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Molecular Basis of Bcl-xL's Target Recognition Versatility Revealed by the Structure of Bcl-xL in Complex with the BH3 Domain of Beclin-1

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Beclin-1, originally identified as a Bcl-2 binding protein, is an evolutionarily conserved protein required for autophagy. The direct interaction between Beclin-1 and Bcl-2 or Bcl-xL provides a potential convergence point for apoptosis and autophagy, two programmed cell death processes. Given the functional significance of the interaction between Beclin-1 and Bcl-2/Bcl-xL, we performed detailed biochemical and structural characterizations of this interaction. We demonstrated that the Bcl-xL-binding domain of Beclin-1 contains a BH3 domain. Therefore, Beclin-1 is a new member of the BH3-only family proteins. The structure of Bcl-xL in complex with the Beclin-1 BH3 domain was determined at high resolution by NMR spectroscopy. Although similar to other known BH3 domains, the Beclin-1 BH3 domain displays its own distinct features in the complex with Bcl-xL. Systematic analysis of all known Bcl-xL/BH3 domain complexes helped us to identify the molecular basis underlying the capacity of Bcl-xL to recognize diverse target sequences.

Keywords: Beclin-1; Bcl-xL; BH3 domain; autophagy; apoptosis

Introduction

Apoptosis, the most intensively studied form of programmed cell death, is a fundamental cellular activity, in which an internally controlled and evolutionarily conserved suicide program is launched to maintain cell homeostasis throughout the entire life cycle of multi-cellular organisms.[1] Apoptosis can be triggered by either an extrinsic pathway through the stimulation of death receptors (e.g. TNF-R1) or an intrinsic pathway *via* the release of signaling factors by mitochondria. The pathways converge at the step of activating the cysteine aspartyl proteases (caspase) that are the executors of apoptosis.[2–6] The Bcl-2 family proteins are key regulators of the intrinsic pathway through the control of the release of cytochrome *c*.[7–13] Members of the anti-apoptotic Bcl-2 family proteins (e.g. Bcl-2, Bcl-xL, Bcl-w, and Mcl-1) contain up to four Bcl-2 homology (BH) domains. In response to certain death signals, a related family of proteins called BH3-only proteins (e.g. Bim, Bid, Puma and Noxa) bind to the anti-apoptotic Bcl-2 proteins and neutralize their anti-apoptotic functions.[14,15] Mechanistically, binding of the BH3-only proteins prevents the anti-apoptotic Bcl-2 family proteins from sequestering pro-apoptotic multi-BH domain proteins such as Bak and Bax.[16]

A series of studies of the 3D structures of the anti-apoptotic Bcl-2 family protein, Bcl-xL, in complex with the BH3 domains from pro-apoptotic proteins (e.g. Bcl-xL/Bak, Bcl-xL/Bad, Bcl-xL/Bim) offered structural insights into the interaction between Bcl-xL and the neutralizing BH3 domain proteins.[14–16] In these complexes, BH1-4 domains of Bcl-xL adopt helical structures and together assemble into a bacterial toxin-like folding structure. The BH3 domain from each binding partner to Bcl-xL forms an amphipathic α-helix, and fits snugly into a hydrophobic pocket formed by the BH1, BH2 and BH3 domains of Bcl-xL.[14–16] Although all known Bcl-xL-binding BH3 domains form amphipathic helices upon binding to Bcl-xL, the amino acid sequence identity of these BH3 domains is not high.

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Abbreviations used: NOE, nuclear Overhauser enhancement; NOESY, NOE spectroscopy; HSQC, heteronuclear single quantum coherence; GST, Glutathione-*S*-transferase.

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Nevertheless, Bcl-xL can interact with these BH3 domain-containing proteins with high affinity.[14,16] Therefore, Bcl-xL is intrinsically adapted to be capable of responding to different BH3-domain binding partners.

Autophagy is a catabolic process in which a cell's own components are cannibalized by a vacuole/lysosome-mediated pathway.[17–19] The outcome of this process is the breakdown of non-vital components and the release of nutrients, ensuring that vital processes can continue. Although autophagy was initially regarded as a pro-survival cellular process that shuffles building blocks of macromolecules to the most fundamental biological processes when nutrients are scarce, recent evidence suggests that autophagy is capable of promoting cell death independent of apoptosis. It has, hence, been designated as the second type of programmed cell death.[19–24] However, the dichotomous functions of autophagy await experimental clarification.

