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Conformation of BCL-XL upon membrane-integration

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Abstract

BCL-XL is an anti-apoptotic BCL-2 family protein found both in the cytosol and bound to intracellular membranes. Structural studies of BCL-XL have advanced by deleting its hydrophobic C-terminus and adding detergents to enhance solubility. However, since the C-terminus is essential for function and detergents strongly affect structure and activity, the molecular mechanisms controlling intracellular localization and cytoprotective activity are incompletely understood. Here we describe the conformations and ligand-binding activities of water-soluble and membrane-bound BCL-XL, with its complete C-terminus, in detergent-free environments. We show that the C-terminus interacts with a conserved surface groove in the water-soluble state of the protein and inserts across the phospholipid bilayer in the membrane-bound state. Contrary to current models, membrane binding does not induce a conformational change in the soluble domain and both states bind a known ligand with affinities that are modulated by the specific state of the protein.

Keywords

BCL-2; apoptosis; nanodisc; structure; NMR

BCL-XL is a major suppressor of apoptosis, a fundamental homeostatic process of programmed cell death that is conserved across a wide variety of organisms and plays key roles in major human diseases. The protein is expressed in a variety of tissues and cell types and overexpressed in many tumors where it acts to promote tumor cell survival, tumor formation and tumor resistance to chemotherapy¹. BCL-XL and the other members of the BCL-2 protein family regulate the mitochondrion pathway to apoptosis through a network of intermolecular interactions that converge at the mitochondrial outer membrane (MOM)^{2, 3}.

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BCL-XL is found both in the cytosol and attached to the intracellular membranes of mitochondria and other organelles^{4; 5; 6}. In healthy cells, it exists in dynamic equilibrium between water-soluble and membrane-associated populations^{7; 8}, whereas, upon apoptosis induction, cytosolic BCL-XL redistributes to mitochondrial membranes with its pro-apoptotic partner BAX⁹. Protein translocation between the cytoplasm and the MOM is regulated by a C-terminal sequence of hydrophobic amino acids that is required for cytoprotective activity¹⁰.

Structural studies of BCL-XL and other BCL-2 proteins have advanced primarily (with the exception of BAX) by deleting the hydrophobic C-terminus and/or adding detergents to optimize protein solubility. The structures of water-soluble C-truncated BCL-XL^{11; 12}, BAX¹³ and BID^{14; 15} provided the initial framework for understanding the functions of the BCL-2 family proteins and for the development of small molecules that promote apoptosis in select tumors¹⁶. Despite their different activities as apoptosis suppressors (BCL-XL) or promoters (BAX, BID), all three proteins adopt a globular fold, composed of six amphipathic α -helices arranged around two central, more hydrophobic α -helices¹⁷. A hydrophobic groove on the surface of the anti-apoptotic family members mediates protein-protein interactions by engaging the conserved third BCL-2 homology (BH3) motifs of their pro-apoptotic counterparts.

To date, the structure of full-length BCL-XL has not been characterized and only limited structural data are available for the membrane-associated states of BCL-XL or any other BCL-2 protein. Structural studies in detergents have provided useful insights about membrane association^{3; 17; 18}. However, detergents have been shown to bind avidly to the BH3-binding groove of BCL-XL¹⁸, induce major unraveling of the BCL-2 three-dimensional structures, as well as influence their ligand binding activities, protein-protein interactions and oligomerization^{3; 17}.

Here we describe the conformations and activities of two functional states of BCL-XL including its complete hydrophobic C-terminus: a water-soluble state and a membrane-integrated state, each present in detergent-free, aqueous or phospholipid bilayer environments. Using nuclear magnetic resonance (NMR) and isothermal titration calorimetry (ITC), we show that the two states differ primarily in the conformation of the C-terminus, which interacts with a conserved surface groove in the water-soluble protein and inserts across the phospholipid bilayer in the membrane-bound protein. Notably, both states are capable of binding a BID BH3 ligand with high affinity and membrane binding does not induce a major conformational change in the soluble domain of the protein.

