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Binding of anti-apoptotic Bcl-2 with different BH3 peptides: A molecular dynamics study

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Abstract

In this work, molecular dynamics simulation and free energy calculations are utilized to study how different BH3 peptides originating from Bax, Bim, Bik and Noxa interact with Bcl-2, one of the main members of anti-apoptotic proteins. The effects of peptide length, sequence and helical content on the binding affinity are discussed, on which a novel BH3-like peptide is designed in silico with an improved binding property.

1 Introduction

B-cell lymphoma 2 (BCL-2) protein family is a collection of proteins that regulate mitochondrial pathway by manipulating the integrity of the outer mitochondrial membrane (OMM). The pathway initiated by mitochondrial outer membrane permeabilization (MOMP) allows downstream pro-apoptotic proteins to go through mitochondrial membrane, diffuse into cytosol and then trigger apoptosis [1]. BCL-2 family members are distinguished as sharing at least one of four conserved Bcl-2 homology (BH1-4) and can be divided into three distinct subclasses [2,3]. One is anti-apoptotic proteins such as Bcl-2, Bcl-xL, Bcl-w, Mcl-1, A1 and Bcl-B which possess BH1 to BH4 domains and promote cell survival through blocking the pro-apoptotic proteins [4]. The pro-apoptotic proteins are divided into two subclasses, namely effector proteins and BH3-only proteins. BH3-only proteins such as Bim, Bad, Puma and Noxa, sense the pro-death signal and convey the signal to effector proteins via activation [5,6]. Upon death stimulus from BH3-only proteins, effector proteins such as Bax and Bak undergo polymerization to form mitochondrial apoptosis-induced channel (MAC) pores within OMM and promote MOMP [7-9].

Although the interactions between these three subclasses have not been fully understood, it has been widely accepted that the coordination between anti-apoptotic and pro-apoptotic proteins is through heterodimerization which is accomplished by binding the BH3 region of pro-apoptotic proteins to the hydrophobic cleft of anti-apoptotic proteins [10-12]. In normal cells, the interplay of anti-apoptotic and pro-apoptotic proteins attains an equilibrium and delivers cell-death signal normally. However, in cancer cells, anti-apoptotic proteins are found overexpressed and the imbalance blocks the activation of effector proteins such as Bax and Bak, thus in turn inhibits apoptosis [6,13]. Since anti-apoptotic proteins are regulated by different upstream and have distinct tissue distribution [14-16], they are responsible for different cancers. Take two main members in anti-apoptotic proteins as example, the expression of Bcl-2 is aberrant in Acute lymphoblastic leukemia and Precursor B-lymphoblastic leukemia, while Bcl-xL is more correlated with Liposarcoma and Brest carcinoma [17]. The unique function of these proteins offers a hint to design small molecules targeting a specific pro-survival member, which has been supported by experimental data showing BH3 peptides derived from different pro-apoptotic proteins have significant difference in binding with different pro-survival proteins [5,18-20].

Binding affinity studies on pro-apoptotic BH3 peptides exhibit a wide range of affinities to the anti-apoptotic protein Bcl-2. Experimental data show similar binding affinity of the 31-mer Bax·BH3 (residue 52-82) (K_D = 35.3 nM) and 36-mer Bax·BH3 (residue 49-84) to Bcl-2, while the shorter 26-mer peptide (residue 52-77) has a significantly poor binding affinity (K_D = 610 nM) with Bcl-2 [21], indicating that the five residues (residue 78-82) at the C-terminus play a significant role in binding with Bcl-2. Peptides with dissimilar sequences also interact differently with Bcl-2. BH3 peptides originating from Bim, Bik and Noxa bind to Bcl-2 with a binding affinity of 2.44 nM, 234 nM and 1620 nM respectively. Regardless of the fact that experimental researches have achieved significant advances in identifying BH3 peptides targeting anti-apoptotic proteins, theoretical studies are still required to provide more detailed information at atomic level on the binding mechanism and hot spots. Since residue 52-56 of Bax·BH3 in crystal structure are missed, in this study 26-mer Bax·BH3 (residue 57-82) and 21-mer Bax·BH3 (residue 57-77), namely Bax26

and Bax21 respectively are chosen to reveal how the five residues at the C-terminus influence the binding of Bax·BH3 with Bcl-2. Moreover, three more 26-mer BH3 peptides originating from Bim, Bik and Noxa (namely Bim·BH3, Bik·BH3 and Noxa·BH3 respectively) are selected to study the sequence dependency of BH3 peptides in binding with anti-apoptotic Bcl-2. All these information aims to provide insight on the design of BH3-like peptides of the next generation with high affinity and specificity.

