



Prospects for Drug Designing: Similar Conserved Interactions of Bim with MCL-1 AND BCL-2

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Abstract:

Structures of antiapoptotic Bcl-2-family proteins that protect the cells against death like Mcl-1 and Bcl-2 bear a surface groove into which the α -helical BH3 regions of certain pro-apoptotic proteins bind. This is done by occupying the hydrophobic pocket formed by the close proximity of their BH1-3 domains. The interactions among these proteins determine whether apoptosis will follow or not. These interactions are important in determining the apoptotic process in the cell. Phylogenetic studies have revealed that the BH domains remain almost conserved in many higher organisms and share functional similarity among them. Similar docking energy values of Mcl-1-Bim complex and Bcl-2-Bim complex support our view of conserved interactions between the BH3 domains of these proteins during apoptosis. These interactions on being disturbed change the apoptotic machinery of the cell thus they bear importance in drug designing.

Keywords: Apoptosis, Mcl-1, Bcl-2, Bim, Docking.

1. Introduction:

1.1 Role of Bcl-2 family in apoptosis.

Apoptosis, or programmed cell death (PCD), is a common and evolutionarily conserved property of all metazoans.¹ Dysregulation of apoptosis can, therefore, contribute to the development of many major diseases including cancer, autoimmunity and neurodegenerative disorders. In most cases, proteins of the caspase family execute the genetic programme that leads to cell death. Bcl-2 proteins are central regulators of caspase activation, and play a key role in cell death by regulating the integrity of the mitochondrial and endoplasmic reticulum (ER) membranes.²

Bcl-2 family of proteins can be divided into three groups, i.e., multidomain antiapoptotic proteins (such as Bcl-2, Bcl-xL, and Mcl-1), multidomain proapoptotic proteins (such as Bax, BAK, and Bok), and BH3-only proapoptotic members (e.g., Puma, Noxa, Bid, and Bim).³ The proapoptotic BH3-only proteins are the most apical regulators of apoptosis induction.⁴ The topology of the Bcl-2 domain in Mcl-1 is very similar to those of other Bcl-2 homologs.

Excluding the N-terminal 151 residues, Mcl-1 shares 24% sequence identity and 35–46% similarity with Bcl-2, Bcl-xL, Bcl-w, and A1.⁵

1.2 BH3 domain involvement in the formation of the binding groove.

The outcome of cell death signaling pathways depends on a complex network involving physical interactions between the pro-survival and pro-death members. A prominent hydrophobic groove is present on the surface of the anti-apoptotic proteins. This groove is the binding site for peptides that mimic the BH3 region of various pro-apoptotic proteins such as Bak and Bad. The BH3 region is responsible for mediating the interactions with anti-apoptotic proteins and the ability of the proteins to promote programmed cell death. Peptides derived from the BH3 regions of pro-apoptotic Bcl-2 family members can bind to anti-apoptotic family members such as Bcl-xL and modulate Bcl-2 regulated apoptotic pathways in living cells.⁶

Structural studies have revealed that the BH3 domain adopts an alpha helical conformation and that the BH3 domain of a proapoptotic member is able to bind to pro-survival members by occupying a hydrophobic pocket formed by the close proximity of their BH1-3 domains.⁵

1.4 Importance of the BH3 domain:

From the functional analysis of genes it is known that the BH3 domain of Mcl-1 lies in exon 1 and this region has motifs for proteolysis. The core positions 1 and 6 in the BH3 domain are crucial for the interaction of pro-apoptotic members with their antagonists; these residues are also present at similar positions in anti-apoptotic proteins. Residues at position 4 might serve to set the pro-apoptotic and anti-apoptotic proteins apart. Although all the position 4 residues have nonpolar side chains, only the three strong survival proteins, Bcl-2, Bcl-xL and Bcl-w, harbour alanines.

The minor differences in the amino acid make-up between the BH3 domains of pro-apoptotic and anti-apoptotic proteins might therefore translate into differences in relative binding affinity and serve not to distinguish binders from nonbinders but to distinguish the stronger binders from the weaker ones. The potency of BH3 domains in cell death has implications for the development of novel therapeutic agents to regulate apoptosis.⁸

Core positions 1 and 6 in the BH3 domain:

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L R R I G D E F Bim
L R R V G D G V Mcl-1
L R Q A G D D F Bcl-2
1 2 3 4 5 6
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The potency of BH3 domains in cell death has implications for the development of novel therapeutic agents to regulate apoptosis.⁸ Mechanisms that abrogate the pro-survival function of Mcl-1 either by diminishing its levels or inactivating its functional BH3 groove have shown promise for the combinational treatment with existing cancer therapies and as single agents in certain malignancies.⁵ The BH3 region of Bim has been used extensively for studies exploring the relationship between sequence, structure, and binding in the Bcl-2 family.¹⁰

1.5 Mcl-1 interaction with other proteins of Bcl-2 family.

Anti-apoptotic MCL-1 is a high priority target for developmental cancer therapeutics due to its emergence as a formidable and pervasive oncogenic protein.⁹ *In vitro* binding studies of recombinant pro-survival members binding to synthetic peptides corresponding to the BH3-domain of multiple pro-death Bcl-2 family members, revealed that like Bcl-2, Bcl-XL and Bcl-w, Mcl-1 interacts with a high affinity to BH3-only Bim, Bid and Puma, however it also selectively interacts with Noxa and Bak⁵

The exact molecular mechanism by which Mcl-1 promotes cell survival is not completely understood but is thought to involve suppression of cytochrome c release from mitochondria, possibly via heterodimerisation with and neutralisation of pro-apoptotic Bcl-2 family proteins, for example, Bim or Bak⁷

1.6 Focus of the study:

Seeing the importance of the anti-apoptotic members of the Bcl-2 family of proteins in regulating apoptosis, the residues of the conserved domain regions are the most obvious drug targets. The interactions of these residues with Bim core conserved BH3 residues have been shown, highlighting that any substitution/mutation in these regions will considerably decrease the stability of the proteins, thus altering the docking energy of interaction between the two proteins and disturbing the anti-apoptotic process.