The C-terminus interacts with the BH3-binding groove of water-soluble BCL-XL

To examine the effect of the C-terminus on the structure and ligand-binding activity of BCL-XL we expressed and purified two versions of the sequence: BCL-XL, the sequence with all C-terminal residues, and BCL-XL_C, the truncated sequence lacking residues 213-233 whose structure has been determined^{11; 12}. Although the hydrophobic C-terminus limits solubility, water-soluble BCL-XL yields a well dispersed solution NMR ¹H/¹⁵N

spectrum (Fig. 1a and S1) that reflects a stable globular fold. Overall, the spectrum is similar to that of BCL-XL C and the assigned backbone chemical shifts show that BCL-XL also forms eight α -helices (Fig. S2). The C-terminus, however, induces a subset of $^1\text{H}/^{15}\text{N}$ signals to shift position compared to the spectrum of BCL-XL C (Fig. 1b and 1c). The most prominent perturbations map to helices $\alpha 2$, $\alpha 3$ and $\alpha 5$, and the $\alpha 7$ - $\alpha 8$ connecting hinge, sites that collectively form the BH3-binding groove of BCL-XL. By contrast, the opposite face of the protein remains largely unaffected. Perturbations of the NMR signals from G94 in $\alpha 2$, G138 at the start of $\alpha 5$, and G196 in the $\alpha 7$ - $\alpha 8$ hinge, are diagnostic of a “closed” conformation of the protein in which the C-terminus interacts with the groove.

The spectral changes induced by the C-terminus are very similar to those induced by the association of BCL-XL with tBID¹⁹. However, the effects of tBID are at least 1.5 times greater in magnitude and relay to a wider range of molecular sites beyond the BH3-binding groove, consistent with tight intermolecular association and the subtle conformational rearrangement of BCL-XL to accommodate the BH3 ligand¹². By contrast, the effects of the C-terminus of BCL-XL are highly localized to the hydrophobic groove, not transferred to other regions of the protein, and smaller than those detected for either the C-terminus of BAX¹³ or the C-terminus of pro-survival BCL-W^{20; 21}, the two BCL-2 proteins with which BCL-XL shares 23% and 44% sequence identity, respectively. In the structures of pro-survival BCL-W, determined for soluble forms of the protein stabilized by point mutations and only limited degrees of C-terminus truncation, the C-terminus folds into the groove, albeit not as a fully formed α -helix^{20; 21}. However, the C-terminus of BCL-XL does not induce a conformational change in the globular domain and does not contribute to forming a tightly bound globular structure as seen in BAX and BCL-W.

The $^1\text{H}/^{15}\text{N}$ spectrum of BCL-XL obtained at 25°C also contains several new, but very broad, signals from residues in the C-terminus (Fig. 1a). Some of these could be assigned to T216, G217, M218, A221, S228, and the W213 indole side chain. However, other signals from the C-terminus, including K233 at the extremity, could not be detected. Interestingly, while G217 yields a weak signal, the signal from M218 is very strong and has chemical shifts consistent with the highly dynamic conformations typically observed for C-terminal residues (e.g. R212 in BCL-XL C).

The profile of peak intensities reflects the presence of a C-terminal tail that experiences varying degrees of dynamics in the intermediate range of the μsec - msec time scale, and is connected to helix $\alpha 8$ by a short, dynamic loop centered at M218. Raising the temperature to 45°C caused the peaks from the C-terminus to disappear (Fig. S1) consistent with the induction of fast amide hydrogen exchange with water and/or dynamics in the unfavorable intermediate range of the NMR time scale.

In cells, BCL-XL homo-dimerization has been proposed to involve the interaction of the C-terminus of one partner with the BH3-binding groove of the other²². *In vitro*, however, protein dimerization or aggregation, which could also result in NMR line broadening or deterioration of the spectrum, are ruled out by size exclusion chromatography, since BCL-XL elutes as a single peak at an apparent molecular weight corresponding to monomeric

protein (Fig. 2a). This suggests that additional factors, present in the intracellular milieu but absent from the NMR samples, exist to support BCL-XL dimerization.

