CHAPTER 1

Introduction

1.1 Overview of tyrosinase

Tyrosinase is important enzyme in both of mammalian melanogenesis and fruit or fungi enzymatic browning. Tyrosinase is a member in type III copper enzyme family involving in melanin production in a wide range of organisms. The malanins formation was starting from tyrosine to L-DOPA which is the rate-limiting step of the enzyme [1-11]. This enzyme is important factor in various functions invoving wound healing, primary immune response, essential for pigmentation, browning in plant and fungi and cuticle formation in arthopod. The enzyme has difference two catalytic mechanisms consisting of the hydroxylation transformation of monophenols to *o*-diphenols (cresolase activity or monophenolase) and the oxidation of *o*-diphenols to *o*-quinones (catecholase activity or diphenolase). These monomers are polymerized to form dark macromolecular pigments (melanins) [12-16].

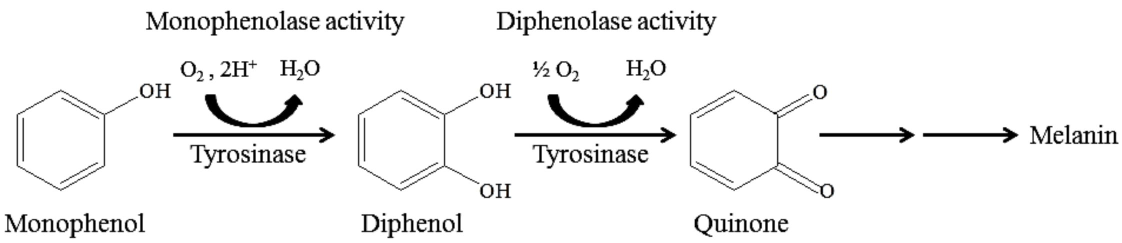

Figure 1.1 Reaction pathways in melanin formation: monophenolase and diphenolase activity by tyrosinase.

The enzyme can be found in plants, animals, fungi and microoganism, and the characterized active site shows two copper ions (CuA and CuB), which are supported by four α-helical bundle and coordinated by six histidine residues surrounding copper site. For this enzyme, the copper is essential for the catalytic activity. The six histidine coordinated by two copper atom is located in active site is well conserved in different species [10].

Tyrosinase can be in various forms. It can be classified base on geometry in three different oxidation states. All of these forms, copper ions are coordinated by six histidine residues. The oxygenated oxy-form of tyrosinase (oxytyrosinase or E_{oxy} :[Cu(II)–O_2^{2-}–Cu(II)]) can be formed by two tetragonal copper (II) ions. In this form, the two copper centers are speculated to be bound with dioxygen as peroxide bridges. This state can bind with both monophenol and diphenol. The oxidized copper (II) containing met-form (mettyrosinase or E_{met}:[Cu(II)–Cu(II)]), consist two tetragonal copper (II) ions as same as the oxy-form. The mettyrosinase is referred as a resting enzymatic form and can react with only diphenol. The reduced deoxy-form (deoxytyrosinase or E_{deoxy} :[Cu(I)–Cu(I)]) contains two copper (I) ions with a coordination arrangement as same as met-form, but without the hydroxide bridge, deoxy-form can be reduced to oxy-form [9-11].

In the food industry, tyrosinase is very important enzyme in controlling the quality of fruit and vegetable storage, processing, and manufacturing. Color and flavor quality can be used to estimate nutritional and market values of foods. Furthermore, in mammals, tyrosinase is essential enzyme for the regulation of melanogenesis. Melanin is end-product in melanogenesis and is producing in melanocytes located in the basal layer of the epidermis such as the eyes, hair follicles and skin. Melanin is important for protection of skin from harmful effect cause by ultraviolet (UV) radiation, vitamin D3 synthesis and the inhibition of photocarcinogenesis. However, the abnormal melanin production causes various dermatological disorders including hyperpigmentations. It is also link to the neurodegeneration associated with the Parkinson disease [17-24].

Tyrosinase activity can be control by tyrosinase inhibitors. In food industry, tyrosinase inhibitors from natural sources are of interest as they are primary considered to be safe. In contrast, in the cosmetic industry, the primary considered due to their skin-whitening effects and preventive effects [24-26]. Tyrosinase inhibitor applied to use in agriculture industries and skin health cosmetics. For example, kojic acid was used as skin whitening agent in cosmetic field, 4-hexylresorcinol was used as tyrosinase inhibitor for shrimp melanosis prevention and fresh and dried fruit slices browning control. Tyrosinase inhibitor is important in cosmetic industry because global market demand has increased for skin whitening agent for individuals who want to obtain lighter color skin. Most tyrosinase inhibitors were tested with mushroom tyrosinase because its commercial availability, but recent research reported significant activity difference between mushroom tyrosinase and human tyrosinase [24-27]. That is to say, this peculiar point of difference should be of concern.

