사람 티로시나제의 3차원 구조 상동 모델링

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Comparative modeling of human tyrosinase - An important target for developing skin whitening agents

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Abstract

human tyrosinase (hTyr) catalyzes first and the rate limiting step in the synthesis of polymerized pigment, melanin which determines skin, hair and eye colors. Mutation of hTyr often brings about decrease of melanin production and further albinism. Meanwhile, a number of cosmetic companies providing skincare products for woman in Asia-Pacific region have tried to develop inhibitors to bright skin color for several decades. In this study, we built a 3D structure by comparative modeling technique based on the crystal structure of tyrosinase from *bacillus megaterium* as a template to serve structural information of hTyr. According to our model and sequence analysis of type 3 copper protein family proteins, two copper atoms of active site located deep inside are coordinated with six strictly conserved histidine residues coming from four-helix-bundle. Cavity which accommodates substrates was like funnel shape of which entrance was wide and expose to solvent. In addition, protein-substrate and protein-inhibitor complex were modeled with the guide of van der waals surface generated by in house software. Our model suggested that only phenol group or its analogs can fill the binding site near nuclear copper center because inside of binding site has narrow shape relatively. In conclusion, the results of this study may provide helpful information for designing and screening new anti-melanogensis agents.

1. Introduction

Tyrosinase (EC 1.14.18.1) is a kind of type 3 copper proteins that catalyses *ortho*-hydroxylation of monophenol such as tyrosine using dioxygen (O_2) (monophenolase activity) and the subsequent oxidation of the diphenolic compound such as dopamine and catechol to the corresponding o-quinone (diphenolase activity) [1][2]. This reaction is the first committed step in the synthesis of melanin from polyphenols. Quinones generated by tyrosinase are converted nonezymatically to unstable intermediates, which then polymerize to melanin. Tyrosinases are widely spreaded in plants, mammals, fungi, and bacteria, and the active site is well conserved. In humans, absence or defect of tyrosinase leads to albinism, a skin disorder characterized by the complete or partial absence of pigment. Although, melanin plays a defense role against UV radiation passing through the skin by absorbing and reflecting, the abnormal production and accumulation of melanins, also lead to several hyper-pigmentary disorders such as melasma, senile lentigo, freckles, pigmented acne scars and post inflammatory hyperpigmentation.

Because tyrosinase mediate rate limiting step in biosynthesis of melanin, inhibition of tyrosinase is one of the promising strategies to inhibit browning of food and to treat hyper-pigmentation

of human skin. Therefore, several de-pigmenting agents or skin whitening agents including natural and synthetic compounds have been developed to reduce or abolish the activity of tyrosinase [3][4].

When accurate three-dimensional structure of validated target proteins is known, structure-based approach for inhibitors may provide hints on how to design and develop novel therapeutic agents. In addition, increase of the computing power of modern computer and sophisticated molecular modeling tools such as docking and pharmacophore-based searching software make it possible to screening millions of compounds virtually in a few days using protein structure. However, the structure of human tyrosinase (hTyr) has not been solved due to expression and crystallization problems. Therefore, in the present study, we predicted and report structure models of hTyr by homology modeling technique using the known crystal structure of type-3 copper protein. This study might be useful to predict the inhibitory activities of candidate compound and to screen virtually the compound database for discovering lead compounds.

2. Materials and Methods

2.1. Software and hardware

In this work, the computational studies were performed by using the following software packages. Homology modeling was performed using MODELLER 9v9 (http://salilab.org) on PC, running on LINUX operating system. Model was evaluated by PROCHECK [5] and Verify-3D [6]. Ligand preparation for complex modeling was done with ISIS Draw (www.acdlabs.com) and converted 3D structure using Discovery studio viewer v2.5 (www.accerlys.com). Interactive visualization and analysis of molecular structures was carried out on Pymol v1.2 (www.pymol.org), and Coot v0.6.2 [7].

