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In silico Design of Anti-Tyrosinase Small Peptides for Skin Lightening Therapeutics

Usha Rani. J[1], Siva Prasad. B[4], Vutukuru. S.S[3] and Chand Pasha[2]*

[1]Department of Microbiology, Little Flower Degree College, Uppal, Hyderabad, India
[2]Department of Microbiology, Nizam College, Osmania University, Hyderabad, India
[3]Department of Biotechnology, Sreenidhi Institute of Science and Technology, Hyderabad, India
[4]Biotechnology Division, Environment Protection Training and Research Institute, Hyderabad, India

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Corresponding Author Email: cpasha21@gmail.com

Abstract

Peptides from natural origin are found to be effective in treating skin related disorders including hyperpigmentation. These peptides have potential to inhibit tyrosinase enzyme function thereby reduce melanin synthesis. Hence present study is aimed at evaluating antityrosinase function of small peptides that were identified from literature and experimental results. 3D structure of human tyrosinase was designed using SWISS MODEL. PROCHECK and Verify-3D tools were used to validate the 3-D model. Flexible docking of small peptides with human tyrosinase protein was carried out using CABS – dock server. Based on Cluster density and average RMSD values, small peptides showed an interaction between human tyrosinase and peptide residues with distances less than 5.5 Å. Thus, it was concluded that small peptides that were screened for antityrosinase activity in the study enable further experimentation in establishing their efficacy in regulating tyrosinase function.

Keywords

Human tyrosinase, CABS- dock, Small peptides, Average RMSD value

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INTRODUCTION

Natural proteins including transforming growth factor (TGF-β) showed anti-tyrosinase activity. It also interacts with MITF thereby interfering with maturation and mitigation of melanosomes [1, 2]. Thus, active sites of proteins that are involved in melanin production can be mimicked with small peptides. These natural proteins and the regulators such as interleukins and bFGFs can serve as templates for designing novel peptides that have potential to inhibit tyrosinase inhibition thereby hypopigmentation. Hence identification of oligopeptides from natural sources that have affinity towards active site of human tyrosinase assists in regulating melanin production. Oligopeptides can be designed to active sites of human tyrosinase thereby modulating its properties. Evidences from the body of literature cited that peptides with 8 and 10 amino acids length resulted in reducing melanin by 27% and 43% respectively. During *In vitro* studies, small peptides containing arginine and phenylalanine along with leucine, valine, cysteine as well as alanine residues showed anti-tyrosinase activity [3].

In silico design oligopeptides help in identifying potential peptide molecules that possess enhanced activity, prolonged in vivo stability thereby facilitating cell penetration and low toxicity when compared to chemical based skin therapeutics. Moreover, the structure of tyrosinase can be modeled *in silico* for delineating target sites of peptide interaction thereby affinity. These kinds of assessments were established for octa-peptide by docking it into catalytic site of tyrosinase [4]. These peptides showed to decrease melanin content by 45%. However, evaluation of efficacy and safety of these kinds of peptides is in progress. In this context, present study aims to *in silico* design of human tyrosinase and its interaction with small peptides and their protein engineering. Small peptides were identified from various experimental sources as well as our earlier experimental analysis conducted on earthworm extract. Further, the small peptides that were identified from earthworm extract were subjected to protein engineering. Subsequently, protein engineered peptide was docked with 3D model of human tyrosinase. Results indicated that the small peptides that were identified from various sources and earthworm extract showed good binding and interaction with human tyrosinase. Outcomes of the study aids in accelerating availability of novel peptide-based therapeutics to treat skin disorders such as hyper-pigmentation.

