MOLECULAR DOCKING AND SIMULATION OF TRANSKETOLASE FROM *Orthosiphon stamineus*

M. L. Ng [*1], Z. Rahmat[1] and M.S Shamsir[1,2]

[1]Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia.

ngmeiling929@gmail.com

zaidahrahmat@utm.my

[2]Innovation and Commercialisation Centre, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia.

shahir@utm.my

ABSTRACT

Orthosiphon stamineus, locally known as Misai kucing is a popular traditional medicinal plant and employed as folk medicine to treat various ailments in Southeast Asia. The pharmacological properties of the leaves have been reported including diuretic, antidiabetic, antihypertensive and so forth. Hence, the plant is commercialized as tea product and launched in the market aiming to benefit human health. Earlier proteome profiling of *O. staminues* leaves identified a multifunctional protein, transketolase. From the literature, transketolase require thiamine diphosphate for catalytic activity and believed that transketolase plays crucial role in prevention of cardiovascular, neurological and diabetes diseases. We generated 3D model of transketolase using I-TASSER and validated followed by docking with thiamine diphosphate using Autodock Vina to identify the key residues in the binding sites and binding mode and further proceeded to simulation at 37°C, 95°C for 50 ns using GROMACS. Docking result elucidated that thiamine diphosphate bound to transketolase through several interactions with residue Gln237, Ser242, Cys246, Arg283 and Phe284. MD simulation result revealed that the protein-ligand complex is stable with reasonable flexibility and compactness based on RMSD, RMSF and radius of gyration at both temperatures.

Keywords: *Orthosiphon stamineus*, transketolase, homology modelling, molecular docking, molecular dynamics simulation

1. INTRODUCTION

Cardiovascular diseases, heart failure and diabetes are the major causes of mortality in people nowadays. There are many drugs produced and available in the market in order to cure these diseases. Nevertheless, the drugs cannot reach the adequate level to control those diseases in patients and may cause side effects on patients in long term [1]. In recent years, computer-aided drug design became an alternative approach to produce drugs with higher effectiveness as it can accelerate new drug discovery and cheaper production. Until now, there are a few computer-aided drug design (CADD) molecules gaining approval from U.S Food and Drug Administration (FDA) and reached clinical stage of drug development [2] using computational methods such as molecular docking and molecular dynamics (MD) simulation. 11β-HSD1 has been identified as an inhibitor to treat diabetes as it can inhibit the production of active cortisol [3] since its presence can prevent insulin secretion from pancreatic-beta cells [4].

Orthosiphon stamineus from the Lamiaceae family is a well-known herb in South East Asia countries and have been extensively used as folk medicine to cure various ailments such as diabetes and arthritis. Moreover, its pharmacological activities have also been proven including antidiabetic, antioxidant, antihypertensive, anti-inflammatory, diuretic and hepatoprotective activities [5]. Meanwhile, transketolase (TKT) is one of the proteins identified from the plant and believed to be a new drug source to prevent certain diseases such as hyperglycemia due to its role in the biochemical reaction. TKT is a thiamine-diphosphate (TPP) dependent enzyme involved in pentose phosphate pathway. Malfunction of TKT highly affect the cardiovascular and nervous system since the cells in both system is very sensitive to deficiency of TKT [6]. Proper-functioning TKT is possible to ensure antidiabetic activities by inhibiting three pathways associated with the pathogenesis of hyperglycemia and able to prevent diabetic retinopathy in diabetic rats [7]. Deficiency of TKT will also cause short stature, developmental delay and congenital heart defects [8]. Moreover, recent studies highlighted that engineered *E. coli* TKT has the ability to

convert L-arabinose in sugar beet pulp into L-*gluco*-heptulose which posseses potential therapeutic value in cancer and hypoglycaemia [9]. Therefore, TKT is a potential therapeutic molecule that can be used to prevent certain ailments such as diabetes and heart diseases. To date, even though TKT from maize, human, bacteria and fungi have been reported, there are no work that provides insight about the structure and the behaviour of TKT from *O. stamineus* at different temperature that could serve as a new therapeutic agent to prevent disease such as diabetes. Hence, three-dimensional (3D) model of TKT from *O. stamineus* was constructed followed by docking with thiamine diphosphate which is crucial for TKT to perform catalytic activity. Simulation of the docked structure provides the key residues in the binding sites and reveals the structural properties to further understand the function and biological activities of TKT. We hope that the insights obtained will be useful to design new drug for diabetes patients and accelerate the drug discovery process.

