PROTEIN-PROTEIN DOCKING STUDIES ON THE SWEET PROTEIN (NEOCULIN) AND THE HUMAN SWEET TASTE RECEPTORS T1R2 AND T1R3.

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Neoculin is a sweet protein capable to alter the sour taste into sweet taste, it is 500 times sweeter than the ordinary sugar. This protein has been discovered in Malaysia under the name of Curculin. There are a number of experimental studies that have been conducted on neoculin but none of the studies focuses on molecular level, in order to understand how the protein interacts with the human sweet taste receptors T1R2 and T1R3. Therefore, in this work, a protein-protein docking study was performed between neoculin and the human sweet taste receptor T1R2 and T1R3. The docking results showed residues that might be important for binding the neoculin with the human sweet taste receptors, particularly T1R3 at the amino terminal domain (ATD). In addition, the current results showed that His11, which is important for the taste modifying ability does not bind directly to the human sweet taste receptors.

Keywords: Noeculin, T1R2 and T1R3, protein-protein docking

INTRODUCTION

Neoculin is a unique protein for its ability to change sour taste into sweet taste, and it's 500 times sweeter than the ordinary sugar with low calories. In 1990 neoculin has been discovered in west Malaysia under the name of curculin and purified from *Curculigo latifolia* fruit [1]. The knowledge of sweet protein can provide a future treatment for diabetes, obesity and other metabolic diseases, especially if it has a taste modifying ability since it can potentially use in the food industry as a replacement for artificial sweeteners [2, 3]. Neoculin together with other types of sweet proteins, sucrose, natural sugar, sweet amino acids, and artificial sweeteners are sensed by the human sweet taste receptors T1R2 and T1R3, which are which are heterodimeric receptors belongs to the family of the G-Protein coupled receptors [4, 5]. Each of the human Sweet taste receptors T1R2 and T1R3 has a large amino terminal domain (ATD), extracellular cysteine rich domain (CRD) and transmembrane helical domain, and they have a multiple ligand binding sites for different types of sweeteners [6]. The protein-protein interactions are very important for it explains the biological activities of the cell. Computational tools come as an effective approach to understand the interactions between the proteins, because they are less expensive and can be accomplished within shorter time compared to the ordinary laboratory methods [7]. Computational approaches such as protein-protein docking can be used to understand the interactions of protein complexes, especially when it is difficult to obtain the information from the laboratory methods, there are many computational tools available to study protein-protein docking [8]. Previous experimental researches has been performed on neoculin in order to identify its important residues, which is responsible for its pH sensitivity using cell-based calcium imagine and human sensory test [5], another research was conducted to demonstrate T1R3 as a major part of the human sweet taste

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receptor could bind with neoculin, using calcium imagine analysis of human and mouse TRs using HEK cells expression method[14], and finally by using (NMR) spectroscopy and alanine scanning mutagenesis, to determine the important neoculin amino acids which are needed for its binding and activation [9]. This research presents a Protein-Protein Docking simulation Studies between the Sweet Protein Neoculin and the Human Sweet Taste Receptors, in order to provide more insight of the neoculin in molecular level, by finding out the important residues of the protein in the important domains, and how it interacts with T1R2 and T1R3.

MATERIALS AND METHODS

Receptor preparation.

T1R2 and T1R2 are the receptors for the docking simulation, since there is no experimental 3D structure for both of them. The homology modeling was carried out to produce the 3D structure of each receptor using MODELLER9.10 [10]. Single template and multi templates homology modeling was running on each of T1 R2 and T1R3 to produce receptor models with reliable quality based on Ramachandran plot analysis.The chosen templates were 2E4Z and 3Q41 for T1R2 with 84.5% residues located in the most favorable region, and 3Q41 is the template for T1R3 with 84% residues located in the most favorable region, respectively [11]. The receptors were prepared before running the docking simulation using protein preparation wizard, which is a tool under Schrödinger Maestro 9.2 [12].

Ligand preparation.