METHODOLOGY

Modeling 3D Structure of Human Tyrosinase

Access to human tyrosinase crystal structure is meagre across various data bases including protein data bank (PDB). Hence present study attempted to design 3D structure of human tyrosinase by SWISS-MODEL (https://swissmodel.expasy.org/interactive/9E8ekD/models/) [5–9]. FASTA sequence of human tyrosinase (P14679) was taken from Uniprot source [10]. The FASTA sequence of human tyrosinase is as follows:

>NIYDLFVWMHYYVSMDALLGGSEIWRDIDFAHEAPAFL
PWHRLFLLRWEQEIQKLTGDENFTIPYWDWRDAEKCDIC
TDEYMGGQHPTNPNLLSPASFFSSWQIVCSRLEEYNSHQ
SLCNGTPEGPLRRNPGNHDKSRTPRLPSSADVEFCLSLTQ
YESGSMDKAANFSFRNTLEGFASPLTGIADASQSSMHNA
LHIYMNGTMSQVQGSANDPIFLLHHAFVDSIFEQWLRR

After designing 3D structure, the model was submitted to PROCHECK [11] and Verify 3D for validating structure [12–14].

Small Peptides

Antityrosinase small peptides and their sequences were selected from various plant extracts as well as other literature sources. However, certain number of small peptides was selected from earthworm extract after Orbi-trap Analysis (A mass spectrometry-based approach) [15]. Moreover, a small peptide corresponding to phytochelatin synthase with sequence CLDQLMADQL [16] was chosen for protein engineering studies. Subsequently, Protein–peptide docking was performed using CABS-dock.

CABS- Docking

Small peptides (Table 1) were docked with 3D model of human tyrosinase using CABS-dock server. As the knowledge about binding of peptides with human tyrosinase, a receptor-based dock can be done [17]. Hence CABS-dock protocol with various programs was run (Figure 1). Subsequently, Small peptide sequences, and 3D structure of the Human tyrosinase receptor were given as input. Analysis of top 10 scored models from the output was analyzed.

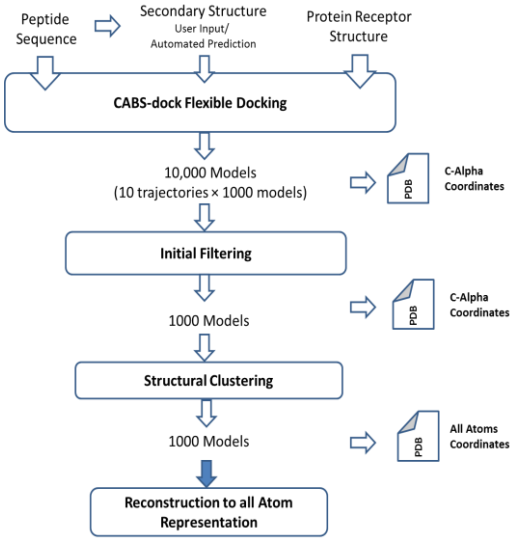

Figure 1 Flow chart of CABS-dock of peptides with human tyrosinase

Table 1. A list of Peptides and their sequences

S.No	Sequence of peptide	Name/ Source	Reference
1	YRSRKYSSWY	Oligo P4	[2]
2	RADSRADC	Oligo P3	
3	VSLLLVG	α- Lactalbumin	
4	MMSFVSLL	α- Lactalbumin	[18]
5	LILVLLAI	Gliadin	
6	LFTEVLDHNECLIITGTEQYSEYTGYAETYR	Oligo68	[19]
7	HGHPFAP	α- MSH	[20]
8	FSLLRY	53 spot	[21]
9	LSIGRL	196 spot	[22]
10	KRTGQYKL	ABFGF	[23]
11	PLLQATLGGGS	PAR2-ANT	[24]
12	MWYRPDLDERKQQKRE	PAR2-ANT	[25]
13	VECYGPNRPQF	Algae	[26]
14	LTEQESGVPVMK	Ostrich egg	[27]
15	CLDQLMADQL	Phytochelatin synthase earthworm peptide	[16]
16	CLSRHVLQACLSRHVLQC	Earthworm Engineered	

