Journal of the Korea Academia-Industrial cooperation Society
Vol. 13, No. 11 pp. 5350-5355, 2012

http://dx.doi.org/10.5762/KAIS.2012.13.11.5350

Comparative Modeling of Human Tyrosinase - an Important Target for Developing Skin Whitening Agents

Jongkeun Choi[1*] and Joo Won Suh[1,2]

[1]**Center for Nutraceutical & Pharmaceutical Materials, Myongji University**

[2]**Division of Bioscience and Bioinformatics, Myongji University**

피부 미백제의 타겟 단백질인 인간 티로시나제의 3차원 구조 상동 모델링

최종근[1*], 서주원[1,2]

[1]명지대학교 농생명바이오식의약소재개발 사업단

[2]명지대학교 생명과학정보학부

Abstract Human tyrosinase (hTyr) catalyzes the first and rate limiting step in the biosynthesis of a skin color determinant, melanin. Although a number of cosmetic companies have tried to develop hTyr inhibitors for several decades, absence of 3D structure of hTyr make it impossible to design or screen inhibitors by structure-based approach. Therefore, we built a 3D structure by comparative modeling technique based on the crystal structure of tyrosinase from *Bacillus megaterium* to provide structural information and to search new hit compounds from database. Our model revealed that two copper atoms of active site located deep inside and were coordinated with six strictly conserved histidine residues coming from four-helix-bundle. Substrate binding site had narrow funnel like shape and its entrance was wide and exposed to solvent. In addition, hTyr-tyrosine and hTyr-kojic acid, a well-known inhibitor, complexes were modeled with the guide of solvent accessible surface generated by in-house software. Our model demonstrated that only phenol group or its analogs could fill the binding site near the nuclear copper center, because inside of binding site had narrow shape relatively. In conclusion, the results of this study may provide helpful information for designing and screening new anti-melanogenic agents.

요 약 사람의 티로시나제는 사람의 피부색을 결정하는 멜라닌 생합성의 첫 번째 반응을 촉매하며, 이 단계는 반응 속도를 결정하는 가장 중요한 단계이다. 따라서, 많은 화장품 회사들은 hTyr의 저해제를 찾고자 하였으나 사람 티로시나제의 3차원구조의 부재로 구조기반의 가상탐색은 불가능하다. 따라서 본 연구에서는 구조기반의 저해제 탐색을 위하여 기존에 그 구조가 알려진 *Bacillus megaterium*의 티로시나제의 3차 구조를 이용하여 인간 티로시나제의 3차원 구조를 상동모델링 법으로 예측하였다. 3차원 구조 분석 결과 인간 티로시나제의 활성부위에 위치한 여섯 개의 히스티딘 잔기가 2개의 구리 원자와 결합할 수 있으며, 이 활성부위는 단백질의 안쪽에 위치함을 알 수 있었다. 기질 또는 저해제가 결합할 수 있는 결합부위는 단백질의 표면에서 안쪽 깊은 곳의 활성부위와 연결되어 있으며 입구 쪽은 넓고 납작했으며 활성부위로 갈수록 좁아지는 깔대기와 같은 모양의 구조를 하고 있었다. 자체 제작 소프트웨어를 활용하여 solvent accessible surface를 만들고 여기에 가장 최적의 위치 및 형태를 갖는 모델을 티로신과 저해제로 가장 잘 알려진 코직산의 결합모델을 제안하였다. 이 결과 티로신과 코직산의 페놀그룹의 히드록시 기능단의 산소가 정확히 구리와 배위결합하는 것을 알 수 있었다. 결론적으로 본 연구 결과는 새로운 미백제를 설계하고 스크리닝하는데 유용한 정보를 제공할 수 있을 것으로 사료된다.

Key Words : Human tyrosinase, Comparative modeling, 3D structure, Kojic acid, Complex structure

This work was supported by a grant from the Next-Generation BioGreen 21 Program (No. PJ008093), Rural Development Administration, Republic of Korea.