Yeast autophagy protein Atg6/Vps30 associates with Vps34 (phosphatidylinositol 3-kinase) and Atg14 to form the phosphatidylinositol 3-kinase complex essential for the nucleation of autophagosomal vesicles.[19,20] Beclin-1, the mammalian ortholog of yeast Atg6/Vps30, also associates with phosphatidylinositol 3-kinase, and has a critical role in mammalian autophagy.[25,26] Recently, Beclin-1 attracted extensive attention, as the protein was shown to be a haploinsufficient tumor suppressor.[27,28] Furthermore, the anti-apoptotic Bcl-2 family proteins, Bcl-2 and Bcl-xL, interact directly with Beclin-1 and subsequently inhibit Beclin-1 dependent autophagy. This finding is particularly interesting, as the interaction between Bcl-2/Bcl-xL and Beclin-1 can be regarded as a point of convergence of apoptosis and autophagy.[29–31] Despite the potential importance of the regulatory role of Bcl-2/Bcl-xL in Beclin-1-dependent autophagy, the structural basis of the Bcl-2/Bcl-xL and Beclin-1 interaction remains obscure.

Here, we report the mapping and biochemical characterization of the minimal Bcl-xL-binding domain of Beclin-1. The solution structure of Bcl-xL in complex with the Bcl-xL-binding domain of Beclin-1 was determined at high resolution by NMR spectroscopy. The complex structure shows that Beclin-1 contains a BH3 domain in its N-terminal region. Detailed biochemical and structural analysis of the interface between Bcl-xL and Beclin-1 provides insights into the versatile targeting properties of Bcl-xL.

Results and Discussion

A short stretch of the sequence containing the BH3-like domain in Beclin-1 specifically interacts with Bcl-xL

We used purified recombinant human Bcl-xL and various fragments of human Beclin-1 to characterize the interaction between the two proteins. The Bcl-xL recombinant protein used throughout this study has its flexible loop (residues 45–84) and the transmembrane domain (residues 197–233) deleted, as such deletions are necessary for preparation and handling of Bcl-xL samples *in vitro*.[16] A 72 residue fragment (residues 104–175) in the N-terminal region of Beclin-1 was found to bind robustly to Bcl-xL in a pull down assay (Figure 1(a)). Deletion of residues 104–131 eliminated interaction between Beclin-1 and Bcl-xL (Figure 1(a)), indicating that this 28 residue fragment of Beclin-1 is required for the protein to interact with Bcl-xL. Next, we purified a thioredoxin-fused Beclin-1 fragment containing this 28 residue fragment (Trx-Beclin-1 (104-131)), and this Trx-Beclin-1 fragment could bind to glutathione-*S*-transferase (GST)-Bcl-xL (Figure 1(b)). We further noted that Trx-Beclin-1 (104-131) binds to GST-Bcl-xL in a similar manner when compared to Trx-Beclin-1 (104-175), suggesting that the 28 residue fragment of Beclin-1 (residues 104–131) is likely to be sufficient for binding to Bcl-xL. Mixing a peptide fragment encompassing Beclin-1 (104-131) (Figure 1(d) and (e)), but not its immediate C-terminal fragment (residues 132–175, data not shown), with Bcl-xL induced large chemical shift changes to a subset of resonances in the ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) spectrum of ^{15}N-labeled Bcl-xL, further supporting that the 28 residue fragment of Beclin-1 (residues 104–131) binds to Bcl-xL specifically. Finally, we measured the binding affinity between Beclin-1 (104-131) and Bcl-xL using the fluorescence polarization method. In this assay, an increasing amount of Bcl-xL was titrated into fluorescence-labeled Trx-Beclin-1 (104-131), and the binding curve was fit with the simple two-component-binding equation. We found that Bcl-xL binds to Beclin-1 (104-131) with a relatively high affinity (K_d ~1.4 μM) (Figure 1(c)). Longer Beclin-1 fragments with extensions in both the N and C termini of this 28 residue peptide did not increase the binding affinity of Beclin-1 to Bcl-xL (data not shown), further demonstrating that the 28 residue peptide fragment identified here represents the complete Bcl-xL-binding domain of Beclin-1.

Amino acid sequence alignment analysis revealed that the Bcl-xL-binding domain of Beclin-1 is highly conserved across species, from flies to humans (Figure 2). Since Bcl-xL is known to bind to its targets *via* their respective BH3 domains, we reasoned that the Bcl-xL-binding domain of Beclin-1 may also encompass a BH3 domain, and Beclin-1 may be a new member of BH3-only family proteins. This hypothesis is consistent with our NMR-based titration experiment showing that the binding of the Bcl-xL-binding domain of Beclin-1 induced chemical shift changes restrained to a region known as the BH3 domain-binding pocket of Bcl-xL (Figure 1(e)). Next, we aligned the amino acid sequence of the Bcl-xL-binding domain of Beclin-1 with those of all previously defined BH3 domains (Figure 2(b)). Except for the residue in the P3 position, the Bcl-xL-binding domain of Beclin-1