2. MATERIALS AND METHODS

2.1 Homology Modeling and Evaluation

The peptide sequences of TKT identified from LC-MS/MS matched and replaced in the sequence. The 3D model of TKT was constructed via I-TASSER server by homology modelling and validated using several evaluation methods such as ERRAT, PROCHECK and VERIFY3D.

2.2 Molecular docking

AutoDockTools (ADT) 1.5.6 were used to prepare the input file for the protein and ligand, thiamine diphosphate (PubChem ID: 1132) and followed by molecular docking using Autodock Vina. The binding model with the lowest binding energy was selected and analyzed based on different interactions and the orientation of the ligand in the binding pocket.

2.3 Molecular Dynamics Simulation

The docked protein-ligand complex was used to perform MD simulation using GROMACS 5.0.4 packages with GROMOS96 54a7 force field [10] at 310 K and 368 K for 50 ns. MD trajectories were analyzed to determine root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg) and hydrogen bond distribution for the system. Salt bridge analysis was also performed using ESBRI program.

3. RESULTS AND DISCUSSION (PLS CHANGE FIGURE FOR MAKING MORE CLEAR)

3.1 Homology modeling and evaluation

TKT from *O. stamineus* was successfully generated using I-TASSER server through homology modeling as shown in Figure 1. The generated 3D model was proceeded to the secondary structure analysis followed by model validation. The secondary structure analysis of TKT was performed using YASARA program and revealed that TKT consists of 40.0% helix, 11.2% sheet, 16.1% turn, 31.3% coil and 1.4% 3_{10} helix. Whilst, the result obtained from several model validation methods such as PROCHECK-97.5% in allowed regions, 2.5% in disallowed regions, ERRAT-86.44%, VERIFY3D-94.24% indicated that the constructed TKT model is in good quality.

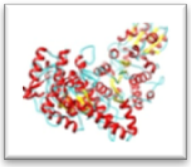

Figure 1. Predicted TKT structure of *O. stamineus* by I-TASSER.

3.2 Molecular docking

In order to find the binding affinities and key interaction of TKT with the ligand, Autodock Vina was used to dock the TPP into the TKT protein model as many diseases such as brain disease, heart disease[6] and diabetes [11] are closely related to lack of functional TKT with the loss of its catalytic properties. Figure 2 showed the docking mode between TKT and its ligand. Gln237, Ser242, Cys246, Arg283 and Phe284 made up the ligand binding pocket from TKT through several interactions including two hydrogen bonds (2.04Å; 3.02Å), a π-sulfur contact (4.74Å); a π-π stacking contact (4.60Å) and a π-alkyl contact (5.05Å) with thiazolium ring of the TPP.

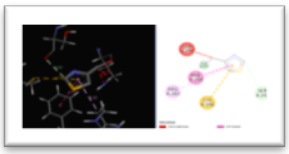

Figure 2. Docking mode of transketolase with its ligand, thiamine diphosphate.

3.3 MD simulation

TKT-TPP complex has RMSD value of about 0.37 nm and 0.7 nm at 310 K and 368 K respectively (Figure 3a). Higher RMSD value indicates the protein undergoes more structural changes and has lower stability. Even though the complex has higher RMSD value at 368 K, the complex is still considered as stable as the RMSD value is lower than 1 and the value was within similar range until end of simulation. From RMSF graph as shown in Figure 3b, higher peak and more fluctuations at 368 K with total of eight loops combined were detected; four loops at 310 K and another four loops at 368 K. Only one loop labelled as L3 and L6 are located at the same protein region at both temperatures. This may indicate that the arrangement of amino acid located at the loop does not change much even when exposed to high temperature and suggests that the function may not be affected. Besides, the curve showed no significant fluctuation at the ligand-binding site of the protein at 368 K which may elucidated that the protein-ligand interaction is static and probably not disrupted at elevated temperature. The compactness of the TKT-TPP complex was considered as low with high graph reading which is about 2.9 nm (Figure 3c) and do not change much at elevated temperature. The average numbers of hydrogen bonds are 562 and 550 for the 310 K and 368 K simulations respectively and shown in Figure 3d. There is only a slight difference in hydrogen bond number between 310 K and 368 K indicating the protein conformation has not change much and the protein function may remain intact. Salt bridge is one of the crucial factors that contributed to protein stability [12] which can be affected even if one salt bridge is disturbed. There are a total of 258 and 295 salt bridges detected at 310 K and 368 K respectively. Additional 37 salt bridges formed at 368 K may play a critical role to maintain protein stability. This finding is supported by Elcock where he mentioned that there are more salt bridges detected in hyperthermophilic proteins compared to mesophilic proteins [13].