1.1.1 Structure of Active Center and Reaction Mechanism

Tyrosinase is in type III copper centre enzyme which contains two copper atoms coordinated by three histidine residues and are involved in some oxygen transporting process and oxidation-reduction reaction. It has three forms determined by differentiated binuclear copper structure of the active site into the enzyme; oxytyrosinase (E_{oxy}), mettyrosinase (E_{met}) and deoxytyrosinase (E_{deoxy}) . The oxytyrosinase has two tetragonal copper (II) atoms. Each copper (II) atoms are coordinating with two strong equatorial and one weaker axial N_{His} ligands. The two copper centers were bound with exogenous oxygen molecule as peroxide and bridges. The optical feature of oxytyrosinase was peroxide bound in mode of confers a distinct $O_2^{2-} \rightarrow$ Cu (II) charge transfer spectrum which can be correlated and includes an extremely intense absorption band at 350 nm. In addition, the oxytyrosinase can be obtained from mettyrosinase by addition of peroxide or by the two-electron reduction of the mettyrosinase to the deoxytyrosinase, followed by the reversible binding of dioxygen. The dioxygen bound as peroxide and give a formal charge of +2 to each of copper atoms. The oxytyrosinase can react with monophenol as well as diphenol compound [28-30].

The mettyrosinase consists of two tetragonal copper (II) atoms similar to the oxytyrosinase. Each copper atoms coordinated with three histidine residues. This state is a resting form of the enzyme. The met-form of type-III protein is strong antiferromagnetic coupling as copper (II) ions are devoid of an electron paramagnetic resonance (EPR) signal, for reason, EPR non detectable. This magnetic coupling requires a superexchange pathway associated with an exogenous bridging ligand or an endogenous bridging amino acid residues [31-32]. The met-form can be converted by addition of peroxide to oxy-form, which can reverse to mettyrosinase when the peroxide is lost. In the absence substrate, as isolated and atmospheric pressure, neutral pH and room temperature was about 85% of the enzyme in mettyrosinase and 15% of the oxytyrosinase. Moreover, the EPR detectable is a half-mettyrosinase has the two coppers in a mix oxidation state [Cu(I)–Cu(II)]. The half-met-form has several spectroscopic studies; it shows electron delocalization between copper atoms and exogenous ligands bridging at two copper centers in the active site [32-33].

The deoxytyrosinase has two copper (I) atoms in the active site coordinating with three histidine residues and arranging similar to the mettyrosinase, but without the hydroxide bridge. Furthermore, the deoxy-form is an analogue of deoxyhemocyanin. It has a bicuprous structure [(Cu(I)–Cu(I)]. Therefore, the bridging ligand must be hydroxide from water, and a similar situation like the case for mettyrosinase [34].

Tyrosinase can react with both monophenol and diphenol compounds. Among these compounds, tyrosine and L-DOPA (3,4-dihydroxy-L-phenylalanine) are common substrate of melanins production. The mechanism of both of monophenolase and diphenolase acivities can be explained. For the monophenolase activity, the monophenol compound can react with oxy-form only. It coordinates to the axial position on one of the copper atoms in the active site of oxytyrosinase. The bound peroxide leads to rearrangement of *o*-hydroxylation of monophenol. The *o*-diphenol will be oxidized to the *o*-quinone. As deoxy-form readies for further dioxygen binding, the oxy-form can be regenerated from oxygen binding [34-35]. The monophenolase activity is present a characteristic lag time which depends on factors such as substrate, presence of a hydrogen donor, and enzyme concentrations. In the kinetic studies, lag time is the time required to reduce met-form deoxy-form by the reducing agent, produce by the small amounts of the oxy-form that usually present in the met-form [28]. In diphenolase activity, the *o*-diphenol can react with both oxytyrosinase and mettyrosinase and oxidized to *o*-quinone. The oxy-form binds with *o*-diphenol and change it to *o*-quinone, yielding the met-form of the enzyme. The latter form was oxidized another *o*-diphenol to *o*-quinone and reduce to deoxy-form. In common, diphenol compound is necessary reducing agent to obtain the deoxytyrosinase which can react with oxygen and continue in the catalytic action.

