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Crystal structure of human Bcl-2 family kin as a novel member of Bcl-2 family mediating apoptosis

세포 사멸을 주관하는 Bcl-2 family의 novel member인 Human Bcl-2 family kin의 결정화 및 구조 규명

2020년 2월

서울대학교 대학원
약학과 의약생명과학전공

오 은 경
Abstract

Crystal structure of human Bcl-2 family kin as a novel member of Bcl-2 family mediating apoptosis

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College of Pharmacy

The Graduate School

Seoul National University

Bcl-2 family proteins govern apoptosis by regulating mitochondrial outer membrane permeabilization (MOMP). The Bcl-2 family proteins contain one or more of the Bcl-2 homology domains (BH1, BH2, BH3, and BH4), and are divided into pro- and anti-apoptotic members. Bfk is a novel member of the Bcl-2 family discovered in an effort to find novel genes with a BH3 domain. Bfk contains BH2 and BH3 regions but no BH1 or BH4 region and lacks a C-terminal transmembrane region. Human Bfk (hBfk) is predominantly expressed in the gastrointestinal tract and shows reduced expression in tumor tissues. The overexpression of hBfk has been known to antagonize some anti-apoptotic Bcl-2 family proteins and to elicit weak pro-apoptotic functions in the gastrointestinal tract.
To elucidate the structure-function relationships of hBfk protein, I have successfully purified the hBfk protein in *E. coli* system and crystallized. Here, I report the crystal structure of hBfk at 2.45 Å resolution using single wavelength anomalous dispersion (SAD) method with selenomethionine derivative crystals to solve the phasing problem. The structure of hBfk consists of 8 alpha helical regions and shows remarkable similarity with other Bcl-2 family proteins (Bcl- X\textsubscript{L}, Bcl-2, Mcl-1, Bax, Bid, etc.) despite low sequence similarity. The structure of hBfk is the most similar with the structure of Bid with a root-mean-square deviation (RMSD) of 2.53 Å over 103 equivalent Ca atoms. Although hBfk is similar with Bid in structure, further studies on cellular functions and binding partners of hBfk are needed.

**Keywords**: Bfk, Bcl-2 family, Apoptosis, BH domain, crystal structure, X-ray crystallography

**Student number**: 2018-27753
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I. Introduction

Apoptosis, the essential process during organ development, tissue turnover, and functioning of the immune system, is a evolutionarily conserved mechanism among organisms. The Bcl-2 family proteins govern apoptosis by regulating mitochondrial outer membrane permeabilization (MOMP), which cause release of cytochrome c and subsequent caspase activation\(^1,2\). The Bcl-2 family proteins contain one or more of the Bcl-2 homology domains (BH1, BH2, BH3, and BH4) and most of Bcl-2 family proteins have a C-terminal transmembrane region. These structurally related proteins consist of pro- and anti-apoptotic members. The anti-apoptotic members (Bcl-2, Bcl-X\(_L\), Bcl-W, and Mcl-1) generally contain all four BH domains as well as C-terminal hydrophobic region and adopt a highly conserved tertiary structure that acts as a BH3 domain-binding groove\(^3,4\). The pro-apoptotic members are divided into two groups. One is pore-formers (Bax, Bak, and Box) which contain multi-BH domains and the other is BH3-only proteins (Bad, Bid, Bim, Noxa, Puma, etc.) which contain only a BH3 domain\(^5\).

Despite low sequence similarities and functional differences, the structures of Bcl-2 family proteins (Bcl-X\(_L\), Bcl-2, Bcl-W, Mcl-1, Bax, Bid, etc.) that have been deposited in Protein Data Bank (PDB) show remarkably high similarities\(^5,6\). In the other hand, BH3 only proteins except Bid are intrinsically disordered and do not share a typical structure of Bcl-2 family proteins. There are various competing models explaining how Bcl-2 family proteins interact with each other. All models suggest that direct binding interactions among the Bcl-2 family proteins are associated with their BH3 domains (Figure 1)\(^7\). However, the exact mechanism that explains how Bcl-2 family proteins regulate apoptosis has not yet been known.