2 Computational methods

2.1 Structure preparation

The crystal structure of Bcl-2 complex with Bax26 is obtained from the Protein Data Bank (PDB) with PDB id 2XA0 [21]. The truncated Bax21 peptide is prepared by manually removing five residues at the N-terminus. Since the long loop connecting $\alpha 1$ and $\alpha 2$ helix in the crystal structure is missing, the C-terminus of $\alpha 1$ and the N-terminus of the $\alpha 2$ are capped with N-methyl group (NME) and acetyl group (ACE) to mimic the backbone of the missing flexible loop. All water molecules in the crystal structure are preserved. Since there is no structural information available for Bcl-2 complexes with Bim, Bik and Noxa, the complex structures are prepared by mutating residues in Bax26 to corresponding sequence respectively. All the structures are prepared using Maestro 8.0 program [22].

2.2 Molecular dynamic simulation

The protein-peptide complexes are solvated in the pre-equilibrated box of TIP3P water [23]. The size of the water box is chosen to have a minimum distance of 10 Å between the atoms of the complex and the extremities of the water box. Sodium (Na⁺) ions are added to neutralise the net negative charge of the system. The AMBER03 force field is employed and molecular dynamics simulations are performed at 300 K with periodic boundary conditions using AMBER10 simulation package [24,25]. The particle mesh Ewald (PME) [26] is used to calculate long-range electrostatic interactions and a 12 Å cutoff is used for the van der Waals interactions. The SHAKE algorithm is used to restrain all bonds involving hydrogen atoms [27]. The minimization phase is divided into two steps. In the first step, the water box is energy minimized for 4000 steps using the steepest decent method followed by 1000 steps of conjugate gradient. A restraint of 500 kcal/mol/Å² is given on the protein complex. In the second step, the protein complex and the solvent are both minimized for 10,000 steps using the steepest decent method followed by conjugate gradient minimization until the energy gradient converges to 0.01 kcal/mol/Å. After minimization, the system is heated from 0 K to 300 K using Langevin thermostat [28] followed by the production run which last for a total of 10 ns at constant pressure with a time step of 2 fs.

It has been suggested that multiple short simulations can produce better quantitative results than a single long simulation due to the difference in exploring conformational space [29]. To get more correct quantitative results, we run three 10 ns trajectories for each complex system with different initial velocity and the trajectories are used in free binding energy calculation and alanine scanning calculation.

2.3 Free binding energy calculations with Sietraj

Sietraj [30,31] is used to calculate the binding free energies of Bcl-2 with the peptides. The solvated interaction energy (SIE) is calculated as the follows:

$$\Delta G_{\text{binding}} = \alpha(E_{\text{Coul}} + \Delta G_{\text{RF}} + E_{\text{vdw}} + \Delta G_{\text{npsol}}) + \text{constant} \quad (1)$$

E_{Coul} and E_{vdw} are the intermolecular Coulomb and van der Waals interaction energy in the bound state respectively. ΔG_{RF} and ΔG_{npsol} represent the change in reaction field energy and non-electrostatic solvation free energy between bound and free states respectively. The sum of E_{Coul} and ΔG_{RF} gives the electrostatic contribution to the binding while E_{vdw} and ΔG_{npsol} can be grouped as the nonpolar contributor to the binding free energy. α (0.104758) is an empirically determined factor to roughly evaluate the enthalpy-entropy compensation. Constant equals to −2.89. For each complex, 1000 snapshots are extracted from the last 5 ns of each molecular dynamics trajectory with an interval of 5 ps.

Since the stable helical nature of a BH3 peptide is directly correlated with its binding affinity and experimentally determined binding affinities for all the BH3 peptides in this study are in nM range, we typically make the approximation that no significant conformational changes occur upon the binding of the BH3 peptide so that the snapshots for all three species (complex, Bcl-2 protein, and BH3 peptide) can be obtained from a single trajectory. This is the 'single trajectory approach' and is what we use in this work.