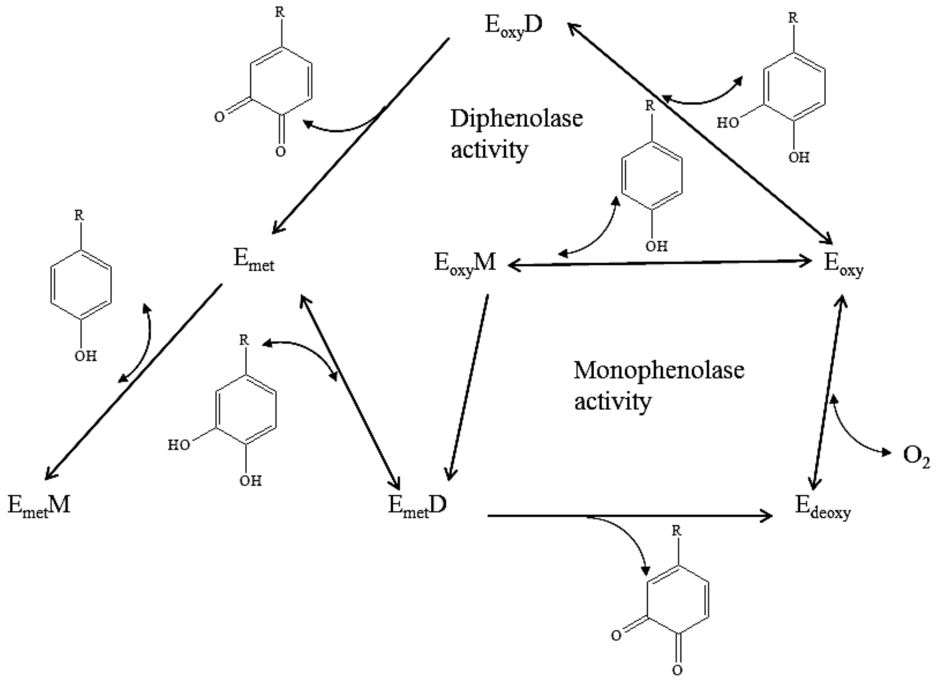

Figure 1.2 The both of monophenolase and diphenolase activity

1.1.2 Tyrosinase Inhibtors

Tyrosinase is the key enzyme to produce melanins, it cause browning in plant and fungi and melasma or some disease in human. Several researches had been study about tyrosinase, especially, controlling tyrosinase. The enzyme can be control by inhibitors. Which can be classifiled as following.

(1) Reducing agents producing chemical reduction of dopaquinone (e.g. ascorbic acid) and is used as a melanogenesis inhibitor because of its efficacy to reverse *o*-dopaquinone to DOPA. It avoids dopachrome and melanin formations.

(2) *o*-dopaquinone scavenger (e.g. most thio-containing compounds) is common melanogenesis inhibitors and produce dopaquinone to form colorless products. The melanogenetic process is slowed down until all of the scavenger is consumed, then it goes back at its original rate.

(3) Alternative enzyme substrates (e.g. some phenolic compounds), their quinoid reaction products absorb in different of spectral range from dopachrome. These phenolics were shown a good affinity of exhibit for the enzyme, the formation of dopachrome was prevented and these could be regard as mistaken inhibitors.

(4) Nonspecific enzyme inactivators (e.g. acids or bases), these are non-specifically denature the enzyme, so inhibiting its activity.

(5) Specific tyrosinase inactivators (e.g. mechanism-based inhibitors) are also called suicide substrates. Tyrosinase was catalyzed and formed covalent bond with these inhibitors, therefore the enzyme was irreversibly inactivated during catalytic reaction. The inhibitors were inhibiting tyrosinase activity by inducing the enzyme catalytic “suicide reaction”.

(6) Specific tyrosinase inhibitors. The compounds reversibly bind to tyrosinase and reduce its catalytic capacity.

Among of these six compound types described before, the “true inhibitors” are only specific tyrosinase inactivators and specific tyrosinase inhibitors. The enzyme bind with true inhibitors for inhibit it activity. Generally, some tyrosinase inhibitors exhibit only weak inhibitory effect due to their reactive and consumable properties toward tyrosinase, or the quinone products.