Recently, new pro-apoptotic members of Bcl-2 family proteins have been identified, but not yet classified due to the different composition of BH
domains in their sequences. Bfk is a novel member of the Bcl-2 family, which was discovered in 2003. Bfk contains BH2 and BH3 domains without C-terminal transmembrane region and appears to have weak pro-apoptotic activity. hBfk is predominantly expressed in tissues of gastrointestinal tract and shows reduced expression during malignant transformation. In addition, there are several experimental results indicating that Bfk correlates with expression of proteins involved in apoptosis of cancer cells. Interestingly, hBfk shows similarities with Bid, a BH3 only protein essentially involved in intrinsic apoptosis pathway, in that the sequences of hBfk and Bid contain a same motif at N-terminus and contain a caspase targeted site for further activation and lack a transmembrane region at C-terminus. Since the structure of Bfk has not been known despite its importance, I started to study on Bfk to elicit the structural uniqueness of Bfk compared with other Bcl-2 family proteins.

I have successfully purified the hBfk recombinant protein in E. coli system and obtained crystals suitable for data collection. Here, I report the crystal structure of hBfk at 2.45 Å resolution using single wavelength anomalous dispersion (SAD) method with selenomethionine derivative crystals to solve the phasing problem. Bfk shows remarkable structural similarity with other Bcl-2 family proteins (Bcl-XL, Bcl-2, Mcl-1, Bax, Bid) and is particularly similar to Bid in sequence and structure.
Figure 1. BH3:groove interaction as a general mechanism of binding between Bcl-2 proteins

Structural (left) and schematic (right) representations of BH3:groove dimers that may be the basis of all interactions between Bcl-2 members. (From Dewson G, Kluck RM. Mechanisms by which Bak and Bax permeabilise mitochondria during apoptosis. J Cell Sci. 2009;122(Pt 16):2801–2808. doi:10.1242/jcs.038166)
Figure 2. Distribution of Bcl-2 homology domains in Bcl-2 proteins

Typical domain structures of Bcl-2 family proteins are shown in boxes, atypical proteins are shown below. (From Amir M. Hossini. Apoptosis induction by Bcl-2 proteins independent of the BH3 domain. Biochemical Pharmacology 76. 1612-1619, doi:10.1016/j.bcp.2008.08.013(2019), with permission from Elsevier)
II. Materials and Methods

2.1. Cloning

Full-length gene of human Bfk (163 amino acids) was purchased from Korea human gene bank (Daejeon, Korea) and was amplified using PCR. The PCR primers were purchased from Cosmogenetech (Seoul, Korea). The amplified product was digested with NdeI and XhoI restriction enzymes (Enzynomics, Daejeon, Korea) and was inserted into the pET-28a(+) vector (Novagen, Madison, WI, USA) to contain an N-terminal hexahistidine tag.

2.2. Overexpression

The recombinant plasmid was transformed into an *Escherichia coli* strain, C43(DE3) (Lucigen, Wisconsin, USA). The transformed cells were grown at 37°C until OD₆₀₀ reached 0.6 in the Luria Broth media containing 30 mg/mL kanamycin. Protein overexpression was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). The cells were incubated for additional 16 h at 20°C, and then collected by centrifugation at 6,000 ×g for 10 min.

For SeMet-derived Bfk, the recombinant plasmid was transformed into an *E. coli* strain, B834(DE3). The transformed cells were grown in M9 minimal media supplemented with extra amino acids containing SeMet.

