Structural insights into TOM-mediated proapoptotic activation of Bax

V.S. URBAN, A.V. DUDKO, V.G. VERESOV

Institute of Biophysics and Cell Engineering of NAS of Belarus, Minsk, Belarus.

e-mail: veresov@ibp.org.by

Most mitochondrial proteins are nuclear encoded, synthesized on cytosolic ribosomes, and imported into the organelle posttranslationally. Nearly all mitochondrial preproteins are imported via the general entry gate, the translocase of the outer membrane or TOM complex [1]. The TOM complex is an assembly of seven subunits acting together at different stages of protein import: the surface receptors TOM20 and TOM70, the general import pore (GIP) consisting of TOM40, TOM22, and the small TOM proteins TOM5, TOM6, and TOM7 [1]. Nearly a decade ago it has been speculated that the TOM complex is involved in tBid-mediated Bax-induced permeabilization of mitochondrial outer membrane resulting in the release of apoptogenic intermembrane space (IMS) proteins, such as cytochrome c, and finally in apoptosis [2 - 4]; however, experimental evidence has been scarce end the results are controversial [5]. Very recently, using computational structural biology tools and the most recent structural information that was obtained through electron-microscopy and cross-linking experiments we have obtained the atomic-resolution structural model of the yeast and human GIP complexes. In the present study, the atomistic model of the GIP complex together with computational structural biology tools are used to gain structural insights into mechanisms of TOM-mediated mitochondrial apoptosis. Because the experimental data show that interaction of the Bcl-2 family proteins Bax and tBid with the TOM40/TOM22 subcomplex of TOM is a key event in TOM-mediated apoptosis, modeling of the 3D-structure of the GIP/Bax and GIP/tBid complexes was performed at first. This was performed in a stepwise fashion with an initial rigid-body global search and subsequent steps to refine these initial predictions. *In the initial stage*, the six-dimensional (6D) rotational and translational space was searched for binding orientations by the Fast Fourier Transform-based program PIPER [6] using the ClusPro docking server [7]. This program tolerates side chain conformational changes and small backbone conformational changes by introducing ‘‘softness’’ to scoring functions, that is, making these scoring functions insensitive to small structural errors. PIPER performs exhaustive sampling on a dense grid, and necessarily samples near-native conformations, regardless of the shape of the energy surface. However, because of the approximate nature of the energy function and the need for tolerating potential overlaps, the PIPER structures that are close to the native conformation do not necessarily have the lowest energies (approximated *in silico* by rapidly computable scoring function). Thus, the detection of near-native structures requires some form of post-processing among high-scored structures obtained upon the initial docking. This refinement post-processing stage was performed by combining the

discrimination-by-clustering strategy embedded in Piper with a local structural refinement by the ROSETTADOCK [8, 9] and HADDOCK [10] programs. A local refinement of ROSETTADOCK includes side chain repacking and a Monte Carlo search of the local rigid-body space of the ligand. The refinement procedure was applied to a set of several top-scored protein complexes obtained at the initial stage using balanced, hydrophobic-favored and electrostatic-favored scoring schemes of PIPER except those that were in obvious conflict with experimental data. ROSETTADOCK refinement was used twice: at the second stage immediately after PIPER ($ROSETTADOCK_1$), and then at the fourh, final refinement stage ($ROSETTADOCK_2$), immediately after HADDOCK refinement used at third stage. HADDOCK [10] applied at the third stage is a docking method using information about the interface region between the molecular components and/or their relative orientations. Unlike many other docking programs, HADDOCK allows for conformational change of the molecules during complex formation, not only of the side chains but also of the backbone, as well as the intermolecular interaction between atoms through water molecules. Here, information about the interface was derived from initial simulations by $ROSETTADOCK_1$ and experimental data. The refinement procedure was applied to a set of several top-scored protein complexes obtained at the initial stage using balanced, hydrophobic-favored and electrostatic-favored scoring schemes of PIPER except those that were in obvious conflict with experimental data. If a certain protein was known to bind to the cytoplasmic part of a receptor, we simulated by PIPER only the protein fragment involving the cytosolic part and near-cytosol membrane part using "masking file" option, while the remainder of the receptor was ignored. Large surface loops, if they existed, were first removed upon PIPER docking and then restored by the Rosetta loop closure protocol [11] being next set as flexible at the HADDOCK stage. Clustering of structures and energy funnels were used to improve the ability of finding the correct structure of the complex. The PRHR docking strategy has resulted in the structure of the GIP/Bax complex possessing low shape complementarity ($\Delta VDWS_{RD}$=-15.9, BSA= 572.7 $Å^2$) where $\Delta VDWS_{RD}$ is the ROSETTADOCK weighted interface Lennard-Jones score, which is calculated by subtracting the corresponding score of complexes from those of the individual chains, and BSA is 'Buried Surface Area'. One salt bridges and one hydrogen bond took place between TOM22 and Bax. Taken together, this resulted in a sufficiently high ROSETTADOCK interface energy score (I_sc) of -5.3 for the highest-ranked $ROSETTADOCK_2$ structure of the complex, suggesting a low binding affinity of GIP towards Bax. In contrast, in the case of the GIP-tBid interaction, a fine shape complementarity ($\Delta VDWS_{RD}$=-162.5; BSA= 3975.4 $Å^2$) took place. Besides, one salt bridge and one hydrogen bond were formed. Together, this resulted in a very low ROSETTADOCK interface energy score value of -11.1, suggesting very high binding affinity between tBid and GIP. Importantly, these results suggest that tBid, which is generated under apoptotic conditions, should form highly stable GIP/tBid complexes.

