CHAPTER 4

Conclusion

The three dimension structure of human tyrosinase was built from bacterial (*Bacillus megaterium*) as a template with high resolution of 2.3 Å and identity percentage at 33.5, similarity percentage at 50.7. The homology model was validated by using PROCHECK and Verify3D. Ramachandran plot shows that 81.6% of residues locate in the most favored regions, 12.6% and 4.1% of residues are locate in additional allowed and generously allowed regions, respectively. The rest 1.7% of residues are in disallowed regions. The residues in disallowed region are Asp59, Leu74, Trp80, Ser152, and Cys174 which are apart from the active area determined from the 3D structure. For Verify 3D, Analysis results 70 % residues of the generated model have score over 0.2; thus, the quality of the predicted model is suitable for further analysis. Binding scaffolds were simulated by using molecular docking and molecular dynamics simulation. The estimated of binding energy from the simulations correlated well with the IC_{50} and K_m value. The obtained models from docking and MD simulation of inhibitors complexing with mushroom tyrosinase indicated that Asn81, Asn260, and Met280 involve inhibitors binding through hydrogen bond interaction while His263 forms pi-pi interaction in the active site. In bacterial tyrosinase, Glu195 forms hydrogen bond with inhibitors and both of His60 and His208 forms pi interaction while Glu230, Ser245, Asn249, Val262, and Ser265 involved in the binding and His252 forming pi interactions in the active site of human tyrosinase. These dock scoring and key residues observed in our study support the previous experimental and computational evident. For mushroom tyrosinase, Studies in which Asn260 and Met280 were proposed to play roles in binding substrate [1] and His263 was observed to form pi interaction in mushroom tyrosinase [2]. Arbutin can interact with Asn205 using hydrogen bonding and His208 using pi interaction in bacterial tyrosinase [3].

The sequence alignment between mushroom and human tyrosinase in comparison with sequence alignment between bacterial and human tyrosinase indicates that bacterial sequence is closely similar to the human sequence. When amino acid appearance of tyrosinase from mushroom, bacterial, and human are compared, the results show cysteines are absent bacterial while both of mushroom and human present. In term of interaction with inhibitors, docking scores suggest that each inhibitor can inhibit tyrosinase from mushroom, bacterial, and human differently. The dock score shows that tropolone is the best inhibitors for mushroom tyrosinase and arbutin is the worst. Human tyrosinase had arbutin as the worst inhibitor as same as mushroom tyrosinase where kojic acid is the best inhibitors. In opposite point of view, the best inhibitor of bacterial tyrosinase is arbutin and the worst is tropolone. For an interaction with enzyme substrate, we found that human and bacterial tyrosinase prefer a binding with L-tyrosine rather than L-DOPA. On the other hand, mushroom tyrosinase can bind with both substrate with essentially the same binding energy value. The comparison of binding structure from mushroom, bacterial, and human indicated that, ascorbic acid located in the active site of bacterial and human tyrosinase can form hydrogen bond with Glu195 (bacterial) and Glu230 (human), which are matching residues in sequence alignment, while this inhibitor located in non-active site in mushroom tyrosinase. Ascorbic acid is reducing agent to reduce *o*-dopaquinone back to L-DOPA for decreasing melanin formation, not necessary at the active site [4-5]. In case of arbutin, mushroom and human tyrosinase use Asn260 (mushroom) and Asn249 (human) to form hydrogen bonding while His263 (mushroom) and His252 (human) form pi interaction, those residues are matching residues in sequence alignment, while bacterial tyrosinase has only pi interaction with His208.