2.3. Purification

The collected cells were resuspended in the buffer A (Table 1) supplemented with 1 mM phenylmethylsulfonyl fluoride and lysed by sonication. Lysed cells were centrifugated at 35,000 ×g for 70 min. The supernatant was filtered with a 0.45 μm syringe filter device (Sartorius, Göttingen, Germany) and
loaded onto a 5 mL-HiTrap Chelating HP column (GE healthcare, Chicago, IL, USA) which was previously charged with 100 mM nickel sulfate and equilibrated with the buffer A. After washing with the buffer A, the retained proteins were eluted by addition of the increasing gradient of buffer B (Table 1). The fractions containing Bfk were further loaded onto a HiLoad 16/600 Superdex 75 prep grade column (GE Healthcare) with the buffer C (Table 1). The purified protein was concentrated with an Amicon Ultra-10K centrifugal filter device (Millipore, Darmstadt, Germany). The purification of SeMet-derived Bfk was same as for the native Bfk.

<table>
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<tr>
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Table 1. Buffers for purification of Bfk

2.4. Crystallization

Initial crystals of Bfk are obtained with the sitting drop vapor diffusion method by mixing 0.5 μL (10 mg/ml) and 0.5 μL crystallization solutions containing 0.1 M Bis-Tris at pH 7.5, 25% polyethylene glycol (PEG) 3,350 (ShotGun 1; Molecular dimension, Cambridge, England) at 22°C. The optimized crystals are obtained with grid screenings with various protein and crystallization solution concentration at 16°C under by the macroseeding using SeedBead. The SeMet-derived crystals was obtained in the same way as for the native crystal.

2.5. X-ray data collection
Prior to expose to x-ray, the Bfk crystals were cryoprotected with the reservoir solution supplemented with 17% (v/v) of glycerol, and then flash-cooled in a nitrogen gas stream at 100 K. X-ray diffraction data were collected using a Pilatus 36M detector system (DECTRIS) at the 11C beamline of Pohang Light Source (Pohang, Korea). The data were collected by oscillating the crystal by 1° to 200°\textsuperscript{13}. The x-ray diffraction data of SeMet-derived Bfk crystal were collected using an Eiger 9M detector system (DECTRIS) at the 5C beamline of Pohang Light Source (Pohang, Korea). The data were collected at the absorption peak wavelength of selenium, and the crystals were oscillated by 1° to 350° during the collection. All the collected data were processed with the HKL2000 program package.

2.6. Structure determination, model building, and refinement

The phase of the SeMet-derived Bfk crystal was solved using the single anomalous diffraction (SAD) method, and the initial model was built with the AutoSol in the PHENIX software package\textsuperscript{14,15}. The model for native Bfk was obtained using the molecular replacement (MR) method by the MolRep\textsuperscript{16} with the initial model as the template structure. All structures were modeled, refined, and validated using the Coot\textsuperscript{17}, Refmac\textsuperscript{18}, and MolProbity\textsuperscript{19}, respectively.
III. Results and Discussion

3.1. Cloning and overexpression

Full length of human Bfk was constructed as a recombinant protein with N-terminal hexa-histidine tag into pET-28a vector to enable selectivity in purification. To find the most suitable E. coli strain for overexpression of Bfk, I tested 6 competent cells for overexpression at 20 °C. Expression test results showed that the Bfk protein was overexpressed to high level and considerably soluble in all 6 strains (Figure 3). First I chose R2 (DE3) competent cells, but a considerable amount of contaminants was followed in whole purification process. Instead, I used C43 (DE3) cells to obtain proteins with high purity. SeMet-derived Bfk was expressed in B834(DE3) cells which are methionine auxotroph.

![Figure 3. SDS-PAGE of expression and solubility test of Native Bfk](image)

A) BL21-CodonPlus(DE3)-RIPL
B) C43(DE3)
C) Rosetta2(DE3) pLysS
D) BLR(DE3)
E) SoluBL21
F) Rosetta2(DE3)
Lane 1: before IPTG induction
Lane 2: total cell lysate after culture for 16 h at 20 °C
Lane 3: supernatant of lane 2 after centrifugation
Lane 4: precipitant of lane 3
M: protein marker