Treatment of BCL-XL C with basic pH, heat or detergent has also been shown to induce the formation of a domain-swapped symmetric homodimer, in which helices $\alpha 6$ - $\alpha 8$ of one monomer participate in forming the prototypical globular structure of the other^{23; 24; 25; 26}. At physiological pH in detergent-free solution, we did not observe dimer formation, even after raising the temperature to 45°C, as evidenced by size exclusion chromatography and the absence of characteristic NMR peak changes associated with conformational reorganization of the $\alpha 5$ - $\alpha 6$ hinge.

The C-terminus of water-soluble BCL-XL is displaced by a BH3 ligand

To assess the influence of the C-terminus on the BH3 ligand binding activity of BCL-XL we obtained NMR spectra and performed ITC experiments for BCL-XL C and BCL-XL in the presence of BID_{BH3}, a peptide spanning residues 80-99 of the BID BH3 motif.

The NMR spectra of BCL-XL C and BCL-XL obtained after adding BID_{BH3} show characteristic perturbations that map to the BH3-binding groove (Fig. 3a, 3b, and S3). In both cases, addition of BID_{BH3} results in virtually identical NMR spectra, representative of the ligand-bound state of the protein. The association of truncated BCL-XL C with its BH3 ligands is well documented¹². For BCL-XL, however, the observation of a stable, ligand-bound complex indicates that the BH3 peptide readily displaces the C-terminus out of the groove into water solution. Although the C-terminus is hydrophobic, the BCL-XL/BID_{BH3} complex remains water-soluble, albeit at lower concentration than for truncated BCL-XL C, as evidenced by the onset of sample precipitation over time. Interestingly, the peak from the W213 indole side chain is only slightly affected by BH3 ligand binding, indicating that the side chain does not interact appreciably with the BH3 binding groove. By contrast, the corresponding site of BCL-W, T172, appears to be tightly bound to the groove²¹. This region of BCL-XL diverges in sequence from BCL-W and structure determination will be needed to shed light on its precise conformation.

ITC experiments (Fig. 3d and 3e), performed by titrating BID_{BH3} into BCL-XL C and BCL-XL, confirm that association is established with the peptide. The thermodynamic binding parameters of BCL-XL and BCL-XL C are very similar (Table S1), indicating that they are dominated by residue-specific interactions between the groove and the ligand. However, the binding affinity of BCL-XL is nearly three times lower than that of BCL-XL C. Weaker peptide binding by BCL-XL is reflected in the much gentler rise of the ITC binding isotherm, compared to the sharper transition observed for BCL-XL C. The data for BCL-XL are consistent with a BH3 binding event that involves competition with the protein's C-terminus, as reported previously for the interaction of BCL-XL with a stapled BH3 peptide²⁷ and for BCL-W, whose affinity for BH3 ligands is also weakened by competition with the C-terminus^{20; 21}.

BCL-XL is homogeneously reconstituted in detergent-free lipid bilayer nanodiscs.

To examine the conformation and ligand-binding activity of membrane-bound BCL-XL and avoid complications associated with the use of detergents, we incorporated the protein in phospholipid bilayer nanodiscs, patches of phospholipid bilayer membrane stabilized by two molecules of an apolipoprotein designated membrane scaffold protein (MSP)²⁸. Nanodiscs provide a detergent-free environment that retains the essential anisotropic physical and chemical properties of biological membranes and have been used to examine the membrane-bound conformation of BAX²⁹. Their open lipid bilayer configuration facilitates functional activity studies by ensuring that embedded proteins are fully accessible to their ligands. The shorter MSP used in this study, MSP1D1 h5, forms smaller nanodiscs (~9 nm diameter) suitable for solution NMR studies of membrane proteins³⁰.

