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Review Article

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Accounting of the Influence of Point Mutations of Bh3-Peptide on the Stability of the Formed Biological Complex on the Example of the Bcl-2 Family Proteins

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Abstract

This article presents a new method that allows one to qualitatively determine the effect of point mutations in peptides on the stability of the formed complex with full-length proteins.

Introduction

This article is devoted to the investigation of the interaction of BH3-peptides with tiapoptotic proteins of the Bcl-2 family, which are regulators of mitochondrial apoptosis pathways. Note that disturbances in the process of apoptosis are a sign of a large number of diseases, such as cancer, sarcomas, carcinomas, lymphomas, and leukemias.

Studies results indicate that peptides have a pronounced protective effect in various diseases and have a modulating effect on various body systems. Unlike chemotherapy drugs, peptides are selective and effective signaling molecules that bind to certain receptors or ion channels, where they cause intracellular effects. Because peptides are highly selective and effective and at the same time relatively safe and well tolerated, they represent an excellent starting point for the development of new therapeutic agents, and their specificity demonstrates excellent safety, tolerability, and efficacy profiles in people with various pathologies [1-3].

We believe that the further development of peptide drugs will be based on the use of computer and mathematical design to find the optimal peptides, taking into account the affinity for their targets, and also to improve their chemical and physical properties. In this chapter, the effect of point mutations in BH3 peptides on the stability of the biological complexes with Bcl-2 will be determined, as well as the qualitative determination of the dissociation constant for binding different BH3 peptides to Bcl-xl proteins.

Let us examine some of the works devoted to the family of Bcl-2 proteins, as well as the study of the affinity of BH3 peptides for Bcl-2 family proteins. In review recent advances in understanding how BCL2 family proteins control MOMP (mitochondrial outer membrane permeabilization) as well as new nonapoptotic functions for these proteins [4]. In it was found that the Bcl-2 protein binds to the Bax protein through two interdependent interfaces, which leads to the inhibition of the proapoptotic oligomerization of Bax [5]. Studies of various interfaces with a large number of involved amino acid residues bring additional clarity to the nature of the interaction of proteins of the Bcl-2 family. In the molecular basis of the binding specificity of proapoptotic BH3 peptides, which contain different motifs, with a pro-apoptotic Bcl-xl protein, was investigated. Various motifs were identified in the BH3 domains of proteins which influenced the binding affinity of the Bcl-xl protein [6].

In this paper, in contrast to the above, we propose a mathematical model for determining the affinity of different BH3 peptides for Bcl-2 family proteins, as well as taking into account the effect of point mutations in peptides on the stability of the biological complex formed by them in dependence at amino acid sequence of protein. To analyze the biochemical processes we use the notion of condition number matrix of the potential energy of the pair electrostatic interaction between peptides. In this physical formulation of the problem, it will characterize the degree of stability of the configuration of the biological complex. In order to choose a more stable biochemical compound between proteins, we select the matrix of potential energy of electrostatic interaction with the smallest value of the condition number [7].

Structure and functions of Bcl-2 family proteins

Proteins of the B-cell lymphoma-2 family (Bcl-2) control their own apoptotic path, regulating the process of permeabilization of the outer membrane of the mitochondria through protein-protein interactions. Structural and biochemical studies have shown the dual role of anti-apoptotic proteins of the Bcl-2 family in inhibiting BH3-only proteins and the activated proteins Bax and Bak. Details of the interactions between the Bcl-2 family proteins are presented in [4]. The proteins of the Bcl-2 family can be divided into 3 groups based on their structure and intracellular functions:

1). One group includes Bcl-2 antagonist/killer (Bak) and Bcl-2 associated X protein (Bax), which are known as apoptosis effectors. Also called multidomain pro-apoptotic BCL2 family proteins, BAX and BAK contain BCL2 homology (BH) domains 1-3 and can

directly permeabilize MOM when activated. Whether Bcl-2-related ovarian killer (Bok) belongs to this same subfamily is not clear. Structurally it is similar to Bak and Bax [8]. however, functionally it does not have the ability to permeabilize the MOM by itself but instead induces apoptosis only in the presence of Bak or Bax [9]. Structural studies have demonstrated that Bak and Bax monomers are globular structures consisting of a central hydrophobic core helix (alfa5) surrounded by eight alpha helices [10-12]. In the Bak monomer, four of these helices (α1, α3, α4, and α6) are long helices that form a circle around the central helix α5 while the others (α2, α7, and α8) are shorter and link either the longer helices or the main structure to the transmembrane (TM) domain which consists of helix α9 [10]. The major structural difference between monomeric Bak and Bax is the orientation of helix α9. In Bax, this helix is buried in a hydrophobic groove formed by helices α3, α4, and α5 [12]. In contrast, the hydrophobic groove of Bak is empty; and the α9 helix of Bak extends away from the remainder of the globular protein.