3.2. Purification

3.2.1. Purification of Native Bfk
The IPTG-induced cells were collected by centrifugation at 6,000 g for 10 min at 4 °C. The cells were 7.0 g for 2.0 L culture. Bfk proteins were successfully purified using two steps of column chromatography. In the first step, the N-terminal hexa-histidine tag was utilized in a Ni$^{2+}$-chelated HiTrap chelating HP column. The elution profile from the HiTrap column is shown in Figure 3 and SDS-PAGE of the column fractions is shown in Figure 4. Upon eluting with a gradient of imidazole, most of proteins were eluted at 90-190 mM imidazole concentration. Most contaminants were removed at this step. Most of the fractions contained a similar amount of contaminants, but oligomeric states of each fractions were different. Two peaks in the elution profile were separately applied to the size exclusion chromatography column, HiLoad 16/600 Superdex 75 prep-grade, for next step of the purification. Consequently, analysis of the size exclusion chromatography showed that the first and second peaks, followed from the affinity chromatography, represented a monomer and a dimer, respectively. The elution profile of the first peak is shown in Figure 5 and SDS-PAGE of the column fractions is shown in Figure 6. The elution profile of the second peak is shown in Figure 7 and SDS-PAGE of the column fractions is shown in Figure 8.
Figure 4. Elution profile of native Bfk from the HiTrap chelating HP column chromatography

Elution was performed with a linear gradient of 30 to 500 mM imidazole.

Figure 5. SDS-PAGE analysis of HiTrap chelating HP column fractions of native Bfk

Lane 1: washing thru collection (3% elution buffer)  Lane 8: fraction #16
Lane 2: fraction #8                            Lane 9: fraction #18
Lane 3: fraction #10                            Lane 10: fraction #20
Lane 4: fraction #12                            Lane 11: fraction #22
Lane 5: protein marker                          Lane 12: fraction #24
Lane 6: fraction #14                            Lane 13: fraction #26
Lane 7: fraction #15
Figure 6. Elution profile of native Bfk from the Superdex 75 column chromatography (1)

Elution was performed with a buffer of 25 mM MES, 200 mM NaCl, pH 6.0.

Figure 7. SDS-PAGE analysis of the Superdex 75 column fractions of native Bfk (1)

Lane 1: fraction #18
Lane 2-7: fraction #20-25
Lane 8: protein marker
Figure 8. Elution profile of native Bfk from the Superdex 75 column chromatography (2)

Elution was performed with a buffer of 25 mM MES, 200 mM NaCl, pH 6.0.

Figure 9. SDS-PAGE analysis of the Superdex 75 column fractions of native Bfk (2)

Lane 1-3: fraction #13-15
Lane 4: fraction #17
Lane 5: protein marker

3.2.2 Purification of SeMet-derived Bfk

The purification of SeMet-derived Bfk was performed in the same manner as the native Bfk. The cells were 6.2 g for 2.5 L culture. In the first step, the N-terminal hexa-histidine tag was utilized in a Ni$^{2+}$-chelated HiTrap chelating HP column. The elution profile from the HiTrap column is shown in Figure 10 and SDS-PAGE of the column fractions is shown in Figure 11. Upon
eluting with a step gradient of imidazole, bound proteins were eluted at 60 mM, 110 mM, 160 mM, and 280 mM imidazole concentration, shown as four peaks in the elution profile. The four peaks of proteins eluted at different imidazole concentrations were separately applied onto the size exclusion chromatography column, HiLoad 16/600 Superdex 75 prep-grade, for next step of the purification. Consequently, analysis of the size exclusion chromatography showed that the first and second peaks, followed from the affinity chromatography, represented a monomer and a dimer, respectively. The elution profile of the first peak is shown in Figure 12 and SDS-PAGE of the column fractions is shown in Figure 13. The elution profile of the second peak is shown in Figure 14 and SDS-PAGE of the column fractions is shown in Figure 15.