The tyrosinase inhibitors can be classified into four different types, including competitive inhibitors, uncompetitive inhibitors, non-competitive inhibitors, and mixed type (competitive/uncompetitive). A competitive inhibitor can be prevents substrate binding by combines with a free enzyme. This inhibitor and substrate are competing for the same site of enzyme. A competitive inhibitor might be a copper ion chelator like tyrosinase substrate analogs, or derivatives of substrate (L-tyrosine or L-DOPA). On the other hand, an uncompetitive inhibitor can binds to the enzyme-substrate complex only. The non-competitive inhibitors can bind to both free tyrosinase and enzyme-substrate complex with the same equilibrium constant. A mixed (competitive and uncompetitive mixed) type inhibitor binds not only with a free enzyme, but also with the enzyme-substrate complex. For most mixed-type inhibitors, their equilibrium binding constants for the free enzyme and the enzyme-substrate complex are different. In addition, the primary criterion of inhibitor is inhibitory strength and IC_{50} value as usually expressed is the inhibitory strength, the concentration of an inhibitor inhibiting half of the enzyme activity. Though, most studies were conducted to discover new tyrosinase inhibitors, kojic acid is common tyrosinase inhibitors and often used as a positive standard. The new tyrosinase inhibitors was described in term of the inhibitory strength as a relative inhibitory activity, which is calculated by dividing the IC_{50} value of kojic acid with that of a newly found inhibitor [6, 36].

1.1.3 Melanogenesis

Tyrosinase is the important enzyme in melanin formation process. This process can be called “melanogenesis”. The precursor substrate of melanogenesis in starting is tyrosin. Melanins pigment contains the heterogeneous polyphenolic polymers with wide colours ranging from yellow to black. The melanin can be found in widely spread in nature. It distribute into two domains of eukaryote and bacteria of, and the phylogenetic tree. Although, melanogenesis was fulfills amount of physiological roles in different organisms as can explain below [37-40].

Plant and fungi. In this part, the melanins cause a significant problem with huge economic impact in agriculture, due to there are reasonable the undesired enzymatic browning appearing during post-harvest storage, which makes the identification of novel inhibitors for inhibit melanin formation very important. In contrast, some case such as cocoa, tea, and raisins need to activity of tyrosinase for the production of distinct organoleptic feature. In case of fungi, melanin was relative importance to the spore formation and the reproductive organs formation. That was tissue protecting after damage and the virulence of pathogenic fungi.

Invertebrates. Here, melanin is important processes in three physiologically and also providing pigmentation. This was about wound healing, defense reactions, sequestration of microbes, encapsulation, cuticular hardneing and the production of toxic intermediates. Melanin production was applied for defense mechanism to encapsulate invading organisms in insects and other arthropods. Moreover, melanin also prevents the loss of blood at a wound site by deposition. At the wound site, melanin can kill invading microorganisms by the being cytotoxic melanogenic quinonoid precursors, and melanin formation also similar to resembles the cuticular hardneing. During cuticular hardneing, the enzyme oxidize N-catecholamines to quinones. These and other reactive metabolites can be formation of the hard cuticle by cross-linking structural proteins. Inhibit or even delay of this process has devastating effect on insects.

Mammals. The degree and distribution of melanin pigments was the most important of many factors in the color of mammalian skin, hair and eyes. In mammals, the specialized group of cells well known as melanocytes, is produced melanin and originate in the neural crest during embryogenesis. These spatial migrate all over the organism during development. Under strict genetic control, the migration patterns may lead to some interesting skin patterns such as in the case of animals like zebras and leopards. Melanosomes located in the dendrites of melanocytes and they are membranous organelles to synthesize of pigments. Melanin pigments play a roles important to several diverse, including camouflage, sexual attraction and regulating body temperature in mammals.

In human, melanin is playing a role important to photoprotective skin from Ultraviolet radiation causes DNA damage lead to formation of reactive oxygen species. Melanin can be causes disorders like vitiligo and albinism when human lack of melanin. Furthermore, melanin has been mostly interest in the involved skin carcinogenesis in malignant melanosomes. In Parkinson's disease, the selective vulnerability of neuromelanins and damage of neurons and their relative is also the great attention. The irregular melanin formation also causes a various abnormal conditions (melasma, freckles, ephelide, senile lentigines, etc).

