Antiapoptotic Action of Bcl-xL through Interaction with the VDAC2/tBid/Bax complex: *in silico* analysis

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Mitochondrial Outer Membrane Permeabilization (MOMP) is a critical step in the intrinsic pathway of apoptosis and is determined by the balance between the proapoptotic and antiapoptotic proteins of the Bcl-2 family. The proapoptotic proteins Bax and Bak are the main executors of MOMP in response to proapoptotic stimuli, allowing proteins in the mitochondrial intermembrane space, such as cytochrome c, to escape into the cytosol where they can induce caspase activation and cell death (1, 2). This process is actively opposed by the Bcl-2 family antiapoptotic members, such as Bcl-xL, Bcl-2, Bcl-w, Bcl-B, Mcl-1 and A-1 (Bfl-1), but the mechanisms are largely obscure. The predominant view is that antiapoptotic proteins act through direct interactions with Bax and BH3-only proteins, such as tBid, in the cytosol or with Bak integrated into the MOM. However, an encounter between the proteins in the cytosol in the required orientations is highly improbable event thus suggesting that the cytosol interaction is unlikely. Currently, it became clear that most of functional interactions of Bcl-2 family members occur at the MOM and that the membrane plays an active role in modulating the interactions between BCL2 proteins (3). Recently, the protein VDAC2 have been found to assist Bcl-2 family proteins Bax, Bak and tBid in MOMP by recruiting Bak to the MOM followed by the tBid-mediated Bak displacement from VDAC2 (4, 5). We suggested that the complex of tBid, known to interact with Bax, with VDAC2 is a receptor for Bax in cells undergone apoptosis, and Bcl-xL performs its antiapoptotic action by binding to the complex VDAC2-tBid-Bax. To test this we first predicted the 3D-structures of the complexes VDAC2-tBid and VDAC2-tBid-Bax and then modeled the binding of Bcl-xL to the VDAC2-tBid-Bax complex using computational structural biology methods. The combination of the global docking program Piper (4) with local refinement by RosettaDock (5) was applied in all cases. We found that tBid binds to membrane-resident VDAC2 with higher affinity than that does Bak (see Table 1), thus explaining why tBid displaces Bak from VDAC2. We detected a moderately strong affinity of Bax binding to the VDAC2-tBid complex and high affinity between Bcl-xL and the VDAC2-tBid-Bax complex (Table 1). The 3D-structures of the complexes VDAC2-tBid-Bax and VDAC2-tBid-Bax-Bcl-xL are shown in Fig.1.

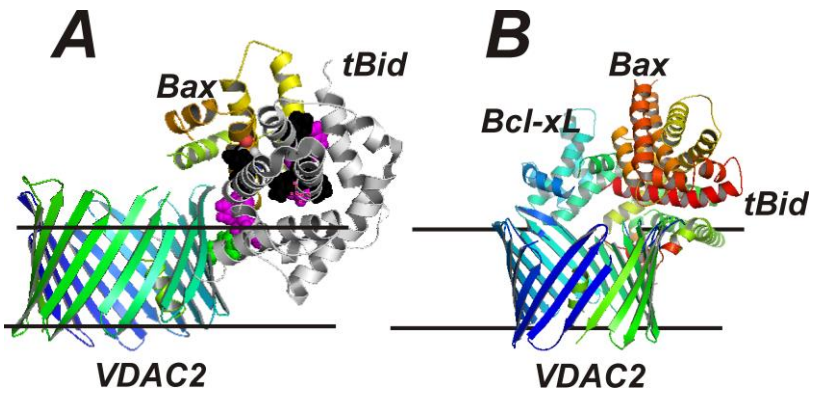

Fig.1. Structural models of the VDAC2-tBid-Bax (A) and VDAC2-tBid-Bax-Bcl-xL complexes.

Table 1**.** The Piper and RosettaDock scoring function values for the highest- rank complexes VDAC2-tBid and VDAC2-tBid-Bcl-xL

Protein complexes	The Piper weighted scores for the highest-ranked structures	The Rosetta scoring function values for refined structures
VDAC2-tBid	-1589.1	-472..9
VDAC2-tBid with Bax	-1197.3	-396.27
VDAC2-tBid-Bax with Bcl-xL	-1497.1	-492..8

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