2). The second group, called anti-apoptotic or pro-survival Bcl-2 family members, includes Bcl-2, Bcl-x large(Bcl-xl), Bcl-2-like protein 2(Bcl-W), Bcl-2-like protein 10(Bcl-B), myeloid cell leukemia 1(Mcl1), and Bcl-2-related protein A1(Bfl-1) (A1 in mouse). These proteins, which contain four BH domains (BH1-BH4), inhibit apoptosis by binding and sequestering their pro-apoptotic counterparts.

The anti-apoptotic Bcl-2 proteins possess a remarkably similar globular structure containing a so-called <<Bcl-2 core>> [13].

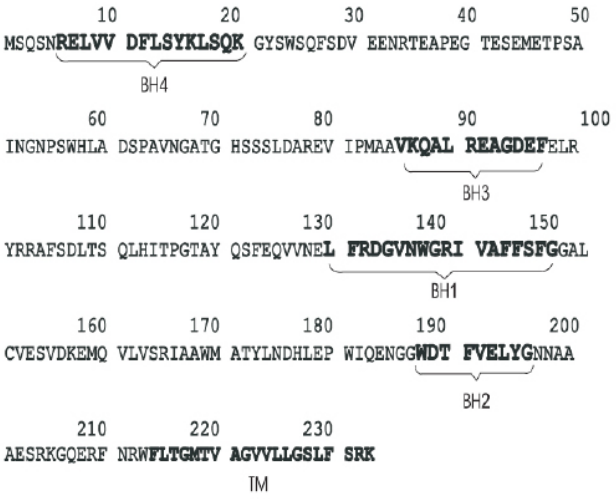

Figure 1: Schema of a Bcl-2 protein. The amino acid sequence Q071817 [14]. Specifying BH domains [15].

This core consists of a bundle of eight alpha-helices that form a hydrophobic groove flanked by the BH1 and BH3 domains. In Bcl-W, the core also includes a short C−terminal helix α-8 attached to the BH2 domain. The hydrophobic groove made by α3−α5 is termed the <<BC groove>> because it binds the BH3 region of binding partners [13,16].

The BC groove is crucial for the biology of anti-apoptotic Bcl-2 family proteins, as it provides an interface for the interaction with the BH3 domain of BH3-only proteins and the apoptotic effectors BAK and BAX. Based on the structure of the BC groove, several BH3 mimics that can occupy this groove and thus inactivate these proteins anti-apoptotic function have been developed and are currently being tested in the clinic [17-19].

3). The final group, termed BH3-only proteins, includes Bim, Puma, Bid, Bad, Noxa, Bik, Bmf, and Hrk. These polypeptides share only a 15-25 residue BH3 domain in common with other Bcl-2 family proteins. This BH3 domain, however, is critical for the interactions of these proteins with other BCL2 family proteins to regulate MOMP.

The most important role of BH3-only proteins such as Bim, Puma, Bid, and Noxa is to act as integrators of various signals to initiate MOMP. The BH3-only proteins are activated by distinct cytotoxic stimuli in various ways, including enhanced transcription and posttranslational modifications [20].

BH3-only proteins can be divided into direct activators and sensitizers [21,22]. As pro-apoptotic signals are received, for example, DNA damage or cellular stress, proteins such as Bid or Bad stimulate and compete with effectors for binding to repressors, and not only neutralize the antiapoptical actions of repressors, but also lead to a proapoptotic effect of the effectors.

The effectors subsequently initiate apoptotic cell death by their ability to integrate into the outer membrane of the mitochondria, which causes the formation of pores in the membranes. This results in the release of apoptogenic factors, such as cytochrome c and Smac/Diablo from the mitochondria into the cytosol [23]. Thus, the concerted action of different Bcl-2 proteins allows one to keep apoptosis under control in a healthy cell, while a disorder in the regulation leads to serious pathological consequences.

The formation of heterodimers between different proteins of the Bcl-2 family determines whether the cell survives or not [6]. The Bcl-2 family is an important therapeutic target due to over-expression in some cancer cells where these proteins contribute to oncogenesis and resistance to chemotherapy. In particular, overexpression of the Bcl-xl and Bcl-2 proteins of apoptosis repressors is associated with the development of various oncological diseases.

The Bcl-xl and Bcl-2 proteins are the most suitable targets for anticancer therapy. Thus, in this paper, interactions of various BH3 peptides with the anti-apoptotic proteins Bcl-xl and Bcl-2 will be investigated.