Figure 10. Elution profile of SeMet Bfk from the HiTrap chelating HP column chromatography

Elution was performed with a step gradient of 50 to 500 mM imidazole
Figure 11. SDS-PAGE analysis of HiTrap chelating HP column fractions of SeMet Bfk

Lane 1: washing thru collection (3% elution buffer)
Lane 2: fraction #3
Lane 3: fraction #5
Lane 4: fraction #9
Lane 5: protein marker
Lane 6: fraction #11
Lane 7: fraction #13
Lane 8: fraction #16
Lane 9: fraction #21
Lane 10: fraction #23
Lane 11: fraction #25
Lane 12: fraction #27
Lane 13: fraction #36
Lane 14: fraction #38
Lane 15: fraction #41

Figure 12. Elution profile of SeMet Bfk from the Superdex-75 column chromatography (1)

Elution was performed with a buffer of 25 mM MES, 200 mM NaCl, 1 mM Tcep, pH 6.0.
Figure 13. SDS-PAGE analysis of the Superdex-75 column fractions of SeMet Bfk

Lane 1-5: fraction #19-23

Figure 14. Elution profile from the Superdex-75 column chromatography (2)

Elution was performed with a buffer of 25 mM MES and 200 mM NaCl, 1 mM TCEP, pH 6.0.
3.3. Crystallization

The native crystals of Bfk were obtained by the sitting-drop vapor diffusion method at 16 °C using a reservoir solution consisting of 0.1 M Bis-Tris at pH 6.0, 28% (v/v) polyethylene glycol (PEG) 3,350 and microseeds made by SeedBead. The crystals of SeMet-derived Bfk were grown at 16 °C using the same reservoir solution with native Bfk.

3.4. X-ray diffraction data collection

X-ray diffraction data of native crystals of Bfk were collected at 100 K using a Pilatus 3 6M detector system (DECTRIS) at 11C beamline of Pohang Light Source (Pohang, Korea) (Figure 16(a)). Native crystals of Bfk belong to the space group P321 with the unit cell parameters of \( a = 114.0 \text{ Å}, b = 114.0 \text{ Å}, c = 27.6 \text{ Å}, \) and \( \alpha = 90^\circ, \beta = 90^\circ, \gamma = 120^\circ \). Table 2 summarizes the
statistics for data collection on the native Bfk. Since there were no suitable models of Bfk homologs for molecular replacement method, the structure of Bfk was determined with SAD method using SeMet-derived Bfk. The X-ray diffraction data of SeMet-derived Bfk crystals were collected with 350 images at the absorption peak for selenium at 100 K on Eiger 9M detector system (DECTRIS) at 5C beamline of Pohang Light Source (Pohang, Korea) (Figure 17). The SeMet-derived crystals belong to the space group P3₂ with the unit cell parameters of \( a = 116.2 \ \text{Å}, b = 116.2 \ \text{Å}, c = 27.7 \ \text{Å}, \) and \( \alpha = 90°, \beta = 90°, \gamma = 120°. \) Table 3 summarizes the statistics for data collection on SeMet-derived Bfk. Since the native crystals were not diffracted well, X-ray diffraction data of SeMet-derived Bfk were selected to obtain a final structure. Because Bfk crystals were prone to decay during data collection, diffraction images from 1 to 120 were used for the rescaling to improve the quality of data. Table 4 summarized the statistics for data collection which was rescaled on SeMet-derived Bfk to use molecular replacement method.

![Figure 17. X-ray diffraction images for native Bfk (a) and SeMet-derived Bfk (b)](image-url)
### Statistics on the native crystal data collection

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**Table 2. Data collection statistics on native Bfk**

<sup>a</sup>Values in parentheses refer to the highest resolution shell (3.26-3.20 Å).

<sup>b</sup>R<sub>merge</sub> = ∑<sub>h</sub>∑<sub>i</sub>|I(h)−<I>(h)>|/∑<sub>h</sub>∑<sub>i</sub>I(h), where I(h) is the intensity of reflection h, h is the sum over all reflections, and i is the sum over i measurements of reflection h.
**Statistics on the SeMet-derived crystal data collection**

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**Table 3. Data collection statistics and phasing statistics on SeMet-derived Bfk**

\(^a\)Values in parentheses refer to the highest resolution shell (3.26-3.20 Å).