2.2. Retrieval of target protein sequence, and sequence alignment

The amino acid sequence of hTyr was retrieved from UniProtKB-Swiss-Prot with accession number P22984 (http://www.expasy.org). In order to find suitable template for homology modeling of hTyr, BLASTp program available on the website of protein data bank (PDB: http://www.pdb.org/) was used to search the crystal structures of the closest homologues by submitting the amino acid sequence of hTyr. Best template was chosen based on the sequence identity and the quality of crystal structure such as resolution and Rfactor value. The first step required for constructing 3D model is the alignment of sequences among templates and target (Fig. 1) which is critical for the accuracy of the structures generated by homology modeling. The sequence of hTyr was aligned to the sequence of the best template, *Bacillus megaterium* tyrosinase (bTyr) using ClustalW program [8] with default parameters.

2.3. Homology modeling and validation of models

Based on the best sequence alignment, comparative modeling was done by means of MODELLER 9v9 with default parameters. Ten satisfactory models were generated initially for hTyr using the crystal structure of Bacillus megaterium tyrosinase as template structure and the best one according to the lowest MODELLER objective function was selected. Two bound cuprous ions at the active site of experimental structure of template were copied into active site of the model structures. Through the procedure mentioned above, an initial model was thus completed.

The refinement of the homology model was carried out through energy minimization to eliminate steric conflicts between the side-chain atoms using Amber 7.0 [9]. After the optimization procedure, the hydrogen atoms were removed and visual inspection was carried out with molecular graphics program Coot and Pymol to peruse the reliability of the alignment and modeling of variable loops of predicted models. Then, the

structures obtained in the manner described above were evaluated by using computational tools including PROCHECK and VERIFY 3D.

2.4 Binding sites analysis and modeling of protein-ligands complexes

PASS (Putative Active Sites with Spheres) was used to search the cavity near the catalytic site, di-nuclear copper center for identifying substrate binding site and characterizing its shape by filling the cavity with the probe spheres [10]. And we could also identify putative functional residues, which surround the binding site, by selecting the residues adjacent to the spheres with maximum distance option of 5Å on graphics program. Those results were used to guide the following modeling of protein-ligand complex structure.

3. Results and Discussion

3.1 Construction of hTyr

Potential templates of hTyr were obtained from PDB site by BLASTp search. Template selection was performed on the basis of sequence similarity, resolution of structure and functional similarity. The amino acid sequence of hTyr was compared with other type-3 copper proteins by Clustal W program. The results showed that tyrosinase from *bacillus magaterium* had the best sequence identities (27% for 116-451 residue), so we used this protein as template. The sequence alignment between target sequence and template is shown in Fig. 1.

At the final stage of homology modeling, the best structure was further checked through the PROCHECK and Verify3D. As shown in Fig. 2, 87.8% of residues were located in the most favored zones, 10.8% in allowed regions, 1.4% in generously allowed regions and There was no residues in disallowed regions. When checked by Verify3D, self compatibility score for modeled structure was 189.95, which is higher than the low score. Moreover, 75% of residues, which had a score over 0.2, were considered reliable. And 3 regions of sequence also exhibited lower scores. This fragment corresponds to sequences that include gaps in the alignment (Fig. 1). However, these fragments are exposed to solvent with no role in substrate or copper binding. In future studies, therefore, we do not intented to focus on this modeled fragment. So, we removed these regions in the final model.

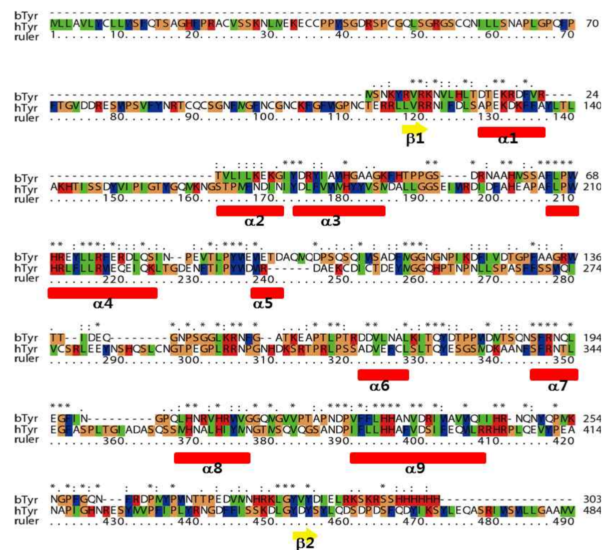

Figure 1. Sequence alignment of hTyr (P22984) with bTyr (PDB code: 3NM8) with Clustal W. Conserved residues are represented by asterisk, semicolon, and dot according to similarity. The secondary structure element for bTyr is demonstrated with yellow arrows for sheet and red box for helices.

