Molecular modelling of human Hexokinase I binding to VDAC1 mithocondrial porin

Spallarossa A[(1)], Pettinati I[(2)], Rosano C[(3)]

[(1)] University of Genova, Dipartimento di Scienze Farmaceutiche,
viale Benedetto XV, 3 - 16132 - Genova
[(2)] Experimental Oncology “B” Laboratory, and
[(3)] Nanobiotechnology, National Institute for Cancer Research (IST),
Largo R. Benzi 10 - 16132 – Genova (Italy)

Motivation

ATP, the main source of energy for mammalian cells survival and proliferation, derives mainly from two sources: glycolysis and Krebs cycle; the latter which uses pyruvate formed from glycolysis in a series of reactions that donate electrons to the respiratory chain complexes in mitochondria, represent the major source of energy in normal cells. In 1924 Otto Heinrich Warburg, a german physiologist, discovered that tumor cells unexpectedly generate energy by glycolisis hence his interpretation of cancer like a mitochondrial dysfunction (Warburg effect). The molecular basis of this high rate of glycolysis involves a number of genetic and biochemical events including overexpression of mitochondrial-bound isoforms of hexokinase (HK type I mainly in the brain and type II mainly in muscles). HKs (both HK-I and HK-II isoforms) play a pivotal role in cancer by promoting cell growth and survival. It has been recently shown that HK-II not only improved the cell’s energy supply in malignat cells, but that it also protected against programmed cell death (apoptosis). HK has also been shown to prevent cytochrome c release and apoptosis in cancer cells Indeed, in vitro and in vivo studies have shown that HK-I and HK-II may play a clear role in protecting against apoptosis through direct interaction with mitochondria and, more specifically, with the Voltage Dependent Anion Channel 1 (VDAC1). VDAC1 is an integral protein of the mitochondrial outer membrane. It is a large channel that transports anions, cations, adenine nucleotides and other metabolites into and out of mitochondria. VDAC1 is a constituent of the mitochondrial PTP (permeability transition pore) which is formed at contact sites between the inner and outer membranes by association of this porine in the outer membrane with another protein, the ANT (adenine nucleotide translocator) in the inner membrane. The rupture of HK:VDAC1 protein complex provides a therapeutic opportunity, as theis association appears to protect tumor cells from mitochondrial outer membrane permeabilization (MOMP), an event that marks the point of no return in multiple pathways leading to cell death. In order to perform an in silico screening of possible small molecules able to inhibit the protein association, we are presenting a computational model of HK:VDAC complex.

Methods

Molecular modelling of human HK-I N-terminal region In order to build a model of the full length enzyme, the N-terminal fragment of HK-I was derived using the HMM-based protein structure prevision SAM-T06. The first 400 atomic positions determined by the server were then superposed to the X-ray coordinates of the enzyme lacking the first 11 residues (PDB code 1QHA; Rosano et al, 1999) to yield to the final full-length protein structure. The model of the full-length protein was refined by different energy minimization cycles using the program Insight II (Discover3 module) (Accelrys, San Diego, CA – USA) and used for the following docking simulations. Docking of ATP to human HK-I In order to validate our procedures we simulated the docking of a molecule of the ATP analogue 5’-adenylylimido-diphosphate (AMP-PNP) to the previously modelled structure of wilt type human HK-I using two different software: Autodock v.3.05 and GOLD. Both the program were able to correctly identify the secondary binding site as experimentally determined by previous X-ray crystallographic studies HK-1:VDAC docking model The HK-I :VDAC complex structure was calculated by the web-service Rosetta using as template proteins the atomic coordinates of human VDAC1 and the full length HK-I previously modelled. The program performed a rigid-body docking based on the two structure of the unbound proteins. Contrary of all the expectations, it appears as evident how the first 15 N-terminal residues of HK-I interact with the inner part of the barrel of VDAC1 and not with the outside walls, within the mitochondrial membrane

Results

Although complexes generated by rigid body docking do not take into consideration any chan-

ge in molecular conformation due to the induced fit, the resulting model of HK-I:VDAC1 chosen may be considered as realistic. The binding mode of the two molecules, in fact, can explain many of the feature of VDAC1 previously discovered and are in agreement with the structural findings made by biophysical and biochemical assays, NMR and X-ray diffraction analysis. The atomic coordinates of the complex have been used, as target protein, for rational drug design. Docking simulations to validate in silico the newly designed small molecules able to inhibit the association process between HK-I and VDAC1 are in progress. The most promising candidates will be eventually chemically sinthesized for further in vitro analysis.

Contact : camillo.rosano@istge.it