*Corresponding Author : Jongkeun Choi
Tel: +82-31-330-6882 email: jkchoi@mju.ac.kr

Received September 4, 2012 Revised (1st October 23, 2012, 2nd October 31, 2012) Accepted November 8, 2012

1. Introduction

Tyrosinases (EC 1.14.18.1) are widely spreaded in plants, mammals, fungi and bacteria. They belong to the type 3 copper protein family containing two copper atoms in their active site. Tyrosinases catalyse *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 to unstable intermediates, which then polymerize to melanin. 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 passed 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 the biosynthesis of melanin, tyrosinase is one of the promising targets to treat hyper-pigmentation of human skin. In addition, The Asia-Pacific region has been the fastest growing area in the global skincare product market and skin-whitening market alone is over US$ 13 billion [3]. Therefore, several depigmenting agents or skin whitening agents including natural and synthetic compounds such as hydroquinone, kojic acid, arbutin, etc., have been developed to reduce or abolish the activity of tyrosinase [4][5][6]. However, it is known that currently available tyrosinase inhibitors have problems in toxicity and efficacy. For instance, although, hydroquinone is a very effective tyrosinase inhibitor, it have serious side effects associated with DNA damage [7].

When accurate three-dimensional structure of validated target proteins is known, it provides hints on how to design new compounds. Structure-based approaches, that screen millions of compounds using 3D structure, may suggest new potent inhibitor candidates. 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 screen millions of compounds virtually in a few days using protein structure. However, the 3D structure of human tyrosinase (hTyr) has not been solved on an experimental base due to protein overexpression and crystallization problems. Therefore, in the present study, we predicted 3D structures of hTyr by homology modeling technique using the known crystal structure of tyrosinase from *Bacillus megaterium* (bTyr). Moreover, we built two hTyr structures bound with ligands including tyrosin and kojic acid. This study might be useful to predict the inhibitory activities of candidate compounds and to screen virtually the compounds 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 [8] and Verify-3D [9]. Ligand preparation for complex modeling was done with ISIS Draw (www.acdlabs.com) and was converted to 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 [10].

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, bTyr using ClustalW program [11] 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 bTyr 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 aforementioned procedure 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 [12]. 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 [8] and VERIFY 3D [9].

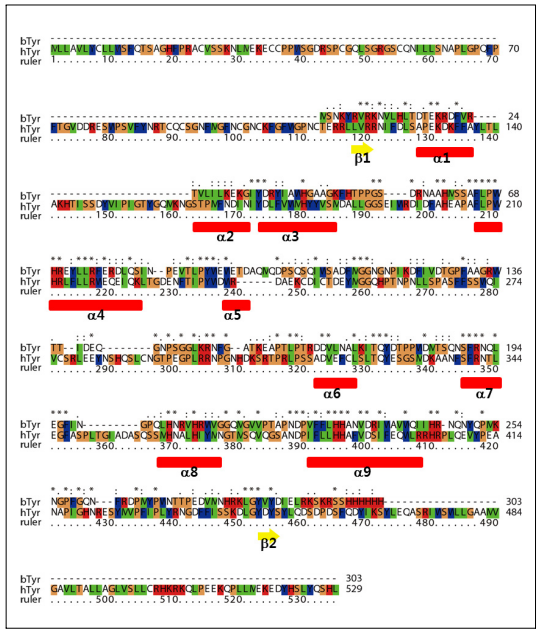
2.4 Binding sites analysis and modeling of protein–ligands complexes

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 [13]. And we could also identify putative functional residues surrounding the binding site, by selecting the residues adjacent to the spheres with maximum distance option of 5 Å 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 3D structure