We tested two methods for obtaining association of BCL-XL with nanodiscs: (i) reconstitution, performed by combining BCL-XL, phospholipids and MSP with detergent and dialyzing extensively to refold BCL-XL during the nanodisc assembly process; and (ii) direct addition, performed by adding folded, water-soluble BCL-XL directly to preformed nanodiscs. In both cases, the nanodisc membranes contained both zwitterionic dimyristoyl-phosphatidyl-choline (DMPC) and anionic dimyristoyl-phosphatidyl-glycerol (DMPG) to mimic the MOM composition.

Reconstitution resulted in tight association of BCL-XL with the nanodiscs (Fig. 2; BCL-XL in ND). Size exclusion chromatography shows that these preparations elute as a single homogeneous fraction (F1), with a volume similar to empty nanodiscs and an apparent molecular weight of 130 kDa, as expected for a nanodisc particle containing a single molecule of BCL-XL, 2 molecules of MSP and 100 phospholipids. Indeed, Western blot analysis shows that F1 contains both BCL-XL and the MSP needed for nanodisc formation.

Conversely, direct addition of folded, water-soluble BCL-XL to preformed nanodiscs (Fig. 2; BCL-XL+ND) did not result in association of the protein with the membrane, despite the presence of negatively charged DMPG, and even when BID_{BH3} was co-added to recruit BCL-XL to the membrane. In this case, BCL-XL eluted as pure protein (F2) and nanodiscs eluted separately in F1. Similar results were obtained for the addition of BCL-XL C to preformed nanodiscs. BID BH3 peptides have been shown to induce membrane association of BCL-XL³¹. The inability of BID_{BH3} to induce quantitative membrane association of BCL-XL may simply reflect a difference of experimental scales: size exclusion chromatography and NMR detect bulk properties and may miss small quantities of membrane-bound protein. Alternatively, it is possible that the higher lateral pressure of nanodisc membranes prevents direct insertion of BCL-XL, and that additional protein factors, specific MOM lipids or MOM curvature morphology are needed to promote spontaneous membrane association of BCL-XL.

Membrane-integrated BCL-XL retains the globular fold of its water-soluble head

The solution NMR ¹H/¹⁵N spectrum of nanodisc-reconstituted BCL-XL reflects a return to an “open” form of the protein, in which the BH3-binding groove no longer interacts with the C-terminal tail (Fig. 1d and 2c). The transition from water-soluble to membrane-bound

BCL-XL is reflected by key NMR spectral changes (Fig. 2d). The peaks from G94, G138, and G196, which occupied “closed” conformation positions in the spectrum of soluble BCL-XL, all return to their positions representative of the “open” conformation, in which the groove is vacant. Notably, the signal from the indole side chain nitrogen of W213, located at the start of the C-terminus, broadens and undergoes a large downfield shift, consistent with localization to a more hydrophobic environment that promotes both stronger hydrogen bonds and dampened dynamics. The peak from M218, which was very sharp in the spectrum of soluble BCL-XL, disappears or shifts dramatically in the spectrum from nanodiscs, consistent with a conformational change associated with membrane-integration. Conversely, the signals from S231 and K233, which were not visible in the spectrum of soluble BCL-XL, now appear as strong peaks, at frequencies consistent with the increased mobility expected for these sites at the very C-terminal end of the protein and predicted to protrude out from the opposite face of the lipid bilayer into the aqueous phase.

