutagenesis of the BCL-X_L protein and the BID peptide and the effects of their interactions.

Table 1

Binding affinities of different BH3 mimics towards BCL-X_L. Assays were saturation titration of the fluorescein-derivatized peptides using fluorescence depolarization measurements

	Peptide	Affinity (nM) (SD)	% change FP
BAK	GQVGRQLAIIGDDINR	390(20)	175
BID11	PDSESQEEMIHNIARXLAQIGDDIDH	250 (8)	250
BID11s	HNIARXLAQIGDDIDH	3600(200)	88
BID1cls	HNIARXLAQIGDDIDH (cyclized X-D)	1600(90)	80

X = K or K cyclized.