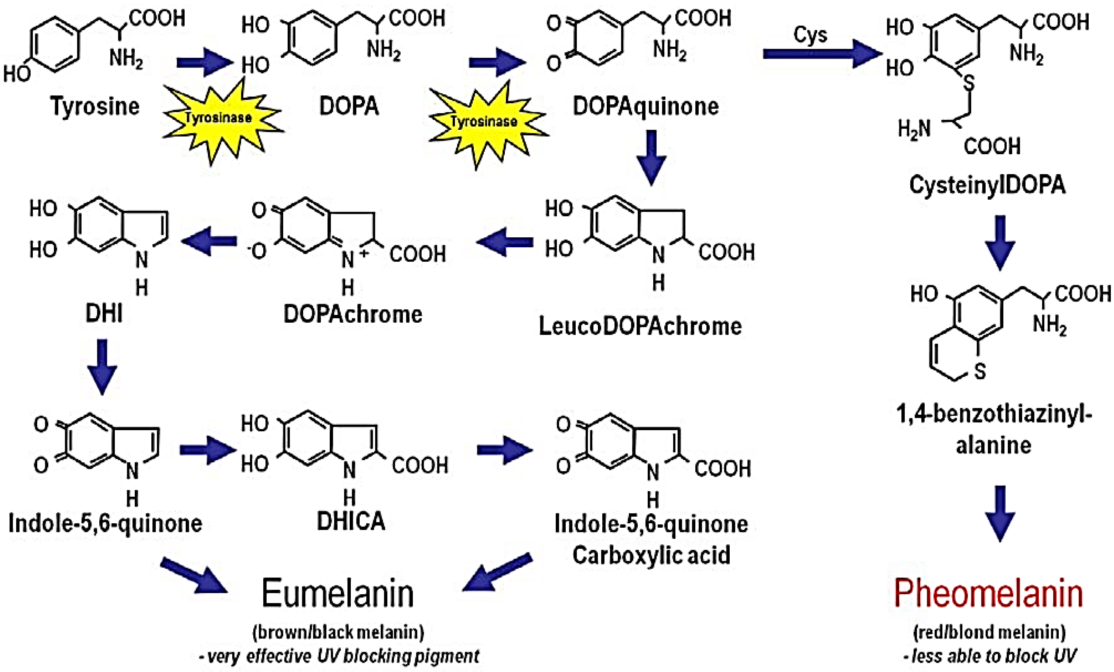

Figure 1.3 Melanin formation pathways [66]

1.2 Homology model, molecular docking, molecular dynamics simulation

There are many principal tools in the theoretical study of biological molecules. But in this work, three methods were used to studies; homology modeling, molecular docking, molecular dynamics simulations. Homology modeling is the technique used to approximate three-dimensional structures of a target protein (or unknown protein) from the available sequence, based on the assumption that the structure of the target protein is similar to the known structures protein of some homologous template proteins. Homology models get a basic idea where the alpha carbons of key residues sit the folded protein, they can guide mutagenesis experiments or suggest structure-function relationships. Homology models can be useful in structure-based drug design, interactions, antigenic behavior, analysis of protein function, and rational design of proteins with increased stability or novel functions [41-43]. The researches about homology model can found in large scale protein and several functions such as homology model of $Na^{+}K^{+}$-ATPase [44],G-protein-coupled receptors [45], and β-Ketoacyl Acyl Carrier Protein Synthase III [46]. The acceptable homology model should be built using template with a minimum percentage of identical residues in a safe zone as shown in **Fig. 1.4**.

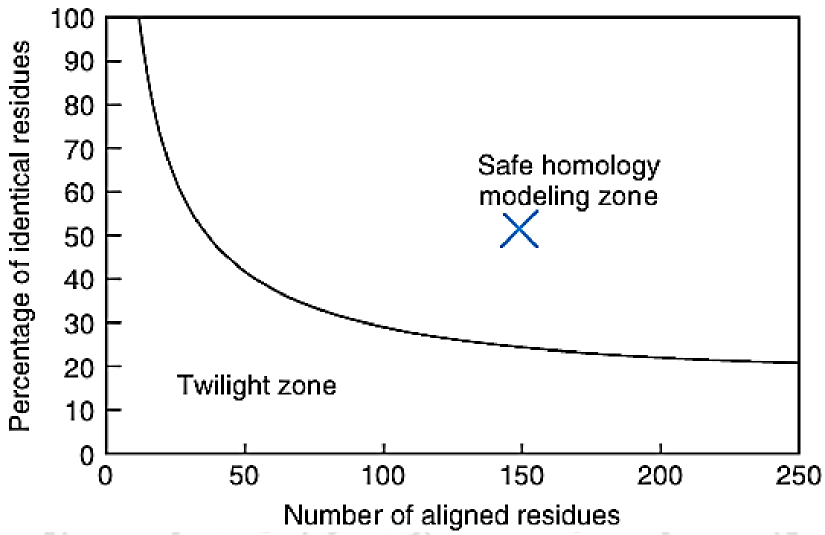

Figure 1.4 The two zones of sequence alignments [67]