The NMR spectrum of nanodisc-integrated BCL-XL is well resolved and so strikingly similar to that of water-soluble BCL-XL C that the majority of resonances from the globular head of the protein can be assigned by direct spectral comparison (Fig. 1d). Differences between the two spectra (Fig. 1e) are significantly smaller than those discussed above for the comparison of water-soluble BCL-XL with BCL-XL C. The most prominent changes map to $\alpha 8$ and its structurally proximal sites (Fig. 1f) indicating that the globular head undergoes a small, localized conformational rearrangement in this region, as the C-terminal tail inserts across the lipid bilayer membrane. Smaller changes are also observed for more distant sites at the polar opposite of $\alpha 8$. Perturbations are seen for I114 and T115 in the $\alpha 3$ - $\alpha 4$ loop, E7 in $\alpha 1$ and F27 in the long $\alpha 1$ - $\alpha 2$ connecting loop, indicating that the globular head also senses the membrane environment, possibly through a loose interaction with the membrane surface.

Despite its overall high quality, the spectrum of nanodisc-bound BCL-XL has distinctly broader resonances than those of water-soluble BCL-XL or BCL-XL C (Fig. S4). Line broadening is consistent with the reduced rotational tumbling motion expected for a 130 kDa protein-nanodisc particle. Raising the temperature from 25°C to 45°C narrows the NMR lines and enables visualization of the majority of peaks from the globular head as well some additional peaks from the tail. However, several signals from C-terminal residues predicted to be membrane-embedded (e.g. G217, G222, G227) remain undetected even at 45°C, as expected for sites with restricted global dynamics in the hydrophobic membrane interior. Resonance assignment and structure determination will benefit from the use of deuterated protein, deuterated phospholipids and high magnetic fields, as shown recently for other integral membrane proteins in nanodiscs ³⁰.

The C-terminal tail of membrane-integrated BCL-XL traverses the membrane

To probe the membrane-inserted conformation of the C-terminus, we examined the NMR spectra of BCL-XL incorporated in phospholipid macrodiscs and liposomes by reconstitution. These lipid bilayer structures are significantly larger (~30 nm diameter) than nanodiscs and require solid-state NMR methods to detect the signals emanating from their

associated proteins³². As macrodiscs undergo a temperature-dependent transition to a planar lipid bilayer phase that aligns spontaneously in a magnetic field, the resulting NMR signals are orientation-dependent and can be used to derive bond orientation restraints for structure determination.

Spontaneous alignment of the BCL-XL macrodiscs was observed at 35°C. The resulting solid-state NMR spectra display the characteristic orientation-dependent features expected for aligned lipid bilayer membranes (Fig. 4a-c and S5). The ¹⁵N spectrum (Fig. 4a, black) is both much narrower and distinctly different from the wide powder pattern observed for BCL-XL in liposomes (Fig. S5b). Most telling, the two-dimensional ¹H/¹⁵N separated local field spectrum exhibits a wheel-like pattern of signals in the range expected for a helix with a membrane-crossing tilt of 20-30° (Fig. 4c). The remaining signals are in the isotropic range of the ¹⁵N chemical shift and ¹H-¹⁵N dipolar coupling. Although highly overlapped, their positions reflect both the presence of motions that average the spin interactions towards their isotropic values, as well as residual specific alignment of molecular sites relative to the membrane, consistent with the observation of both isotropic intensity and a powder pattern in the ¹⁵N spectrum from liposomes (Fig. S5b).

Notably, while the ¹⁵N spectrum obtained by cross-polarization has abundant signals from backbone and side chain nitrogens (Fig. 4a, black), essentially no signal was detected with an experiment designed to sense exclusively mobile sites (Fig. 4b), indicating that the entire protein is motionally restricted on the μ sec time scale of the NMR experiments. Similar results were obtained for the protein in liposomes (Fig. S5d and S5e). Thus we conclude that the globular head and C-terminal tail of BCL-XL are both tightly associated with the large, slow-tumbling membranes of macrodiscs and liposomes.