2.4 Alanine scanning calculation

Alanine scanning calculation is a mean to analyze the contribution of side chains from each residue on the binding free energy by mutating the residue into alanine. In this research, Sietraj is used to perform alanine scanning on each residue of BH3 peptides and their contributions on the binding free energy are then analyzed by calculating the difference in binding free energy ($\Delta\Delta G$) before and after the mutation. In addition, the polar and non-polar contribution of the key residues is estimated to determine the main factor that leads to the relatively high contribution.

2.5 Helical content analysis

Beside the length and amino acid sequence of the peptide, the stability of the helix might be another factor that impacts the binding affinity of protein and peptide. Helical content is an indicator to evaluate the stability of α helix. In this analysis, only amino acids with dihedral angles in the range of $(\varphi, \psi) = (-60 \pm 20, -40 \pm 20)$ are considered as helical [32].

3 Results and discussion

3.1 Binding of Bcl-2 with Bax·BH3 peptides

The weak binding affinity due to five residues less at the C-terminal of Bax-BH3 peptide prompts us to explore the contribution of the five residues on the binding of Bax-BH3 with Bcl-2. The binding free energy calculated by Sietraj for Bcl-2 complex with Bax26 and Bax21 is -18.29 kcal/mol and -16.40 kcal/mol respectively, which is in good agreement with the experimental drop on binding affinity from -10.69 kcal/mol to -8.50 kcal/mol due to the missing of five residues at the C-terminal of Bax-BH3 peptide. This positive correlation laid a good foundation for the next alanine scanning analysis. As shown in Fig. 1, among the five truncated residues at the C-terminal, R78 has the most significant contribution to the binding with Bcl-2 due to the strong hydrogen bonding interaction between the guanidine side chain and the backbone oxygen of Glu200 as shown in Fig. 2a. Through the further comparison of the data in Fig. 1, two more residues R64 and D68 are identified with significantly less contribution when five residues are truncated. Detailed interaction analysis in Fig. 2 shows that in the absence of the five residues, both the hydrogen bond between the guanidine side chain of R64 and the backbone oxygen of Glu136, and the salt bridge between the carboxylate side chain of D68 and the guanidine side chain of Arg146 disappear.

Fig. 1 Alanine scanning results for Bax26 and Bax21.

Fig. 2 (A) Hydrogen bond stability between R78 and Glu200; (B) hydrogen bond stability between R64 and Glu136; (C) salt bridge stability between D68 and Arg146.

Since different degree of the deformation of Bax-BH3 peptides is observed during the simulations, helical contents in function of simulation time are plotted for the common part in both Bax26 and Bax21 to explore the possible relationship between helix stability and binding affinity. As we can see from Fig. 3, lower helical content curve for Bax21 refers to less stability of the peptide, which may cause the breaking of the R64-Glu136 hydrogen bond and the D68-Arg146 salt bridge, leading to weak binding affinity with Bcl-2.

Fig. 3 Helical contents for Bax26 and Bax21.

3.2 Peptide sequence on binding affinity

The above analysis of Bax26 and Bax21 indicates a possible relationship between helical stability of Bax-BH3 peptides and the binding affinity with Bcl-2 protein. In order to prove it further, three more BH3 peptides from

different proapoptotic proteins, namely Bim-BH3, Bik-BH3 and Noxa-BH3 are used to explore how amino acid sequence influences the binding of BH3 peptides with Bcl-2 protein. Since the complex structures of the three chosen peptides are constructed by introducing mutations on the structure of Bcl-2/Bax-BH3, extensive simulations are required generally to relax the complex structure completely. In order to avoid the statical errors accumulating at long MD simulation, multiple short-MD-trajectory strategy starting from the same initial protein structure with different initial atom velocities is used to simulate the complex structures between Bcl-2 and BH3 peptides, namely Bim-BH3, Bik-BH3 and Noxa-BH3, respectively.

Binding affinities of the four Bax-BH3 peptides calculated with Sietraj method in comparison to the experimental data are plotted in Fig. 4, indicating a generally good correlation with the experimental results and improve the credibility of the subsequent detailed analysis.

Fig. 4 Correlation between the experimental and the calculated binding affinities of the four BH3 peptides.