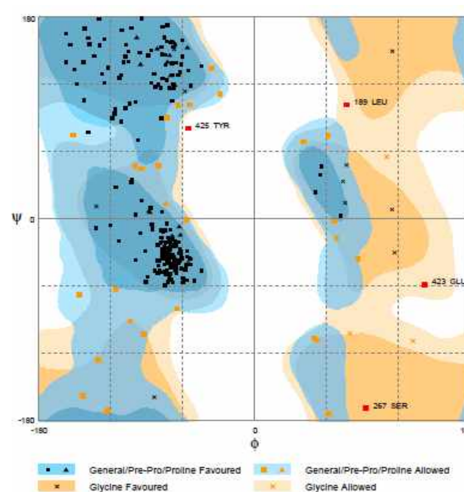

Figure 2. Ramachandran plot of hTyr model built using PROCHECK [10] implemented in CCP4 package.

3.2 Substrate binding site

Although, overall structure and especially 4-helix -bundle of initial structure are similar to template proteins, the conformations of side chains of catalytic histidine residues did not take orientation properly to coordinate Cu atoms. So we search proper configuration of side chain using rotamer library implemented in Coot. Bond distance between N atoms and cooper atoms are 2.5-2.8Å. It is a good criterion in refining structure. The binding properties and catalytical activities are depend on the shape of binding site determined by the side chain of second shell residues surrounding active site. In the case of hTyr, as shown in Fig. 4, the entrance region of binding site is widely opened to solvent so that it can be accommodate various size of substrates.

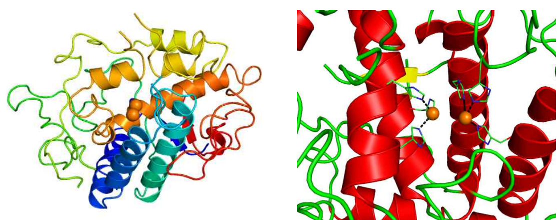

Figure 3. Overall structure and catalytic site of hTyr

3.3 The complex structures of tyrosinase-tyrosine and tyrosinase-kojic acid

In order to estimate reaction mechanism in terms of structure view, hTyr and tyrosine complex structure was built manually using van der waals surface as a guide (Fig. 4). The inside of binding cavity is 3Å wide and 0.5Å thickness which is perfectly fit to aromatic ring such as phenol and tyrosine. Moreover, because the shape of binding site near Cu atoms is sharp, only one atom can approach and interact with Cu atoms directly. Therefore, as shown in Fig. 4 A, the phenol group of tyrosinase may perfectly fill the binding site.

To explore binding scheme of well-known inhibitor, kojic acid, we also built a structure complexed with Kojic acid, a fungal metabolite widely used as a skin-whitening agent in cosmetics industry. As shown in Fig. 4B, kojic acid take similar orientation with tyrosine in the binding site and its oxygen atoms take position to coordinate with Cu atoms.

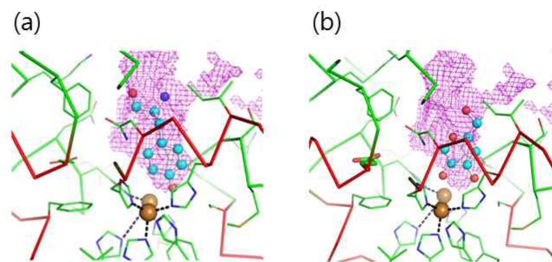

Figure 4. tyrosinase-ligands complexes.
(a) tyrosine coordinated with CuB (b) kojic acid

4. References

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