Exchange of the surrounding H₂O for ²H₂O produced selective obliteration of the ¹⁵N spectra of BCL-XL in macrodiscs (Fig. 4a, blue). A significant portion of the 100-140 ppm intensity disappeared, including signals from Arg and Lys side chains, as expected for the water-exposed globular head of the protein. By contrast, the residual spectrum observed between 70-120 ppm persisted for days and, therefore, is attributed to the formation of a transmembrane helix with strong hydrogen bonds that resist exchange. Similar selective reduction of the NMR signal was observed for BCL-XL in liposomes (Fig. S5d), while for nanodisc-bound BCL-XL, exposure to ²H₂O caused the solution NMR spectrum to disappear completely within one day (Fig. S4d), reflecting the water solubility of the globular head and the inability to detect signals from the membrane-embedded C-terminus, in either H₂O or ²H₂O.

The combined NMR data for BCL-XL in nanodiscs, macrodiscs and liposomes show that the protein forms two domains, a water-soluble head tethered to the lipid bilayer by a C-terminal transmembrane α -helix. The data do not reflect membrane surface association of the C-terminal tail. Complete structure determination will be needed to determine the precise conformation of the C-terminus in membrane-integrated BCL-XL. The ability to detect solid-state NMR spectra of BCL-XL in macrodiscs and liposomes reflects a tight coupling of the head with the transmembrane helix. On the other hand, most of the peaks from the C-

terminus are absent from the solution NMR spectra of BCL-XL in nanodiscs, indicating that the transmembrane helix remains more motionally restricted than the head.

Membrane-integrated BCL-XL binds a BH3 ligand with high affinity

Addition of BID_{BH3} to nanodisc-integrated BCL-XL induced spectral changes very similar to those observed for BCL-XL and BCL-XL C after addition of the same peptide (Fig. 3c and S3). However, consistent with transmembrane insertion of the C-terminus, peaks from S231, K233 and the W213 side chain, remained completely unaffected by the BH3 peptide. Notably, ITC measurements (Fig. 3f) show that the affinity of membrane-integrated BCL-XL for the BH3 peptide returns to the higher range observed for BCL-XL C, as expected for a structure where the globular head is tethered to the lipid bilayer membrane by a transmembrane helix and the ligand no longer needs to compete with the C-terminus for the binding groove.

Compared to their water-soluble states, membrane-bound BCL-XL and BCL-W have been proposed to display diminished affinity for their BH3 ligands due to a loosening of the groove structure in the hydrophobic environment^{26; 33; 34}. According to current models, membrane binding by water-soluble BCL-2 proteins induces major conformational changes, while BH3 ligand binding by membrane-integrated BCL-2 proteins causes ulterior structural changes and ligand release³. The data in Fig. 3 show that the opposite is true. The measured affinity of membrane-bound BCL-XL for BID_{BH3} is actually 1.6 times greater than that of the isolated water-soluble head, represented by BCL-XL C. It will be important to examine whether this enhancement also holds for other BH3 ligands and reflects the presence of cooperative interactions of the protein and ligand with the lipid bilayer membrane. This finding could have important implications for the design of BH3 mimetics that target pro-survival BCL-2 proteins.

Conformation of membrane-integrated BCL-XL

The effects of dynamics are likely to play an important role in the function of BCL-XL. Nevertheless, the structural model in Fig. 4d provides a useful instantaneous view of the membrane-bound protein. The model was derived from the NMR and ITC data. It was generated by restraining the water-soluble head and the transmembrane helix by rigid-body refinement of each domain against its assigned NMR frequency range measured for BCL-XL in aligned macrodiscs. The transmembrane helix adopts a ~25° tilt in the lipid bilayer, placing F210, W213 and F214 at the lipid-water interface as expected for membrane interfacial aromatic residues, and enabling S231 and K233 to protrude into the aqueous milieu on the other side of the membrane. The position of the water-soluble head is such that helices α 1- α 8 all adopt orientations within ~35° of the membrane surface, consistent with the observation of near isotropic frequencies in the spectra from aligned macrodiscs. Its orientation is likely to be modulated by additional motions relative to the C-terminal tail. However, it must be such that the hydrophobic groove remains exposed to the aqueous phase and able to readily engage its partners, in line with the ITC data.