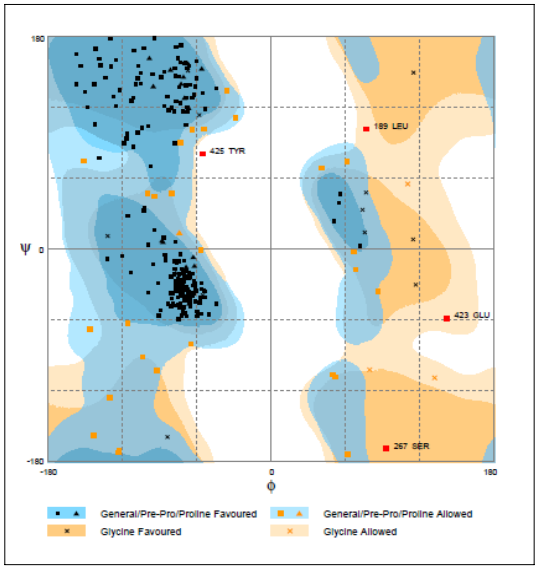
Potential templates of hTyr were obtained from PDB site by BLASTp search. Template selection was performed on the basis of sequence similarity, resolution of structure and functional similarity. The amino acid sequence of hTyr was compared with other type-3 copper proteins by Clustal W program. The results 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.



[Fig. 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.

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 were 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 exhibiting lower scores were fragments corresponds to gaps in the sequence 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 intent to focus on this fragment. So, we removed these regions in the final model.



[Fig. 2] Ramachandran plot of hTyr model built using PROCHECK [8] implemented in CCP4 package.

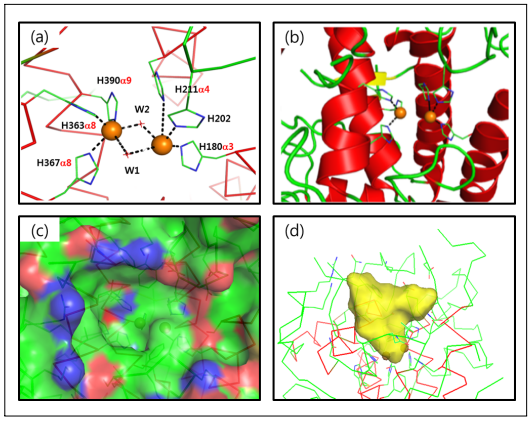
3.2 Overall structure

The sequence analysis revealed that hTyr consists of 4 domains, signal peptide (1-18), luminal domain (19-476), transmembrane helical domain (477-497) and cytoplasmic domain (498-529) with 6 N-linked glycosylation site (86, 111, 161, 230, 337 and 371 ASN). The predicted structure of hTyr is a catalytic domain of hTyr (116-451) and a kind of α/β proteins (two strands and 9 helices) with dimensions of 56 Å x 41 Å x 36 Å. In our model, we could not model N-terminal (1-115), C-terminal (452-529) and several gap regions (138-161, 284-289 and 351-357) due to absence of template resulting low credibility of model. Except this region, overall structure of hTyr is compact and its secondary structure is composed of primarily α-helices. There is short 2 stranded β sheet originated from N (118-120) and C-terminal (447-449) in our structure. A four-helix-bundle (172-185, 206-225, 340-370, 384-403) sustaining the catalytic di-copper nuclear center is located in the center of molecule and surrounded by helices. And two copper atoms of active site is respectively coordinated by three histidine residues (180, 202 and 211 for CuA, 363, 367 and 390 for CuB) contributed from the middle of α3, α4, α8 and α9 helices.

[Fig. 3] Overall structure of hTyr predicted by MODELLER.

3.3 Substrate binding site

Although, overall structure and especially 4-helix-bundle of initial structure are similar to that of template protein, the conformations of side chains of catalytic histidine residues did not take orientations properly to coordinate Cu atoms. Hence, we searched proper configuration of side chains using rotamer library implemented in Coot. Bond distances between N atoms and cooper atoms are 2.5-2.8 Å. It is a good criterion in refining structure. The binding properties and catalytical activities depend on the shape of binding site, which is usually 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 accommodate various size of substrates. That may be the reason why tyrosinases have broad substrate specificities [14]