Figure 3. a)RMSD graph of TKT versus time at both 310 K and 368 K. b)Graph of RMSF versus residue for ligand-bound transketolase at 310 K and 368 K. c)The radius of gyration graph versus time (50 ns) for ligand-bounded transketolase at 310 K and 368 K. d)The graph of number of hydrogen bond versus time for thiamine diphosphate-bounded transketolase at 310 K and 368 K. Black line=310 K, Red line=368 K.

4. CONCLUSION

The TKT model from *O. stamineus* was successfully constructed and docked with TPP. Gln237, Ser242, Cys246, Arg283 and Phe284 residues are determined as the possible ligand binding sites and the TKT-

TPP complex is stable with reasonable flexibility and compactness at both 310 K and 368 K which means that this complex can withstand high thermal stress condition. These results are expected to provide some useful insights to design new therapeutic molecule for diabetes and accelerate the drug discovery process. For future works, it is interesting to make comparison between *O. stamineus* TKT and TKT from other species to find out the speciality of *O. stamineus* TKT.

ACKNOWLEDGEMENT

The authors would like to thank Universiti Teknologi Malaysia (UTM) for the financial assistance under RUG vote no. 00K59.

REFERENCES

1. Bibi, S., Sakata, K. (2016). Current Status of Computer-Aided Drug Design for Type 2 Diabetes. Current Computer Aided Drug Design, 12, 2, 167-177.
2. Talele, T. T., Khedkar, S. A., Rigby, A. C. (2010). Successful applications of computer aided drug discovery: moving drugs from concept to the clinic. Current Topics in Medicinal Chemistry, 10, 1, 127-141.
3. Lagos, C. F., Vecchiola, A., Allende, F., Fuentes, C. A., Tichauer, J. E., Valdivia, C., Solari, S., Campino, C., Tapia-Castillo, A., Baudrand, R. (2014). Identification of novel 11β-HSD1 inhibitors by combined ligand-and structure-based virtual screening. Molecular and Cellular Endocrinology, 384, 1, 71-82.
4. Pereira, C., Azevedo, I., Monteiro, R., Martins, M. (2012). 11β-Hydroxysteroid dehydrogenase type 1: relevance of its modulation in the pathophysiology of obesity, the metabolic syndrome and type 2 diabetes mellitus. Diabetes, Obesity and Metabolism, 14, 10, 869-881.
5. Adnyana, I. K., Setiawan, F., Insanu, M. (2013). From Ethnopharmacology To Clinical Study of *Orthosiphon stamineus* Benth. International Journal of Pharmacy and Pharmaceutical Sciences, 5, 3, 66-73.
6. Martin, P. R., Singleton, C. K., Hiller-Sturmhofel, S. (2003). The role of thiamine deficiency in alcoholic brain disease. Alcohol Research and Health, 27, 2, 134-142.
7. Hammes, H.-P., Du, X., Edelstein, D., Taguchi, T., Matsumura, T., Ju, Q., Lin, J., Bierhaus, A., Nawroth, P., Hannak, D. (2003). Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. Nature Medicine, 9, 3, 294-299.
8. Boyle, L., Wamelink, M. M., Salomons, G. S., Roos, B., Pop, A., Dauber, A., Hwa, V., Andrew, M., Douglas, J., Feingold, M. (2016). Mutations in TKT are the cause of a syndrome including short stature, developmental delay, and congenital heart defects. The American Journal of Human Genetics, 98, 6, 1235-1242.
9. Subrizi F., Cárdenas-Fernández M., Lye G. J., Ward J. M., Dalby P. A., Sheppard T. D., Hailes H. C. (2016). Transketolase catalysed upgrading of L-arabinose:the one-step stereoselective synthesis of L-glucoheptulose. Green Chemistry, 18, 3158-3165.
10. Schmid, N., Eichenberger, A. P., Choutko, A., Riniker, S., Winger, M., Mark, A. E., van Gunsteren, W. F. (2011). Definition and testing of the GROMOS force-field versions 54A7 and 54B7. European Biophysics Journal, 40, 7, 843-856.
11. Zhao, J., Zhong, C. J. (2009). A review on research progress of transketolase. Neuroscience Bulletin,25, 2, 94-99.
12. Kumar, S., Nussinov, R. (1999). Salt bridge stability in monomeric proteins. Journal of Molecular Biology, 293, 5, 1241–1255.
13. Elcock A. H. (1998). The stability of salt bridges at high temperatures: implications for thermophilic proteins. Journal of Molecular Biology, 284, 2, 489-502.