The model also shows that the α 1- α 2 connecting loop abuts the membrane surface providing an explanation for the small perturbations (relative to BCL-XL C) observed for the NMR

signals from $\alpha 1$ and the $\alpha 1$ - $\alpha 2$ loop upon nanodisc integration. Whether these perturbations reflect protein-membrane interactions important for stabilizing the membrane-bound state of the protein or promoting further membrane integration of the water-soluble head, downstream in the apoptosis program, remains to be tested.

Membrane integration of the head has been proposed to occur in response to the alteration of the side chain electrostatics caused by acidic pH^{35;36}. Helices $\alpha 5$ and $\alpha 6$ are long enough to span the MOM and are thought to be important for membrane insertion¹¹, in a manner analogous to the pore-forming domains of bacterial toxins³⁷. Helix $\alpha 1$ has also been proposed to insert based on NMR studies of BCL-XL C in detergent micelles¹⁸. Previous solid-state NMR studies with BCL-XL C suggested a shallow insertion of the globular head in the lipid bilayer³⁸. However, since these experiments were all performed in the absence of the C-terminus, additional manipulations of sample conditions (low pH, detergent, and ultrafiltration to concentrate the protein with liposomes) were required to promote the association of BCL-XL with the membrane.

Conclusions

In summary, we have reconstituted two functional states of BCL-XL in aqueous solution and bound to membranes. Both states retain BH3 ligand binding activity, with affinities that are modulated by the specific state and environment of the protein. Single atomic sites can be resolved by NMR to obtain information about structure and ligand-binding activity in detergent-free conditions. In addition to providing atomic-resolution molecular information about BCL-XL, the results illustrate the power of performing NMR experiments with functional, detergent-free, membrane-integrated proteins. This approach will be applicable to other BCL-2 family proteins, enabling parallel structural studies and activity assays to be carried out on identical native-like samples.

Water-soluble BCL-XL adopts a conformation where the C-terminus interacts with the BH3-binding groove on the surface of the globular head but is also sufficiently dynamic to be easily displaced by a BH3 ligand. The competitive effect of the C-terminus modulates the BH3 ligand affinity to a level lower than observed for the truncated protein BCL-XL C. Membrane-bound BCL-XL forms a C-terminal transmembrane α -helix that anchors the water-soluble globular head to the lipid bilayer membrane, leaving the hydrophobic groove exposed to the aqueous environment and functionally accessible to its binding partners.

Significantly, membrane-integration does not alter the globular structure of BCL-XL and does not abrogate its BH3 ligand binding activity. The BH3 binding affinity of membrane-bound BCL-XL is not only retained, but appears to be enhanced, suggesting that further characterization of the membrane-integrated states of pro-survival BCL-2 proteins will provide useful insights for the design of BH3 mimetics, especially for more refractory targets.

The present results are likely representative of cytosolic and MOM-anchored BCL-XL, as it exerts its cytoprotective function in the early stages of the apoptosis program, before outright MOM permeabilization and commitment to cell death. As transmembrane insertion

of the C-terminus is predicted to be irreversible - due to the energy cost associated with removing a hydrophobic helix from the lipid bilayer - the conformation of BCL-XL during retrotranslocation with BAX, to and from the MOM, is likely to be closer to the water-soluble state of the protein and involve a transient interaction with the membrane surface; this will be facilitated by the dynamic properties of the C-terminus. As the apoptosis program progresses towards cell death, the water-soluble head of BCL-XL may become further integrated in the membrane.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Structural information is described for water-soluble and membrane-bound BCL-XL.
- The protein is in detergent-free environments and includes the complete C-terminus.
- The C-terminus modulates conformation and ligand binding affinity of soluble BCL-XL.
- Membrane-bound BCL-XL has a transmembrane C-terminus.
- Membrane-bound BCL-XL maintains globular fold and has enhanced binding affinity.