Interestingly, upon application of PRHR strategy to the interaction between Bax and the GIP/tBid complex, we found significant shape complementarity between Bax and the GIP/tBid complex ($\Delta VDWS_{RD}$=-47.6; BSA= 2713.4 Å^2). Besides, two salt bridges and two hydrogen bonds took place between tBid and Bax. Taken together, this resulted in a low ROSETTADOCK interface binding score (Isc) of -8.0 for the highest-ranked ROSETTADOCK structure of the complex.

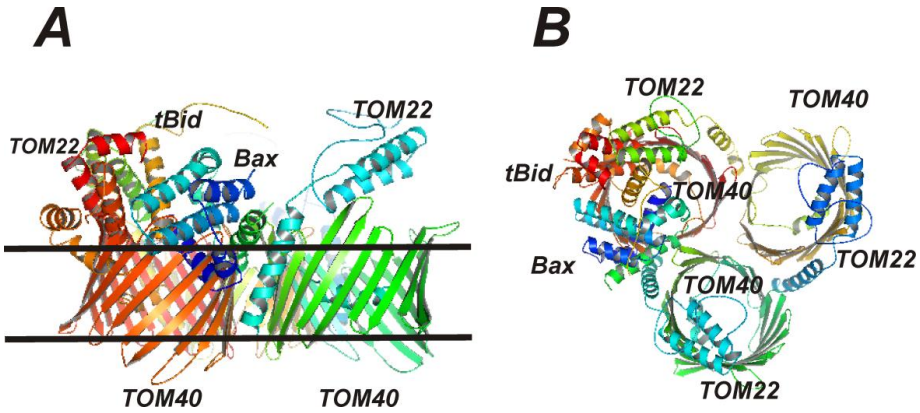

Fig.1. Structural model of the GIP/tBid/Bax complex. The side view (A) and the top view (B) of the complex are given.

The model of the GIP/tBid/Bax complex is shown in Fig.1. The most important results of simulations are presented in Table 1. Collectively, these results suggest that during apoptosis once tBid is formed due to Bid proteolysis by caspase- 8, the GIP/tBid complex will be formed prone to recruit Bax to mitochondria with high affinity. Collectively, our results explain in terms of 3D-structures of interacting proteins one of the key issues of mitochondrial outer membrane permeabilization and mitochondrial apoptosis – why the recruitment of Bax to the mitochondrial outer membrane in response to apoptotic stimulus occurs.

Table 1. The ROSETTADOCK interface energy scores (I_sc), Buried Surface Area (BSA), ROSETTADOCK van der Waals interaction energy score ($\Delta VDWS_{RD}$), ROSETTADOCK desolvation energy score ($\Delta solv_{RD}$), number of intermolecular salt bridges (N_{sb}) and intermolecular hydrogen bonds (N_{HB}) for the highest- rank complexes between GIP and Bax, between GIP and tBid and between GIP/tBid-Bax

Protein pairs	Isc	BSA Å^2	Evdw kcal/ mol	Edesolv kcal/ mol	N(sb)	N(hb)
GIP-Bax	-5.3	572.7	-15.9	2.1	1	1
GIP-tBid	-11.1	3975.4	-162.5	1.8	1	1
GIP/tBid-Bax	-8.0	2713.4	-47.6	2.3	2	2

1. M.Bohnert, N.Pfanner, M. van der Laan (2015) Mitochondrial machineries for insertion of membrane, *Curr Opin Struct Biol*., **33:**92-102.

2. G. Bellot et al. (2007) TOM22, a core component of the mitochondria outer membrane protein translocation pore, is a mitochondrial receptor for the proapoptotic protein Bax. *Cell Death Differ*., **14**:785-794

3. M. Ott et al. (2007) The mitochondrial TOM complex is required for tBid/Bax-induced cytochrome c release. *J Biol Chem.* **282**:27633-27639

4. P. F. Cartron et al. (2008) Bax inserts into the mitochondrial outer membrane through different mechanisms. FEBS Lett. 582(20):3045-51 (2008)

5. L. K. Sanjuán Szklarz et al. (2007) Preprotein transport machineries of yeast mitochondrial outer membrane are not required for Bax-induced release of intermembrane space proteins. *J Mol Biol*.368:44-54.

6. D. Kozakov et al. (2006) PIPER: an FFT-based protein docking program with pairwise potentials. *Proteins* **65**: 392-406

7. S. R. Comeau et al. (2004) ClusPro: an automated docking and discrimination method for the prediction of protein complexes. *Bioinformatics* **20**: 45-50.

8. J. J. Gray et al. (2003) Protein-protein docking with simultaneous optimization of rigid-body displacement and side-chain conformations. *J Mol Biol.* **331**: 281-299

9. S. Lyskov, J. J. Gray. (2008) The RosettaDock server for local protein-protein docking *Nucleic Acids Research* **36**(Web Server Issue) W233-W238

10. S. J. de Vries, M. van Dijk, A.M.J.J. Bonvin (2010): The HADDOCK web server for data-driven biomolecular docking. *Nature Protocols* **5**: 883-897

11. D. J. Mandell, E. A. Coutsias, T. Kortemme (2009) Sub-angstrom accuracy in protein loop reconstruction by robotics-inspired conformational sampling. *Nat Methods* **6**: 551-552