MOLECULAR MODELING OF FULL-LENGTH 3D-STRUCTURE OF THE PROTEIN BCL-W AND MECHANISMS OF ITS ANTIAPOPTOTIC ACTION

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The proteins of Bcl-2 (B-cell lymphoma 2) family (Bcl-2 proteins) are important cell death regulators, whose main function is to control the release of cytochrome c from mitochondria in the intrinsic apoptotic pathway [1]. This family comprises nearly 20 both pro- and anti- apoptotic proteins, which share one or more short regions of sequence homology, the Bcl-2 homology regions (BH1, BH2, BH3 and BH4), which interact in various ways to induce or prevent pore formation in the outer mitochondrial membrane. The mammalian Bcl-2 antiapoptotic protein subfamily includes Bcl-2, Bcl-xL, Mcl-1, Bcl-w, A1/Bfl-1, NR-13 (human homolog nrh), Bcl-B and Bcl2-L-10. The most consistent correlate of antiapoptotic proteins survival function in intact cells is suppression of cytochrome c release. The antiapoptotic protein Bcl-w provides pleiotropic resistance against multiple death stimuli, is up-regulated in colorectal cancer, and plays an essential role in spermatogenesis. However until now, antiapoptotic action of the Bcl-w is not completely understood from an structural standpoint. In accordance with current concept of mechanistic action of Bcl-2 and Bcl-xL, following synthesis, these survival factors are targeted to either ER or mitochondrial membranes, into which they integrate through their hydrophobic C-terminal tail [1]. In this state, they probably sequester either directly Bax and Bak or BH3-only proapoptotic proteins required for the activation of Bax, Bak and/or caspases by a hydrophobic pocket that is formed from the four conserved domains (BH1–4) of these proteins [1]. The recent structural [2, 3] and functional [4] studies suggest that the current model describing the mechanistic action of Bcl-2-like proteins might not entirely apply to the survival factor Bcl-w. In contrast to Bcl-2 and Bcl-xL, which most probably have their hydrophobic C-termini exposed and therefore need rapid membrane targeting and/or insertion [1], Bcl-w has its C-terminal tail folded back into its hydrophobic pocket and, similar to Bax, can be soluble [2,3]. Functional studies showed that Bcl-w is active while weakly associated with mitochondria [4]. It was also hypothesized that in apoptotic cells, a BH3-only protein neutralizes the survival activity of Bcl-w by binding to its 'hydrophobic pockets', thereby releasing its C-terminal domain and allowing its inser-

tion into the membrane [4]. Yet, the structural understanding of antiapoptotic action of Bcl-w is incomplete largely due absence of full-length structure and availability of high resolution data on Bcl-w complexes with proapoptotic proteins. Earlier it was suggested that Bcl-w sequesters both BH3-only proteins and Bax. In this study we tested this hypothesis by modeling Bcl-w full-length structure and 3D-structures of complex Bcl-w -tBid.

Modeling of the three-dimensional structure of Bcl-w was carried out basing on the crystal structure of a truncated form of Bcl-w (residues 1–183) [2] and using the program MODELLER 9 version 3 [5] to model the structure and position of the C-terminal tail (residues 184- 193) of the protein.The crystal structure of Bax (Protein Data Bank code 1F16) has been used as a template upon homology modeling. The modeling of the linker between N-terminal part (1-183) and C-terminal tail (184-175) has been carried out by the kinematic loop closure Rosetta protocol [6]. The accuracy of the model was improved by several LBFGS minimizations by the program Tinker [7]. The stereochemical quality of the final model was assessed using the validation program PROCHECK [8]. Molecular modeling of the complexes Bcl-w-Bax anchored to the mitochondrial outer membrane by helix α9 and Bcl-w -tBid in solution has been carried out by the program Piper [9].

The final structure of full-length human Bcl-w is shown in Fig.1.

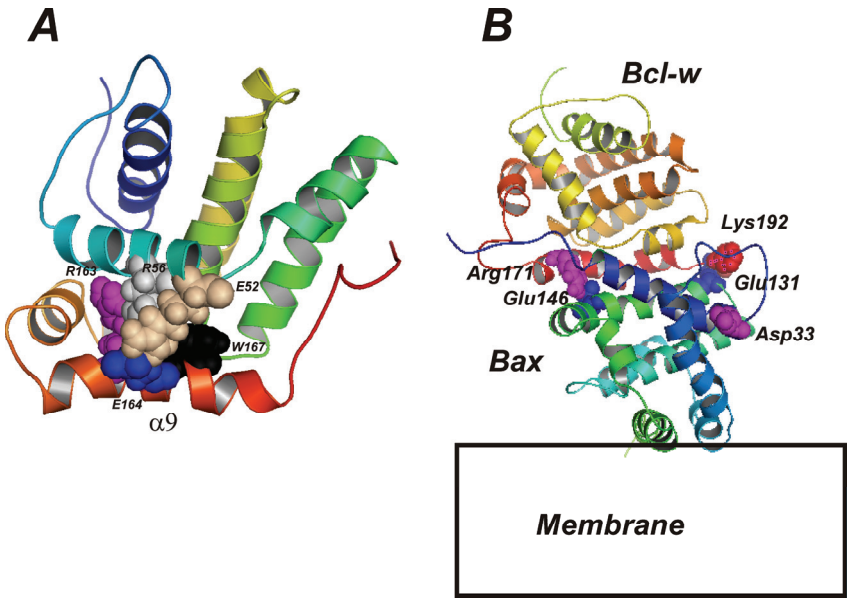

Figure 1 – *A*: The final structure of full-length human Bcl-w. Residues forming polar contacts are shown as spheres; *B*: Complex of MOM-associated Bax with Bcl-w

The formation of two salt-bridges (Arg163-Glu52 and Glu164-Arg56) and one hydrogen bond (Trp167-Glu52) has been established. In addition, several hydrophobic interactions seem as possible. These results suggest that Bcl-w helix α9 should have a high affinity for the canonical Bcl-w hydrophobic groove, while Bcl-w will associate as a peripheral protein to the mitochondrial outer membrane rather than inserting into it. Molecular docking simulations showed that Bcl-w sequesters MOM - associated Bax (Fig.1B) with an interface formed by salt-bridges between Bcl-w Arg171, Lys192 and Bax Glu146, Glu131, respectively, preventing by this sequestration Bax activation by tBid. A significant affinity between Bcl-w and tBid was also established with the main contribution from hydrophobic interactions between Bcl-w hydrophobic convex formed by helices α3, α4 and α9 and tBid hydrophobic groove formed by tBid helices H3, H6, H7, H8.

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