\(^b\)Rmerge = \(\sum h\sum i l(h) - \langle l(h)\rangle / \sum h\sum l(h)i\), where \(l(h)\) is the intensity of reflection \(h\), \(h\) is the sum over all reflections, and \(i\) is the sum over \(i\) measurements of reflection \(h\).
**Statistics on the rescaled SeMet-derived data collection**

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<td>50.0-2.45 (2.49-2.45) $^a$</td>
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<tr>
<td>Total/unique reflections</td>
<td>47845/15406</td>
</tr>
<tr>
<td>Redundancy</td>
<td>3.1</td>
</tr>
<tr>
<td>Rmerge$^b$</td>
<td>7.1 (78.4)$^a$</td>
</tr>
<tr>
<td>Data completeness (%)</td>
<td>99.1 (90.9)$^a$</td>
</tr>
<tr>
<td>$&lt;$I$&gt;$/$\sigma$(I)</td>
<td>12.3 (1.1)$^a$</td>
</tr>
</tbody>
</table>

$^a$Values in parentheses refer to the highest resolution shell (3.26-3.20 Å).

$^b$Rmerge = \[\sum_h \sum_i |I(h)i - \langle I(h) \rangle| / \sum_h \sum_i I(h)i\], where $I(h)$ is the intensity of reflection $h$, $h$ is the sum over all reflections, and $i$ is the sum over $i$ measurements of reflection $h$. 

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Table 4. Statistics on the rescaled SeMet-derived data collection
3.5. Structure determination

A homologous template model search using XtalPred web site (http://xtalpred.godziklab.org/XtalPred-cgi/xtal.pl) revealed that Bfk shares no significant sequence similarity with proteins deposited in PDB. The top hit was a human Bid (PDB accession 2bid) with only 15% identity. Since Bfk could not be solved by the molecular replacement method with a lack of homologous models, X-ray diffraction data of SeMet-derived Bfk were used to solve a phase problem in SAD method. All 7 selenium atoms were found by program AUTOSOL of PHENIX software package. The phases were further improved by density modification using the program RESOLVE, yielding an initial model. Phasing statistics are summarized in Table 5. The model of SeMet-derived Bfk was refined using Coot and Refmac. The statistic of data was improved with rescaled data of SeMet-derived crystal. Final structure of Bfk with the rescaled data was solved by molecular replacement with the program MolRep and refined with Coot and Refmac. The data of Native Bfk were not used to determine the structure of hBfk.
## Model refinement

<table>
<thead>
<tr>
<th>Resolution range (Å)</th>
<th>38.05-2.45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rwork / Rfree (%)</td>
<td>21.1/24.6</td>
</tr>
<tr>
<td>No. of non-hydrogen atoms</td>
<td>3326</td>
</tr>
<tr>
<td>Average B factor (Å)</td>
<td>43.0</td>
</tr>
</tbody>
</table>

### R.m.s. deviations from ideal geometry

| Bond lengths (Å) | 0.0089 |
| Bond angles (°)  | 1.1292 |

### Ramachandran plot

| Favored / Outliers (%) | 99.1/0 |
| Poor rotamers (%)      | 0      |

Table 5. Model refinement on SeMet-derived Bfk
3.6. Overall structure of hBfk

Figure 18. Overall structure of hBfk

Bfk consists of 8 alpha helices shown as ribbon representations colored in teal. BH2 and BH3 domains are shown in orange and yellow, respectively.

The structure of human Bfk was determined at 2.45 Å resolution with three molecules in the asymmetric unit. Bfk consists of 8 alpha helices (α1-α8), which adopt a canonical Bcl-2 fold. Bfk contains a central hairpin arrangement consisting of α6 (residues 108–126) and α7 (residues 131–144), which is flanked by α1 (residues 7–23), α3 (residues 50–63), α8 (residues 146–155) on one side, α4 (residues 69–81), and α5 (residues 84–102) on the other side. There is no electron density for the extend region connecting α2 to
α3 (residues 32-42), suggesting that it is a flexible random coil like other Bcl2 family proteins.