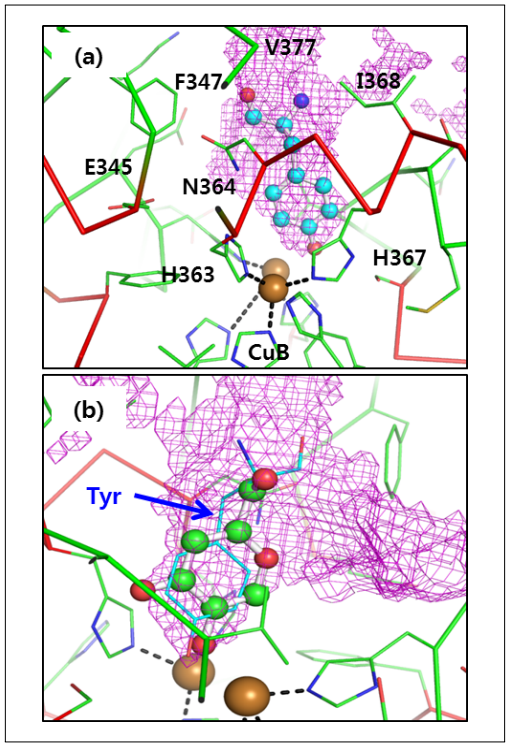


[Fig. 4] Catalytic (a, b) and substrate binding (c, d) site of hTyr. CuA and CuB atoms were showed with orange ball. The shape of binding site was represented with yellow surface in (d).

3.4 The complex structures of tyrosinase–tyrosine and tyrosinase–kojic acid

In order to figure out how substrates bind to tyrosinase, hTyr and tyrosine complex structure was built with a molecular graphics program, Coot using solvent accessible surface as a guide (fig. 5a). The inside of binding cavity is 3 Å wide and have 0.5 Å of thickness. That is perfectly fit to aromatic ring such as phenol and tyrosine. Moreover, because the shape of binding site near Cu atoms is narrow and sharp, only one atom can approach and interact with Cu atoms directly. Therefore, as shown in fig. 5a, the phenol group of tyrosine 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 which is a fungal metabolite and widely used as a skin-whitening agent in cosmetics industry. As shown in fig. 5b, kojic acid take similar orientation with tyrosine in the binding site and its oxygen atoms take position to coordinate with Cu atoms just like tyrosine. Enzyme kinetic studies revealed that kojic acid was a competitive inhibitor against monophenolase activity and showed a mixed inhibition on diphenolase activity. Interestingly, it was reported that all of the slow binding inhibitors contained α-hydroxyketone group [15]. In terms of reaction mechanism, one as well as two bonds between copper atoms and hydroxyl groups of diphenol is possible. However our model demonstrated that only one hydroxy group could be coordinated with copper atom and two electrons of substrate might be eliminated from hydroxyl groups sequentially. In the case of α-hydroxyketone group of inhibitors, it could not donate a pair of electrons and therefore, reaction might be stopped in this state.



[Fig. 5] Tyrosinase-ligands complexes of (a) tyrosine and (b) kojic acid which were built manually using solvent accessible surface (pink mesh) as a guide. The compounds binding to hTyr were represented with ball-and- stick model. Blue stick models also represented tyrosine for comparison in (b).

In conclusion, we constructed a three-dimensional model of hTyr and its complex structures associated with tyrosine and kojic acid. and demonstrated the shape of binding site and ligand specificities, It was evident that hTyr model had reasonable quality when tested with PROCHECK. Its quality also confirmed by Verify 3D test. From the refined model, we found out that binding site has a flat funnel-like shape of which entrance exposed to solvent region is 10 Å width. We believed that our model can be used for designing new candidate compounds and virtual screening to search millions of compound libraries.

References

[1] R.H. Holm, P. Kennepohl, E.I. Solomon, "Structural and functional aspects of metal sites in biology", Chem. Rev., Vol.96, pp.2239–2314, 1996.

[2] H. Claus, H. Decker, "Bacterial tyrosinases", Syst. Appl. Mcrobiol., Vol.29, pp.3-14, 2006.