2. Materials and methods:

2.1 Binding Site Prediction: Q-site finder, Pocket finder and CastP are used to find the amino acids involved in the formation of binding sites within the BH3 domain region of Mcl-1.

2.2 Disorder prediction: The disorder prediction was done using RONN, Globplot, DisEmbl and I-mutant tools. The I-mutant tool is the predictor of protein stability changes upon mutation. Its results gave the effect of substitutions on the stability of these residues. The disorderness among the conserved domain regions was done to identify the

potential mutations possible within them, which may lead to the destabilization of the protein thus hindering its anti-apoptotic function.

2.3 Structural Studies: The 3Dimensional structure of the protein was retrieved from the Protein Data Bank PDB. The tools used to identify the structure were HH Pred and Phyre from Expsy server.

2.4 Docking Studies: Argus Lab was used to study the binding interactions of the core conserved residues of the BH3 domains of Mcl-1 and Bcl-2 anti-apoptotic proteins with the core conserved BH3 residues of a pro-apoptotic protein Bim.

3. Results and discussion:

3.1 Sequence Retrieval: The FASTA sequences of the Mcl-1 and Bim sequences were retrieved from the Uniprot Database. All the other sequences of various species were retrieved from the NCBI database.

3.2 Structure Analysis: HH Pred and Phyre from Expsy server results gave the PDB id 2PQK which is a human Mcl-1-Bim complex. A chain is the Mcl-1 while B chain is the Bim protein.

3.3 Disorder prediction:

We have found the BH3 domain to be disordered and thus is highly prone to mutations, which is also indicated by the RONN results in which maximum residues show decrease in stability if replaced with any other amino acids.

I-Mutant results show that substitutions in these sites are responsible mainly for the decrease in the stability of the protein.

Table 3.1 Values of Disorder Prediction of the BH3 domain of Mcl-1 by RONN

L - 0.59	R - 0.58
R - 0.57	V - 0.56
G - 0.55	D - 0.55
G - 0.54	V - 0.53

Table 3.2 showing I-Mutant results

Position	Amino Acid	Amino Acid Replaced	Effect on stability
213	L	Any	decrease
214	R	Any	decrease
215	R	Any	decrease
216	V	Any	decrease
217	G	E Any other	increase decrease
218	D	E Any other	Increase decrease
219	G	Any	decrease
220	V	Any	decrease

3.4 Binding Site Prediction: From the results of Q-site finder, Pocket finder and CastP tools for Mcl-1 domain residues present in the binding site were identified to be A 209 L 210 L 213 R 214 G 217 and D 218.

3.5 Docking Analysis: The optimization of human Mcl-1(2PQKA), Bim (2PQKB) and Bcl-2 (1G5M) proteins was done using the ArgusLab 4.0.1 software.

Table 3.2: Shows the Docking energy values obtained through ArgusLab (Values indicated in bold indicate very near values.)

Bim BH3 core residues (2PQKB)	Docking of Bcl-2 BH3 core binding site residues LRQAGDDF (1G5M)	Docking of Mcl-1 BH3 core binding site residues LRRVGDGV (2PQKA)
L 10	-6.47 kcal/mol	-6.56 kcal/mol
R 11	-5.24 kcal/mol	-5.35 kcal/mol
R 12	-5.34 kcal/mol	-4.63 kcal/mol
I 13	-5.59 kcal/mol	-5.63 kcal/mol
G* 14	-4.69 kcal/mol	-4.70 kcal/mol
D 15	-4.97 kcal/mol	-5.45 kcal/mol

5. Conclusion:

Targeting the Bcl-2 family of proteins is a promising strategy for treating cancer. Upregulation of antiapoptotic Bcl-2 proteins is one way to avoid cell death.⁹ Despite high overall structural conservation, differences in this groove afford binding specificity that is important for the mechanism of Bcl-2 family function. With the antiapoptotic Bcl-2 family members attracting significant attention as therapeutic targets, these structures contribute to our growing understanding of how specificity is achieved and can help to guide the design of novel inhibitors that target Mcl-1.¹⁰

The study shows similar core residue-residue interactions among the Bcl-2 family proteins Mcl-1 and Bcl-2 through docking studies. On the basis of the above results, we can say that these interactions being highly conserved in nature are responsible for the normal process of anti-apoptosis, but any mutation/substitution in these core conserved residues will significantly affect the stability of the protein and its loss of function finally leading to the unavailability of Mcl-1 for binding during the anti-apoptosis process.

These conserved interactions could be used to design similar BH3 mimetic compounds of our interest which bear similar interaction energies like the original proteins but have substituted/mutated residues to produce the desired outcome in promoting or controlling the apoptosis of the cells. Thus these results bear importance for future drug designing, where the anti-apoptotic machinery has to be halted to check the uncontrollable growth of cancerous cells.

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