Docking is a method to predict the position and orientation of small molecule (ligand) when bound to each other (protein receptor or enzymes) to form a stable complex [47]. The strength of association or binding affinity may be predicted from the preferred orientation between two molecules using, for example, scoring functions. Docking is useful for predicting the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Therefore, docking is very important in drug discovery process. Molecular docking is significance in the biological and pharmaceutical, determined by efforts have been directed towards improving the methods used to predict docking [48]. Previous work about docking of several proteins such as Dengue virus type 2 protease non-competitive inhibitors [49], RNA-Drug [50] and DNA-intercalators [51] have been reported.

Molecular dynamics (MD) is a microscopic method, these enables one to estimate thermodynamic variables defined by ensemble averages. MD is based on a numerical solution of *Newton's* second law or the *equation* of motion under the assumption of the validity of classical mechanics. This simulation is a complex system, modulled at atomic level, that whose physical movements of atoms and molecules in the context of N-body simulation. The most MD simulation also applied in materials science, chemical physics and the modelling of biomolecules, this method was calculates the time dependent behavior of a molecular system. MD simulations have information detailed on the fluctuations and conformational changes of proteins and nucleic acids, which that are regularly used to observe the structure, dynamics and thermodynamics of biological molecules and their complexes. MD simulations also used in the determined of structures from x-ray crystallography, NMR experiments and other experimental techniques and theory, to understand the dynamics was important to connect between protein function and protein structure [52-54]. Previous work about MD simulations of several enzymes such as Acetylcholinesterase [55], NADH Cytochrome B5 Reductase [56] and Rhodanase [57] have been reported.

1.3 Review of Tyrosinase Crystal Structures and Inhibitory Activities

Tyrosinase was first investigated in 1895 by Bourquelot and Bertrandin from mushroom (*Russula nigricans*) [58], it widely distributed in nature. The best-characterized tyrosinases are of tyrosinase from actinobacteria *Streptomyces glausescens* and fungi *Neurospora crassa.* Generally, *A. bisporus* has been used as model of mammalian tyrosinase in melanogenesis activity test since 2009 [6]. The comparison sequence alignment of human tyrosinase by BLAST on NCBI database (http://www.blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) found that, top three of percentage identity of human tyrosinasse (consider only tyrosinases, May 2016) is tyrosinase from *Bacillus megaterium* (bacterial), *Streptomyces Castaneoglobisporus* (actinobacterial) and *Agaricus bisporus* (mushroom) with score 29, 28 and 22, respectively [59]. In this thesis mostly was focus on three part studies of tyrosinase (3D structure of tyrosinase, Kinetic studies of tyrosinase and Docking of tyrosinase) from mushroom, bacterial and human.

3D structure of tyrosinase

The first crystal structures of tyrosinase and one of the best-characterized tyrosinases from actinobacteria *Streptomyces glausescens* in 2006 [9]. The crystal structures have high resolution at 1.2-1.8 Å. These structures contain four α-helixs as a core of enzyme, have two copper located in helical bundles at catalytic site. Its contain copper-bound and metal-free tyrosinase in a complex with ORF378 as a "caddie" protein, because caddie protein assists with transportation of two copper (II) ions into the tyrosinase catalytic site. The caddie protein contains has one six-stranded β-sheet and one α-helix. The crystal structures suggest that, the caddie protein covers tyrosinase around the hydrophobic molecular surface and it was interferes a substrate tyrosine binding to the catalytic site of tyrosinase. Both of tyrosinase and catechol oxidase as a member in the type 3 copper protein family but in catechol oxidase, it lacks monooxygenase activity. The comparison structure and function of tyrosinase and catechol oxidase show that, the differentiated of catalytic activity is based on the structural, observe that a large vacant space is present just above the active site of tyrosinase and that one of the six histidine residues on the two copper ions is highly flexible.

The first high resolution the crystal structure of *A.bisporus* tyrosinase complex with tropolone was reported in 2011 [60]. It has resolutions at 2.3 Å, based on the structures of a plant catechol oxidase from *Ipomoea batatas* and a actinobacteria tyrosinase from *Streptomyces castaneoglobisporus*. These structure was most popular in melanogenesis studies. The crystal structure contain a heterotetramer with molecular mass of 120 kDa, have subunits of two heavy (H) and two light (L) polypeptide chains. The complex have two H subunits of ~392 residues and fold similar to other tyrosinases and two L subunits of ~150 residues, has a lectin-like fold. The H subunit contains a dinuclear copper center in the deoxy-state, each copper atom is coordinated with three His ligand. The side chains of these histidines have hydrogen bonds or, in the case of His85, It has a thioether bridge with the side chain of Cys83. Tropolone binds closely the two copper centers without directly coordinating with the copper ions in the active site. The crystals structure of *A. bisporus* tyrosinase with a lectin-like fold was also present, but potential carbohydrate binding sites were not conserved.