[3] http://www.asianscientist.com/features/skin-whitening-products-asia-2012/

[4] A. Rescigno, F. Sollai, B. Pisu, A. Rinaldi, E. Sanjust, "Tyrosinase inhibition: general and applied aspects" J. Enzyme Inhib. Med. Chem., Vol.17, pp.207-218, 2002 .

[5] Y.J. Kim, H. Uyama, "Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future" Cell. Mol. Life Sci., Vol.62, pp.1707-1723, 2005.

[6] T.-S. Chang, "An updated review of tyrosinase inhibitors" Int. J. Mol. Sci. Vol.10, pp.2440-2475, 2009.

[7] J.P. Germanas, S. Wang, A. Miner, W. Hao, J.M. ready, "Discovery of small-molecule inhibitors of tyrosinase", Bioorg. Med. Chem. Lett., Vol.17, pp.6871-6875, 2007.

[8] R.A. Laskowaski, M.W. McArther, D.S. Moss, J.M. Thornton, "PROCHECK a program to check sterio-chemical quality of a protein structures", J. Appl. Crystalogr., Vol.26, pp.283-291, 1993.

[9] D. Eisenberg, R. Luthy, J.U. Bowie, "VERIFY3D: Assessment of protein models with three-dimensional profiles", Methods Enzymol., Vol.277, pp.396-404, 1992.

[10] P. Emsley, B. Lohkamp, W.G. Scott, K. Cowtan, "Features and development of Coot", Acta Crystallogr. D Biol. Crystallogr., Vol.66, pp.486-501,2010.

[11] J.D. Thompson, D.G. Higgins, T.J. Gibson., "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice", Nucleic Acids Res., Vol.22, pp.4673-4680, 1994.

[12] D.A. Case, T.E. Cheatham 3rd, T. Darden, H. Gohlke, R. Luo, K.M. Merz Jr, A. Onufriev, C. Simmerling, B. Wang, R.J. Woods. "The Amber biomolecular simulation programs", J. Comput. Chem., Vol.26, pp.1668-88, 2005.

[13] G.P. Brady Jr., P.F. Stouten, "Fast prediction and visualization of protein binding pockets with PASS", J. Comput. Aided Mol. Des., Vol.14, pp.383-401, 2000.

[14] L. Rassaei, J. Cui, E.D. Goluch, S.G. Lemay, "Substrate-dependent kinetics in tyrosinase-based biosensing: amperometry *vs.* spectrophotometry", Anal. Bioanal. Chem., Vol.403, pp.1577-1584, 2012.

[15] E.J. Land, C.A. Ramsden, P.A. Riley, M.R.L. Stratford, "Studies of *para*-quinomethane formation during the tyrosinase-catalyzed of 4-alkylcatechols", Arkivoc, Vol.2008, pp.258-267, 2008.

Jongkeun Choi [Regular member]

- Feb. 1993 : Seoul National Univ., Department of Agricultural Chemistry, B.S.
- Feb. 1995 : Seoul National Univ., Department of Agricultural Chemistry, M.S.
- Aug. 2006 : Seoul National Univ., School of Chemistry, Ph.D.
- Sep. 2011 ~ current : Myongji Univ., Center for Nutraceutical and Pharmaceutical materials, Assistant Professor

<Research Interests>
Structural Biology, Cheminformatics, Functional Food & Cosmetics

Joo-Won Suh [Regular member]

- Feb. 1979 : Seoul National Univ., Agricultural Chemistry, B.S.
- Sept. 1986 : University of California, Davis, Food Science and Technology, M.S.
- Sept. 1989 : University of California, Davis, Microbiology, Ph.D.
- 2011 ~ current : Center for Nutrceutical and Pharmaceutical Materials, Next-generation, Director
- 1990 ~ current : Myongji University, Division of Bioscience and Bioinformatics, Professor

<Research Interests>
Microbiology, Agricultural biotechnology
Functional Food, Pharmaceutical materials