MOLECULAR MODELING OF FULL-LENGTH 3D-STRUCTURE OF THE PROTEIN A1/Bfl-1 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]. They comprise both pro- and anti-apoptotic proteins, which interact in various ways to induce or prevent pore formation in the outer mitochondrial membrane. Due to their central function in the apoptotic machinery, Bcl-2 proteins are often deregulated in cancer. To this end, many anti-apoptotic Bcl-2 proteins have been identified as important cellular oncogenes and attractive targets for anti-cancer therapy. A1 is the smallest member of the BCL-2 family and has been shown to retard apoptosis in various cell lines. In a physiological context, the antiapoptotic protein A1 is mainly expressed in the hematopoietic system, where it facilitates survival of selected leukocytes subsets and inflammation. However, A1 is overexpressed in a variety of cancer cells, including hematological malignancies and solid tumors, and may contribute to tumor progression. The function of the anti-apoptotic Bcl-2 family member A1 is poorly understood due to the lack of appropriate loss-of-function mouse models, redundant effects with other Bcl-2 pro-survival proteins upon overexpression and the lack of full-length structure (only truncated form of A1 with residues 1-24 and 31-149 is available [2, 3]). In this study, we present a molecular modeling study of full-length A1 (FL-A1) and structural basis of its antiapoptotic action.

Modeling of the three-dimensional structure of A1 was carried out basing on the crystal structure of a truncated form of A1 (residues 1–149) [2] and using the program MODELLER 9 version 3 [4] to model the structure and position of the C-terminal part (residues 144-175) 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 loop (24-31) and the linker between N-terminal part (1-143) and C-terminal part (144-175) has been carried out by the kinematic loop closure Rosetta protocol [5]. The accuracy of the model was improved by several LBFGS minimizations by the program Tinker [6]. The stereochemical quality of the final model was assessed using the valida-

tion program PROCHECK [7]. Molecular modeling of the complexes of A1 with Bak integrated into the MOM by helix α9 and with tBid has been carried out by the program Piper [8].

The final structure of human A1 is shown in Fig.1.

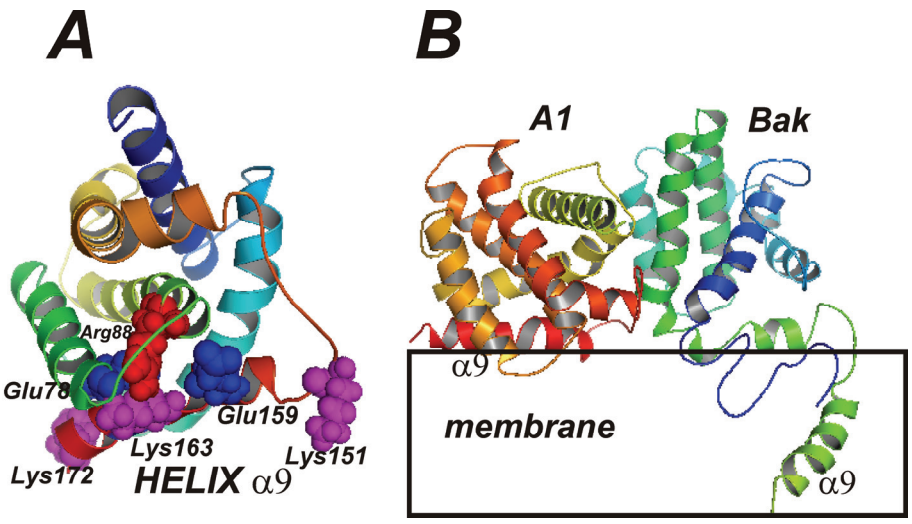

Figure 1 – The full-length structure of the protein A1 (A) and complex of A1 with Bak peripherally associated with MOM (B). A: Glu78 and Arg88 of canonical binding groove were predicted to form salt-bridges with Lys163 and Glu159, respectively

The formation of one intramolecular salt-bridge (Lys163-Glu78) was predicted and one more salt bridge (Arg88-Glu159) was shown to be likely. In addition, several hydrophobic interactions were possible, implicating residues Phe-157, Leu-158, Gly-162, Ile-164, Cys-165, Leu-168, Ser-169, and Leu-171, from helix α9 and Ser-43, Val-44, Val-48, Asn-51, Leu-52, Val-74, Lys-77, Thr-91, and Phe-148 from hydrophobic cleft. These results suggest that helix α9 should have a high affinity for the hydrophobic cleft and associate as a peripheral protein to the mitochondrial outer membrane rather than inserting into it. These results are in contrast with the data from [10] where low affinity for the conventional hydrophobic cleft and coexistence of two conformations were hypothesized: one with helix α9 buried into the cleft and the other with α9 exposed. To establish the mechanisms of antiapoptotic action of A1, the interaction of A1 with tBid in solution and the interaction of A1 with Bak constitutively integrated into the MOM by helix α9 was investigated by molecular docking. Because helix α9 of A1 is known to play a key role in preventing apoptosis [9], among 30 models obtained by molecular docking of A1 to Bak, only those where helix α9 of A1 is in contact with either Bak cytosolic part or with the MOM were analysed. No models were found with direct contact be-

tween Bak and A1 helix α9. Among those interacting both with MOM by helix α9 and with Bak, one with best score was considered as the most plausible model responsive for antiapoptotic action of A1. A significant affinity between A1 and tBid was also established with the main contribution from two salt bridges formed by Glu124, Glu6 of A1 and Arg168, Arg88 of tBid, respectively.

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