RESULTS AND DISCUSSION

Modeling 3D Structure of Human Tyrosinase

Protein PDB Id 5m8q.2.A is used as template for designing 3D model of human tyrosinase. The 3D model [Fig 2 (A)] showed 2.85 A RMSD with sequence similarity 0.44 and coverage 1.00. QMEAN value of the model is -3.35. Z- score value of model is <1 [Fig 2 (B and C)]. Sequence identity was found to be 46.55% [Fig 2 (D)]. Results of PROCHECK are presented in [Fig 2 (E)]. It showed 84.4% residues in most favored regions (red), 12.2% in additional allowed regions (yellow), 3.4% in generously allowed regions (light yellow) and 0 0.0% in disallowed regions(white) respectively. Verify 3D analysis resulted in high quality structure indicated by 86.69% of the residues of the built model that scored over 0.2 (If the generated model is having more than 70% of the residues have a score of greater than or equal to 0.2, then the protein structure is considered to be of high quality). Thus, the quality of 3D model of human tyrosinase was evaluated.

Docking Small Peptides with 3D Model of Human Tyrosinase Using CABS – dock

CABS – dock server is used to predict interaction of small peptides with human tyrosinase. The CABS-dock server [17] resulted top 10- scored models for each sequence. The peptides with more cluster density and RMSD value < 5.5 were tabulated (Table 2). The results show that small peptides possess good interaction with human tyrosinase. RMSD analysis of small peptides indicated their potential to interact with human tyrosinase effectively. However, a small peptide, corresponding to phytochelatin synthase with sequence CLDQLMADQL (RMSD 2.5) that was subjected to protein engineering (CLSRHVLQACLSRHVLQC), resulted in RMSD of 1.6. Furthermore, its RMSD is found to be similar to experimentally validated small peptides (Table 2, S.No. 1). It indicates that the engineered small peptide can be considered as a candidate for inhibiting human tyrosinase function. It also serves as a proxy for conducting experimental analysis and formulation of skin lightening agents.