In addition, the first structures of tyrosinase from *Bacillus megaterium* was propose in 2011 [11] the same with crystal structure of *A.bisporus* tyrosinase, The crystal structure was determined at a resolution of 2.0–2.3 Å. This structure contains a dimer in the asymmetric unit, each copper ions in active site are coordinated by three His ligand. The overall structure of monomeric is based on the structure of *Streptomyces castaneoglobisporus* (actinobacteria) tyrosinase, but it lacks an accessory Cu-binding "caddie" protein. The crystal structure was study under several different conditions showing various occupancies and positions of the copper atom in catalytic center. The results show that, pathway by which copper is accumulated in or lost from the enzyme. Moreover, they suggest that residues R209 and V218, the residues place on the second shell of residues surrounding the catalytic center, play roles important to substrate binding based on their position and flexibility. The crystal structure complex of *B. megaterium* tyrosinase with kojic acid is the first structure with a binding ligand.

Kinetic studies of tyrosinase

The kinetic studies of tyrosinase had been reported and test in various condition. The most of these studies use L-tyrosine and L-DOPA as substrates of tyrosinase. Especially, L-DOPA the late-limiting step of tyrosinase. Jeon *et al* [27] reported different mechanism between human and mushroom tyrosinase by studied about inhibitory effects on L-dopa oxidation of tyrosinase by skin-whitening agents chase of temperature, pH, and biological activity (K_m and IC_{50} valued) were reported human tyrosinase and mushroom tyrosinase. The results show that, optimum temperature for L-DOPA oxidation of human and mushroom tyrosinase was 50 ºC and 40 ºC, respectively. From this, human tyrosinase has optimum temperature for L-DOPA oxidation higher than mushroom tyrosinase. The optimum pH of human and mushroom tyrosinase is 7.5 and 7.0, respectively. The K_m value for L-DOPA for human and mushroom tyrosinase was reported to be 0.31 mM and 1.88 mM, respectively. Human tyrosinase has a higher affinity for L-DOPA. Inhibitory effect of several tyrosinase inhibitor revealed that suggested ascorbic acid was the best inhibitor for inhibition of human and mushroom tyrosinase as determined by the lowest IC_{50} values.

Inhibition studies have been carried out in several class, Espin *et al* [61] reported a kinetic study of the inhibition of mushroom tyrosinase by tropolone. They concluded that, tropolone was a slow-binding inhibitor in isoforms of mushroom tyrosinase. When the concentrations of tropolone increase, it activated a progressive decrease in both of initial velocity and the final steady-state rate in the progress curves of product accumulation. The enzyme-inhibitor complex in rapid form undergoes a slow reversible reaction. When add $CuSO_4$, the inhibition of the different isoforms can be reversed. The inhibition by tropolone was evaluated by nonlinear regression fits described by the kinetic parameters. The differentiated of isoforms with tropolone was incubate experiments, demonstrated that inhibitor can bind to the "oxy" form of tyrosinase only, justifies by mechanism previously proposed about the inhibition of tyrosinase by slow-binding inhibitors.

Docking studies of tyrosinase

Tyrosinase is the key enzyme in melaogenesis therefor important in cosmetic industry. So several reported was studies binding structure of tyrosinases and theirs inhibitor by using docking simulation, e.g. Docking study of mushroom tyrosinase. Senol *et al* [62] search inhibitory potential of ascorbic acid against tyrosinase. ascorbic acid or vitamin C is water-soluble vitamin and had a high antioxidant potential. Several researches have published inhibitory effect of ascorbic acid and its derivatives on tyrosinase. Although, some of those research was performed on the ascorbic acid effect against tyrosinase on various substrates have been reported conflict results. Docking study was indicated that ascorbic acid is strong inhibitor of tyrosinase interacting with four amino acid (histidine 263, serine 282, phenylalanine 264, and valin 283) in the active site of tyrosinase. Ascorbic acid had hydrogen bindings with Cu1 and Cu2 with distances of 3.57 and 3.41 Å, respectively, through its O5 atom.