2 . Numerical calculation results & Conclusion

This part of the article presents the results of the numerical calculations of the interaction of BH3 peptides with Bcl-xl and Bcl-2 proteins, as well as an analysis of the effect of point mutations in BH3 peptides on their ability to form stable biological complexes with the pro-apoptotic Bcl-xl protein.

2.1 Interaction of modified BH3-peptides of Bax protein with Bcl-2 protein taking into account the replacement of amino acid residues.

In this section, the binding of BH3 peptides of the Bax protein was numerically simulated, taking into account various changes in amino acid residues with the Bcl-2 protein. The obtained result will allow us to determine the influence of point mutations in the BH3 peptides of the Bax protein on the stability of the complexes formed with the Bcl-2 protein.

In [24], the binding structure of the Bcl-2 protein to the Bax protein region was determined. The Bax protein peptide forms an amphiphilic α−helix and binds to the BH3-binding hydrophobic groove of the Bcl-2 protein. The intermolecular interaction between the Bcl-2 protein and the Bax protein peptide is mediated by hydrophobic and ionic interactions. Some of the main amino acid residues from the protein Bcl-2 are the amino acid residues in the region from 107a.a. to 146 a.a. as well as the amino acid residue in the 200 a.a. region. The amino acid sequences of the BH3-peptide of the Bax protein with the performed amino acid residue substitutions, as well as the dissociation constants for binding each peptide to the Bcl-2 protein are shown in Table 7.4.

Table 1: List of amino acid sequences of BH3 peptides of the Bax protein with amino acid substitutions and dissociation constant for each peptide when interacting with the Bcl-2 protein

Location of point mutations in the region $Bax_{(49–84)}$	amino acid sequence	K_d, nmol
Bax(49−84)$_{(wt)}$	QPPQDASTKKLSECLRRIGDELDSNMELQRMIADVD	15.1
mBax(61A,R64A,R78A)	QPPQDASTKKLSACLARIGDELDSNMELQAMIADVD	787
mBax(E61A)	QPPQDASTKKLSACLRRIGDELDSNMELQRMIADVD	95.2
mBax(R64A)	QPPQDASTKKLSECLARIGDELDSNMELQRMIADVD	129
mBax(D68A)	QPPQDASTKKLSECLRRIGAELDSNMELQRMIADVD	1040
mBax(E69A)	QPPQDASTKKLSECLRRIGDALDSNMELQRMIADVD	476
mBax(R78A)	QPPQDASTKKLSECLRRIGDELDSNMELQAMIADVD	57.1

Interaction of the BH3 peptide of the *Bax*(*wt*) protein with the Bcl-2 protein was taken as the main interaction of the BH3 peptide with the Bcl-2 protein. When point exchanges of amino acid residues in the BH3 peptide of the Bax protein are performed, we assume that the main interactions of these modified BH3 peptides fall on the same sections of the Bcl-2 protein as the *Bax*(*wt*) BH3-peptide of the wild type when interacting with the Bcl- 2 and the point replacements of the amino acid residues do not essentially change the binding site with the Bcl-2 protein, but have a significant effect on the affinity of complex formation. When analyzing the interaction of modified BH3 peptides with Bcl-2, the sites identified as the main sites in the interaction of the wild-type BH3 peptide with Bcl-2 will be analyzed.

(Figure 2) shows the numerical results obtained for the interaction of all Bax(*wt*) and modified BH3 peptides of the Bax protein with Bcl-2 at low values of lg (cond (w)). The results of numerical simulation of the interaction of the BH3-peptide Bax (*wt*) with Bcl-2 on the graph are presented by a black square, while the results of the interaction of all other modified BH3 peptides of the Bax protein with Bcl-2 are represented by empty figures.

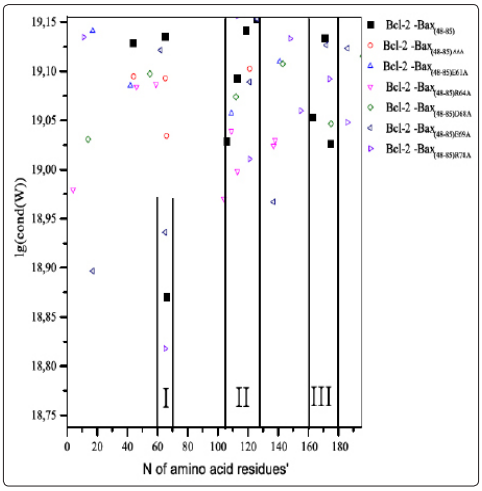

Figure 2: Results of the numerical calculation of the interaction of the Bax3 peptides of the Bax protein with the Bcl-2 protein (fill figures) and the Bax3 peptides of the Bax protein, in which the amino acid residue substitutions (empty figures) were performed, with the Bcl-2 protein in the region of the smallest values of lg (cond (W)), $\epsilon = 1$

For further analysis of the data, we identified three significant areas with the lowest values of lg (cond (W)) in the interaction of the BH3 peptide *Bax* (*wt*) with the Bcl-2 protein in the range of lg (cond (w)) from 18.75 to 19.15. Recall that each point on the graph represents the first a.a. when binding two amino acid sequences.