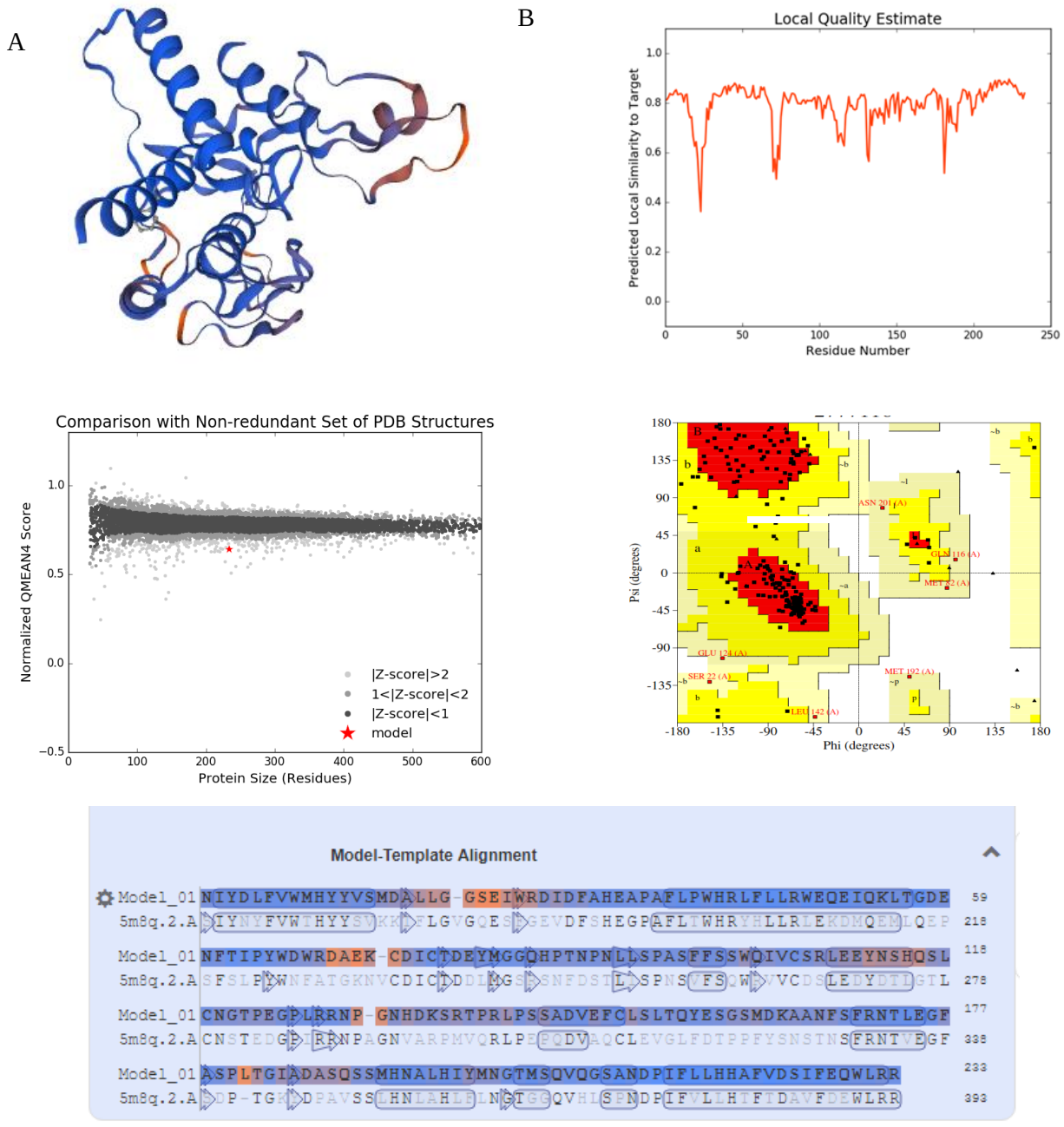

Figure 2 (A) 3D structure of Human Tyrosinase built by SWISS – MODEL (B) Local quality estimate of the 3D model (C)Z-Score analysis of 3D model of Human Tyrosinase (D) Sequence alignment Template 5m8q.2.A and Human Tyrosinase (E) Ramachandran Plot of 3D model of Human Tyrosinase

Table 2 List of small peptides with human anti- tyrosinase potential that were identified by using CABS-dock

S.No.	Peptide Sequence	Average RMSD	Cluster Density
1.	YRSRKYSSWY	1.68699	62.2411
2.	RADSRADC	3.25996	29.755
3.	VSLLLVG	1.22449	70.2335
4.	MMSFVSLL	4.76678	39.2298
5.	LILVLLAI	4.72971	77.1718
6.	LFTEVLDHNECLIITGTEQYSEYTGYAETYR	3.76616	28.9419
7.	HGHPFAP	5.47405	34.4453
8.	FSLLRY	1.72615	72.4157
9.	LSIGRL	3.62448	59.0429
10	KRTGQYKL	4.02058	49.7441
11	PLLQATLGGGS	4.01862	52.5056
12	MWYRPDLDERKQQKRE	3.03141	42.2245
13	VECYGPNRPQF	1.98133	28.4674
14	LTEQESGVPVMK	3.31301	38.3337
15	CLDQLMADQL	2.59713	40.8143
16	CLSRHVLQACLSRHVLQC	1.67695	38.1645

CONCLUSION

In recent times, peptide-based therapeutics are gaining prominence for treating hyper-pigmentation via tyrosinase inhibition. In this pursuit, *in silico* analysis of peptide interaction with human tyrosinase paves a way for identification of small peptides that can be used as tyrosinase inhibitors. Thus, 3D structure of human tyrosinase and its interaction with peptides indicated that small peptides isolated from various sources including earthworm extract exhibit good antityrosinase binding. However, further studies are warranted to validate their *in vivo* efficacy and safety.

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