Docking study of mushroom tyrosinase and kojic acid analog had been publish. Lima and co-worker [63] interest in kojic acid because the function of kojic acid as a macrophage activators. This work studied about kinetic, molecular docking, MD simulations and binding free energy to provide insights into the activity of inhibitor against tyrosinase. The docking study showd the influence of eight amino acid residues

(Met280, Asn260, His61, His85, His94, His259, His263 and His294) and the interactions established between theses residues and inhibitor.

In addition, mushroom tyrosinase was docking with tyrosol and its analog [64]. The effects and the mechanisms governing the effects of tyrosol and its analog was reported. Tyrosol is the major active components of a flowering herb in the Crassulaceae family (*Rhodiola rosea*), it shows anti-aging, anticancer, anti-inflammatory, hepatoprotective, and anti-oxidative. Docking study indicated that, the OH moiety of tyrosol forms one hydrogen bond with Ser282 and pi interaction with His263. Furthermore, the acetic acid moiety of 4-hydroxyphenylacetic acid form one hydrogen bond with Ser282 and three pi interactions with His61, His263, and copper ion, these result showed 4-hydroxyphenylacetic acid had the stronger interaction with the active site and possessed potent inhibitory activity. Moreover, The acetic acid moiety of 2-hydroxyphenylacetic acid form one hydrogen bond with Gly281 and its OH group form two hydrogen bonds with Val283 and Ser282 and pi interaction with His263. The result indicated that 2-hydroxyphenylacetic acid had also possessed good potential inhibitory activity on tyrosinase compared with 4-hydroxyphenylacetic acid.

Number of docking study of bacterial tyrosinase is much less than mushroom tyrosinase and one of those studies is about molecular docking studies of a phlorotannin, dieckol isolated from *Ecklonia cava* with tyrosinase inhibitory activity [3]. This work used the crystal structure of *Bacillus megaterium* tyrosinase (PDB ID: 3NM8) for analysis of binding structrure and the results show dieckol interacts with Gly46, His208, and Met215. Another of docking study of bacterial tyrosinase focused on synthesis design, docking studies and antioxidant activity of some chalcone and aurone derivatives were reported [65]. Chalcones and aurones are found to possess high antioxidant activity and are known to be inhibitors for tyrosinase involved in melanogenesis. Molecular docking was performed use crystal structure of tyrosinase from *B. megaterium*. Docking results showed that eight aminoacid residues (Ile39, Gly143A, Ile139, Lys47, Lys47, Ala44, Gly43, and Gln142) of tyrosinase were highly conserved and important in substrate binding or catalysis.

In this thesis, 3D structures of tyrosinase and binding structure with four common inhibitors: arbutin, ascorbic acid, kojic acid and tropolone will be simulated for the prediction of potent inhibitors of tyrosinases. The human tyrosinase model was generated using bacterial tyrosinase template. The objective of our work is to find out of pre-screening tyrosinase inhibitor discovery for therapeutic and cosmetic purposes. Therefore, structural information with the different models of tyrosinase, bacterial, mushroom, and human will be investigated been. as bacterial tyrosinase has more similar structure to human tyrosinase and it should be used in both experimental and in silico screening of candidate molecules with potential tyrosinase inhibitory activity. The docking of tyrosinase will be performed and key amino acids in binding pocket will be highlighted.

1.4 Research Plan, Methodology and Scope

1.4.1 Literature on tyrosinase and its inhibitors will be review

1.4.2 Modeling of homology model will be constructed

- Homology model of human tyrosinase will be built from its template
- The obtained model will be evaluated and refined to generate the homology model

1.4.3 Molecular docking of protein-ligand will be performed

- The 3D-structure of tyrosinase binding with each inhibitor(kojic acid, ascorbic acid, arbutin and tropolone) will be generated
- Docking result will be analyzed in term of binding structure and activity

1.4.4 Molecular dynamic simulation (MD)

- The binding structure of tyrosinases and their inhibitors in periodic system will be generated.
- MD simulation result will be analyzed

1.4.5 Result refinement will be carried out

1.4.6 Manuscript will be prepared and this work will be presented at an appropriate conference

1.4.7 Discussions, conclusion, and thesis writing

1.5 Research Objective

1.5.1 To model 3D structure of human tyrosinase using bacterial tyrosinase template

1.5.2 To predict bioactivity of tyrosinase from three different sources (bacterial, mushroom, and human tyrosinase) via molecular docking

1.5.3 To compare/analyze active site and binding mode of each complex regarding to experimental evident

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