Structure modeling, molecular screening and docking of mammalian AMPK and its plant homolog KIN10 for new ATP-competitive inhibitors

Raevsky A.V.[1], Kapov P.A.[2], Blume Ya.B.[3]

Institute of Food Biotechnology and Genomics NAS of Ukraine, Osipovskogo str., 2a, Kiev-123, 04123, Ukraine

rayevsky85@gmail.com [1], karpov.p.a@gmail.com [2], yablume@univ.kiev.ua [3]

Recently, mammalian microtubule cytoskeleton was identified as a sensitive target of AMP-activated protein kinase (AMPK).[PMID:23316058] It was shown that phosphorylation of the microtubule (MT) plus end protein CLIP-170 by AMPK is required for MT dynamics and the regulation of directional cell migration.[1] Now, mammalian AMPK are the one of the most interesting targets associated with MT growth and responsible for cell activation under metabolic stresses such as nutrient starvation, heat shock, ischemia/hypoxia, etc. [PMID:12203120] It serves as an energy sensor and is considered as promising drug target for treatment of type II diabetes and obesity. [PMID: 19273282]

In present, our attention to AMPK is caused by the role of their plant homologue - SnRK kinase in cytoskeleton regulation. Sucrose nonfermenting 1-Related protein Kinase (SnRK) is homologous of SNF1 and AMP-activated protein kinases (AMPK), which widely exists in plant and involves in a variety of signaling pathways. Particularly, SnRK1 plays important roles in the transcription regulation, signaling and plant development. [PMID: 17766403] A self-regulation kinase domain in the N-terminal is the common structural characteristic of SnRK protein kinase family.[2] The region is a highly variable, and can interact with other protein. Compared with other protein kinase, there is a conserved amino acid-threonine in the activation region. Subunit of a probable heterotrimeric complex consisting of an alpha catalytic (KIN10 or KIN11) subunit, and a beta (KINB) and a gamma (KING or SNF4) non-catalytic regulatory subunits.

Is probable that catalytic subunit of the probable trimeric SnRK-related complex, may play a role in a signal transduction cascade regulating gene expression and carbohydrate metabolism in higher plants [PMID: 10220464; 11387208]. Based on these data we decided to build alpha subunit (KIN10) and identify probable ATP-competitive inhibitors of this plant kinase.

First of all a template structure of AMPK from *Rattus norvegicus* [PDB ID 2Y94] was derived from Protein Data Bank. Basing on the sequence (UniProt Q38997) of Kin10 from *Arabodopsis thaliana* a complete 3D-structure model was reconstructed. Next part of preparation assumed several

steps of energy minimization and structure relaxation. Initially our model was optimized during 20 ns with Generalized Born Implicit Solvent calculation method of molecular dynamics in Gromacs 4.5 package to increase a number of conformational states. The MD trajectory was analysed and clustered in 15 separate groups basing on the RMSD values with an average structure for each cluster. After validation of the structures with Molprobity server one of them was selected. It was relaxed during 80 ns in explicit solvent environment with amber99sb forcefield. RMSF for separate residues was calculated with g_rmsf tool.

The conformation of active site can be in open or closed state so it had to be optimized for screening. To cope with this task a molecule of staurosporine from mamalian complex [2Y94] was docked in the active site of relaxed KIN10 model. For this purposes we applied GoldScore/ASP protocol of CCDC Gold 5.0 software. To estimate stability of the ligand pose a short molecular dynamics simulation of 5 ns was carried out for both mamalian and plant kinase complexes with a ligand. A topology file for staurosporine was generated with Antechamber tool of AmberTools 12 package. The comparison of both dynamics was based on ligand and protein RMSD and number of hydrogen bonds. Several frames of the most stable complexes were extracted from the trajectory and visualized in PyMol 1.5.

The last step assumed a screening search of a library (~3000 compounds) against plant homologue to find putative inhibitors of KIN10. Dock 6 was used as a screening tool to find 96 compounds with conjectural inhibitory activity and CCDC Gold was applied for more exhaustive docking to reduce this number to the end point number of 7 compounds. All these compounds are falling in the volume of a staurosporine, have good enough scores for the next stage of the study as prospective AMPK inhibitors.

1) Q.A. Acton (2012) *Advances in Cytoskeleton Research and Application*. 2011 Edition. ScholarlyEditions. – 100 p.

2) D. Xue-Fei; C. Na; W. Li; Z. Xiao-Cui; Q. Bo; Li Tian-Lai; Z. Guo-Liang (2012) The SnRK Protein Kinase Family and the Function of SnRK1 Protein Kinase, *Int. J. Agric. Biol.*, 14: 575-579.