As reported previously, residue P3, P7, P10, P14 and P18 in BH3 peptides have been identified to be critical to the hydrophobic interactions with anti-death Bcl-2 proteins. From the alanine scanning in Fig. 5, the residues from different BH3 peptides in these positions show relatively high contributions to the binding which further address the importance of these five hydrophobic residues. Among these hydrophobic residues, P3 and P18 show quite similar contribution to the binding in the four BH3 peptides, indicating a high tolerance for hydrophobic residues in the hydrophobic sites H1 and H5. Residue P7, P10 and P14 in Noxa-BH3, on the other hand, show much less contribution compared to the residues in other BH3 peptides. Of these, residue P7 and P10 are highly conserved in the four BH3 peptides. As for the residues at the P14 position in the BH3 peptides, valine in Noxa-BH3 contributes the least probably due to its short side chain not enough to stretch out to occupy the H4 site.

Fig. 5 Alanine scanning results for BH3 peptides.

Residues besides the hydrophobic residues mentioned above also contribute significantly to the binding with Bcl-2 protein. Briefly, the region that residue P6 is pointing to is surrounded by the carboxylate side chain of Asp111,

$$=$$

favorable to the binding, verifying the almost zero contribution of alanine in Bik·BH3.

Fig. 6 together with the helical content for Bax-BH3 (Bax26). Again, there is some correlation between the helix stability and the binding affinity to Bcl-2. The more stable the helix, the better the binding affinity to Bcl-2, probably

Fig. 6 Helical contents for BH3 peptides.

3.3 In-silico design of BH3-like peptide

Based on the information obtained concerning how peptide length and peptide sequence affects binding affinity, a new peptide with the sequence in [Table 1](#) is proposed by combining the individual residue from the four BH3 peptides that contributed the most to the binding at each position. The same procedures for the explicit simulation and binding free energy calculation determine the binding free energy of the new peptide with Bcl-2 to be -19.63 kcal/mol, which is the strongest among the BH3 peptides in this study. The alanine scanning results in [Fig. 7](#) match our expectation with critical contributions of the side chains at the P3, P6, P7, P8, P10, P14 and P18 positions to the binding of the novel peptide with Bcl-2 protein. Although the test on the new peptide is quite tentative without further experimental results and folding simulations to support it, this study aims to provide insights for de novo design of BH3-like peptides that specifically inhibit Bcl-2. With the information of energetic contribution of individual side chain at each position from the BH3 peptides available, we can design various peptides on the basis of maintaining helical stability by making some adjustments in the specific position to increase the contribution of its side chain to the binding affinity or even improve the specificity for particular protein over other proteins of BCL-2 family.

Table 1 The peptide sequences of Bax26, Bax21, Bim·BH3, Bik·BH3, Noxa·BH3, and the in silico designed peptide. The sequences are numbered from 1 to 26.

[illegible]

In Silico peptide	I	R	L	S	Q	R	L	R	R	I	G	D	E	F	N	L	C	Y	R	S	R	R	M	I	A	D
Bax21	K	K	L	S	E	C	L	R	R	I	G	D	E	L	D	S	N	M	E	L	Q	-	-	-	-	-

Fig. 7 Alanine scanning result for the in silico designed peptide.

4 Conclusion

In this work, Bax26 and 5-residue truncated Bax21 were investigated via molecular dynamics simulation to explore the underlying mechanism for the truncation-induced binding affinity drop and the possible link between helical stability and binding affinity emerges. To further examine the link, three more BH3 peptides, namely Bik-BH3, Bim-BH3, and Noxa-BH3 were also studied. Similarly, calculating the relative binding affinities that are in agreement with the experimental results makes the analysis possible. And the introduction of more BH3 peptides reproduces the correlation between helical stability and binding affinity. Although only four BH3 peptides are not sufficient to produce any solid conclusion, this study can still provide insights on the *de novo* design of BH3-like peptides against anti-death Bcl-2 protein.

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Graphical abstract

Highlights

- 5-residue truncation induced binding affinity decrease for Bax-BH3 was investigated.
 - The relative binding affinities for BH3 peptides was successfully reproduced.
 - The helical stability of BH3 peptides may have some correlation with their binding with Bcl-2 protein.
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