Figure 19. Ribbon diagram of the structure
The view on the left has the same orientation as Figure 18 while the middle view has been rotated 180° about the vertical axis and the right view 90° about the horizontal axis.

3.7. Structural comparison with other Bcl-2 family proteins
Although the overall sequence identities between Bfk and other Bcl-2 family members are low, ranging from 13% (Mcl-1) to 20% (Bcl- X_L), their three-dimensional structures are similar with Cα RMSD value of 2.53 Å (Bid, 103 residues) to 3.65 Å (Bcl-2, 98 residues) (figure 20). Among the Bcl-2 family proteins of which structures have been deposited in PDB, the structure of Bfk shows the highest similarity to that of Bid and Bcl-X_L with the RMSD value of 2.53 Å and 2.54 Å, respectively. In the other hand, the RMSD value of Bcl-X_L with Bid is 3.37 Å. It is well known that Bid suffers conformational changes after cleaved by caspase and helices (6, 7, and 8) of Bid are expected
to be inserted into the mitochondrial outer membrane⁶. In addition to the structural similarity between Bfk and Bid (figure 21), it is worth noting that their sequences contain the same motif (ECIxNxLxxxFL) at N-terminus and a caspase targeted site (figure 22). Besides, unlike other Bcl-2 family proteins, Bfk and Bid do not have a transmembrane region in their C-terminus. However, Bid contains a N-terminal disordered region and much longer disordered loop between α2 and α3 than Bfk (figure 23). Therefore, further studies on cellular functions and binding partners of hBfk are needed for a better understanding.

Figure 20. Structural superposition of Bcl-2 family proteins.
The structures of Bcl-2 family proteins (Mcl-1, Bax, Bcl-2, Bcl-X₁, and Bfk)
are superposed and represented as a ribbon diagram. Mcl-1, Bax, Bcl-2, Bcl-X_l, and Bfk are colored in purple, yellow, grey, orange, and teal, respectively.

Figure 21. Structural superposition of Bfk and Bid

The overall structure of Bfk (turquoise) and Bid (salmon) is superposed.
Figure 22. Sequence alignment of Bcl-2 family proteins.

BH1, BH2, BH3, and BH4 domains are indicated with rectangular boxes colored in green, orange, yellow, and sky blue, respectively. Bfk shares partial homology with Bid in N-terminal region, indicated in purple rectangular box.
Figure 23. Sequence alignment of Bfk and Bid

Sequence and secondary structure of Bfk and Bid are shown.
4. References


국 문 초 록


hBFK 단백질의 구조-기능적 상관관계를 규명하기 위해 대장균 발현 시스템에서 hBFK를 성공적으로 정제하고 결정화했다. Phase problem을 해결하기 위해 Selenomethionine-derived crystal을 이용하여 SAD 방법으로 hBfk의 결정구조를 2.45 Å 해상도로 규명하였다. hBfk는 8개의 알파 나선으로 구성되어 있으며, 낮은 시퀀스 유사성에도 불구하고 다른 Bcl-2 family proteins(Bcl- X_l, Bcl-2, Mcl-1, Bax, Bid 등)과 상당한 유사성을 보인다. hBid는 hBfk와 103개의 Cα 원자에 대해 2.53 Å의 RMSD값을 가짐으로써 Bcl-2 family proteins 중 가장 낮은 RMSD 값을 보였다. 이 연구를 통해 hBfk의 구조가 밝혀졌지만, 세포사멸 과정에서의 hBfk의 역할을 규명하기 위해서는 hBfk의 세포 기능과 결합 파트너에 대한 추가 연구가 필요하다.
주요어: Bfk, Bcl-2 family, 세포사멸, BH domain, 결정 구조, X-ray 결정학

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