The first area lies in the interval from 60 a.a. to 70 a.a., the second region lies in the region from 105 a.a. to 130 a.a., and the third area from 160 a.a. to 180 a.a.

For each of these areas, we calculated the number of hits for each interaction of the

BH3 peptide with Bcl-2 and plotted the result in the form of a histogram, see (Figure 3). As can be seen from the histogram, the greatest number of hits-8, is typical for the interaction of the BH3-peptide *Bax*(*wt*) with Bcl-2. The number of all other hits for modified BH3 peptides Bax c Bcl-2 corresponds to a smaller number of hits of the minimum values of lg (cond (W)) in these regions. Also, from the given histogram 2.1, the interaction of the BH3-peptide Bax (49−85) with the Bcl-2 protein is characterized by the most frequent hit of the lg (cond (W)) values in the selected regions in comparison with the other modified BH3 peptides of the Bax protein, in which the amino acid residues were substituted.

Thus, six modified BH3-peptides of the protein Bax (49−85) in which the amino acid residue substitutions were performed bind to the Bcl-2 protein worse than the BH3- peptide *Bax* (*wt*), while the site Bax (49−85) is prone to form the most stable biological complexes with the Bcl-2 protein compared to all other Bax (49−85) peptides in which one or more amino acid residues were replaced by the amino acid residue of alanine (A). This result is in good agreement with the previously performed experimental article, which indicates that the dissociation constant of mutant peptides upon binding to

the Bcl-2 protein is higher than when bounding to the natural site of Bax(49−85))[24].

The influence of point mutations on the stability of the formed biological complexes was studied on the example of the interaction of the BH3 peptides of the Bax protein in

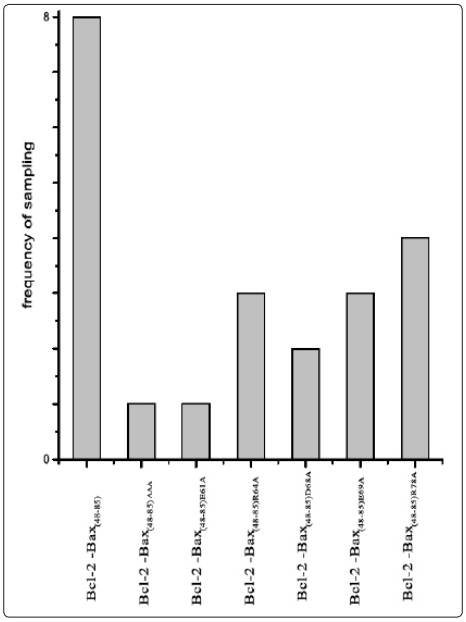

Figure 3: Frequency of BH3 peptides Bax in the three designated regions of the Bcl-2 protein, $\epsilon = 1$

which point replacements of amino acid residues were made with the whole Bcl-2 protein. The formation of the biological complex of BH3-peptide of the protein Bax (49−85) with Bcl-2 was taken as the main interaction. The numerical results of the interaction of the protein Bax (49−85) with Bcl-2 were compared with the remaining results of the interaction of BH3 peptides Bax with the Bcl-2 protein taking into account the substitution of amino acid residues. As a result, the Bcl-2 protein regions with the largest number of minimum values of lg (cond (W)) were found in the interaction with Bax (49−85). The subsequent analysis of these regions revealed that the other modified BH3 peptides contain much less than the minimum values of lg (cond (W)) in the previously designated regions.

Thus, it is possible to use the obtained result to determine the binding site of the peptide with the whole protein in order to determine the stability of the formation of the biological complex by any modified BH3 peptide of the Bax protein in which the amino acid residues have been replaced with the Bcl-2 protein.

The results of the work were obtained using computational resources of Peter the Great Sainte-Petersburg Polytechnic University Supercomputing Center (www.spbstu.ru). The calculations were performed in MATLAB computing environment 2017a, operat-ing system CentOS Linux 7.

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