In silico prediction of preferred substrate of some HAD-Related Acid Phosphatase of A. thaliana

Javad Zamani Amirzakaria[1], Mohammad Ali Malboobi[1]*, Sayed-Amir Marashi[2], Tahmineh Lohrasebi[1]

[1]Department of Plant Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

[2]Department of Biotechnology, Faculty of Science, University of Tehran, Tehran, Iran

Abstrac: Monoesteric phosphatases, commonly known as acid phosphatase (APs) enzymes, catalyze the hydrolysis of phosphoric ester bonds of various substrate types including phosphorylated sugars, lipids, proteins and nucleic acids. These enzymes play central roles in phosphate acquisition through absorption, recycling and scavenging of this essential element from both internal and external resources Arabidopsis thaliana has 58 acid phosphatases, divided to 5 groups including: Purple Acid Phosphatase(PAP), HAD-Related Acid Phosphatase(HRP), Phospholipid Phosphatase(PLP), Histidine Acid Phosphatase(HAP) and SurE-Related Acid Phosphatase(SAP).These enzymes do not show strong substrate specificity but preferably bind to a substrate. Understanding preferable substrate of APs is important to unravel pathway and response mechanism of plants in phosphate starvation condition and to design rationally genetic manipulations for improving plant tolerance to stresses.

Method:

In present work we only took A.thaliana HAD acid phosphatases into account and detected those involved in root and phosphate starvation condition, using microarray data analysis. Homology modeling was applied to predict 3D-structure of proteins. Molecular docking was used to dock minimized protein structures with ligands obtained from genome scale metabolic networks of A.thaliana and screen preferred substrate for each protein based on binding energy, binding mode and distance between phosphoric ester and cofactor (Mg2+ localized in active site of HAD acid phosphates) . Molecular dynamic simulation was performed to refine protein-ligand complex models.

Results:

Our study indicated that 4 HAD acid phosphatases including: AT1G04040, AT4G29260, AT4G29270 and AT5G44020 are involved in root phosphate metabolism of A.thaliana under phosphate starvation and preferably bind to Pyridoxamine-5′-phosphate(ZINC1532807) , Sphingosine-1-phosphate(ZINC08860500), phosphoadenosine-5'-phosphosulfate(ZINC12494889), Inosine 3'-monophosphate(ZINC13549616), respectively. All Protein-ligand complexes showed backbone RMSD in the range of 3.2-3.5 for a period of 100 ns, asserting stable behavior of complexes during simulation.

Conclusion:

We have modeled A.thaliana HAD acid phosphatases, involved in phosphate starvation condition and assigned their preferred substrates. This framework can be used to detect other preferred substrate of A.thaliana Aps. These new recognized substrates are expected to make the foundation for rational design for plant metabolic engineering.

[3] [1]. Allen, K.N., Dunaway-Mariano, D., 2004. Phosphoryl group transfer: evolution of a catalytic scaffold. Trends Biochem. Sci. 29, 495–503. doi:10.1016/j.tibs.2004.07.008

[4] [2]. Lu, Z., Dunaway-Mariano, D., Allen, K.N., 2005. HAD superfamily phosphotransferase substrate diversification: structure and function analysis of HAD subclass IIB sugar phosphatase BT4131. Biochemistry (Mosc.) 44, 8684–8696. doi:10.1021/bi050009j

[5] [3] Irwin, J.J., Sterling, T., Mysinger, M.M., Bolstad, E.S., Coleman, R.G., 2012. ZINC: A Free Tool to Discover Chemistry for Biology. J. Chem. Inf. Model. 52, 1757–1768. doi:10.1021/ci3001277

[6] [4]. Mintz-Oron, S., Meir, S., Malitsky, S., Ruppin, E., Aharoni, A., Shlomi, T., 2012. Reconstruction of Arabidopsis metabolic network models accounting for subcellular compartmentalization and tissue-specificity. Proc. Natl. Acad. Sci. 109, 339–344. doi:10.1073/pnas.1100358109