Figure 1. Effects of the C-terminus and membrane integration on the structure of BCL-XL
 (a, d) Solution NMR $^1\text{H}/^{15}\text{N}$ correlation spectra recorded at 14.09 T for water-soluble BCL-XL (blue), water-soluble BCL-XL ΔC (black), and nanodisc-bound BCL-XL (red), at 25°C (a) or 45°C (d). (b, e) Total difference (ΔHN) in amide ^1H and ^{15}N chemical shifts, relative to the spectrum of BCL-XL ΔC , induced by the C-terminus of water-soluble BCL-XL at 25°C (b), or by integration of BCL-XL in nanodiscs at 45°C (e). Peaks from BCL-XL ΔC residues 203-211 were not detected at 45°C. (c, f) Ribbon and surface views of the globular head of BCL-XL, represented by the solution NMR structure of BCL-XL ΔC (PDB: 1BXL), showing sites affected by the C-terminus (blue; $\Delta\text{HN} > 0.05$ ppm) or by nanodisc integration (red; $\Delta\text{HN} > 0.025$ ppm). The C-terminus is depicted in dark blue (dashed line). BCL-XL

Figure 2. Mapping the transition from water-soluble to membrane-integrated BCL-XL
(a) Size exclusion chromatography analysis of BCL-XL reconstituted in nanodiscs (in ND), BCL-XL added to preformed nanodiscs (+ND), BCL-XL added to nanodiscs with BID_{BH3} (+ND + BID_{BH3}), or empty nanodiscs (ND). Molecular weights (kDa) of the markers used for calibration are shown at top. **(b)** Western dot blots of size exclusion chromatography fractions F1 and F2 probed with anti-His antibody to detect BCL-XL, or anti-ApoA1 antibody to detect nanodisc MSP. **(c)** Selected regions of the solution NMR ¹H/¹⁵N correlation spectra of water-soluble BCL-XL (blue) and nanodisc-integrated BCL-XL (red). **(d)** Total difference (ΔHN) in amide ¹H and ¹⁵N chemical shifts induced by integration in nanodiscs relative to the spectrum of water-soluble BCL-XL.

Figure 3. Association of BCL-XL with BID_{BH3}

(a-c) Solution NMR ¹H/¹⁵N correlation spectra recorded at 14.09 T for water-soluble BCL-XL C at 25°C, water-soluble BCL-XL at 25°C and nanodisc-integrated BCL-XL at 45°C, obtained with or without BID_{BH3}. Pink circles represent peak positions for peptide-bound BCL-XL C. (d-f) ITC titrations of BID_{BH3} into BCL-XL, showing calorimetric data (top) and integrated heat (bottom) as functions of BID_{BH3}/BCL-XL molar ratio. The data shown were corrected for non-specific binding by subtracting control ITC titrations performed with peptide and buffer alone or empty nanodiscs in buffer alone. Solid lines are the best fits of the binding isotherms to a single-site binding model, used to extract the values of the dissociation constant (K_d). Titrations were performed as described ¹⁹.

Figure 4. Conformation of membrane-bound BCL-XL

(a-c) Solid-state NMR spectra recorded at 11.74 T for BCL-XL in aligned macrodiscs at 35°C. (a, b) One-dimensional ^{15}N spectra obtained with cross polarization (CP) or insensitive nuclei enhanced by polarization transfer (INEPT) experiments, for samples in H_2O (black) or $^2\text{H}_2\text{O}$ (blue). (c) Two-dimensional $^1\text{H}/^{15}\text{N}$ separated local field (SLF) spectrum showing the wheel-like pattern of peaks expected for a transmembrane helix with 25° tilt (blue circle). (d) Ribbon and surface views of the structural model derived from the NMR data. The globular head is represented by the structure of soluble BCL-XL C (PDB: 1BXL). Sites affected by nanodisc integration (red) were derived from Fig. 1. The dashed yellow line delineates the vacant BH3-binding groove. The C-terminal transmembrane helix (blue) adopts a 25° tilt relative to the membrane normal (n).