The sweet taste protein neoculin was downloaded from the protein database bank (PDB) with ID (2D04), the 3D structure model of neoculin was delivered to the protein database bank by X-Ray diffraction at 2.76Ao resolution [13], as shown in figure2. Neoculin contains 908 amino acids, which is made up of 8 chains A, B, C, D, E, F, G and H. However, only the chains A, D, G, and H have been chosen according to the PDB information, because they are involved in the protein active sites. The receptors were prepared using protein preparation wizard [12].

Docking Simulation.

The protein-protein docking between the sweet protein neoculin and the human sweet taste receptors T1R2 and T1R3 were carried out using Schrödinger Maestro 9.2, which is a graphical user interface (GUI) [14].

RESULTS AND DISCUSSION

One of the major experimental studies was carried out to recognize the important region of the human T1R2-T1R3 required for neoculin binding, and it shows that neoculin is mainly bound to the amino terminal domain (ATD) of T1R3[15]. The present docking results shows agreement with this finding. Table 1 shows the important residues in the particular chains of neoculin, which appear frequently in the docking results. Figure1 shows the location of the important residues in the sequence alignment between the chains of neoculin using clustalw [16], which are being circled and these residues are mostly located in the highly conserved regions. The docking results shows that all residues that bind the neoculin with T1R3 are important which are shown frequently, and those residues can be found particularly at chain D and G of neoculin as Figure 2 and Figure 3 show the important residues and how they bound to the receptors, which is presented together with their hydrogen bond and its length. Those residues are Arg110, Arg111, Asn107, Gln8 and Lys90 from chain D of neoculin and Arg111, Asn107, Gln90 from chain G of neoculin are bound to the ATD, which is located between the residues 1 to 498 of the receptor.

Human sweet taste Receptor	Neoculin Chain	Residures
T1R2	A	Lys33, Tyr97, Thr83, Ser2
T1R2	D	Cys77, Arg111, Gly79, Asp80, Ala85, Arg110
T1R2	G	Cys109, Arg110, Asn107, Gly105, Ser2, Gln90, Gln8
T1R2	H	Asp91, Asn2, Asp80, Arg110, Arg111, Asn61, Trp78, Arg93
T1R3	A	Ser111, Arg110, Cys109, Asn107, Gln90, Ser20, Asn80
T1R3	D	Asp91, Arg110, Arg111, Asn107, Gln8, Lys90, Lys33, Asn36, Ala20
T1R3	G	Asn80, Arg110, Asn107, Ser20, His36, Gln90, Asp91
T1R3	H	Tyr97, Arg111, Arg93, Lys90, Lys33

Table 1. *The Important resdiures of neoculin that appers frequently in the docking results.*

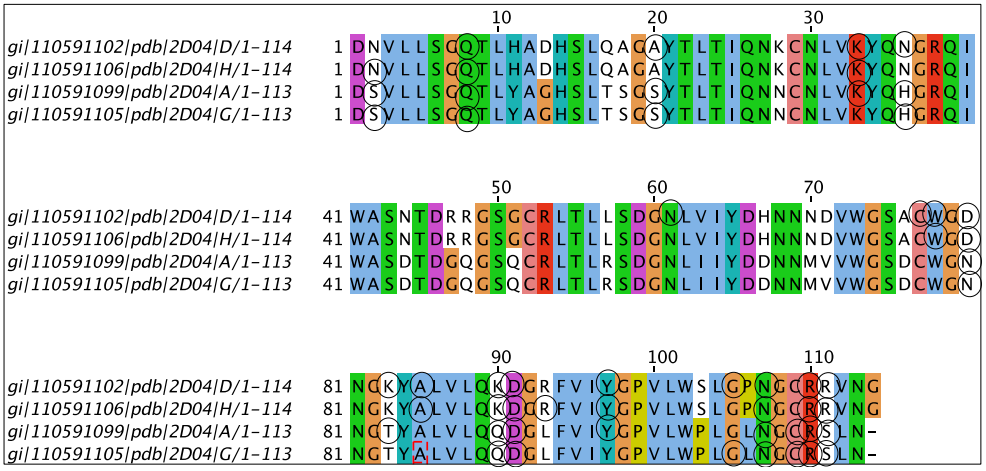

Fig. 1. *The alignment between the neoculin domains A, D, G and H together with the important residues which are circulated.*

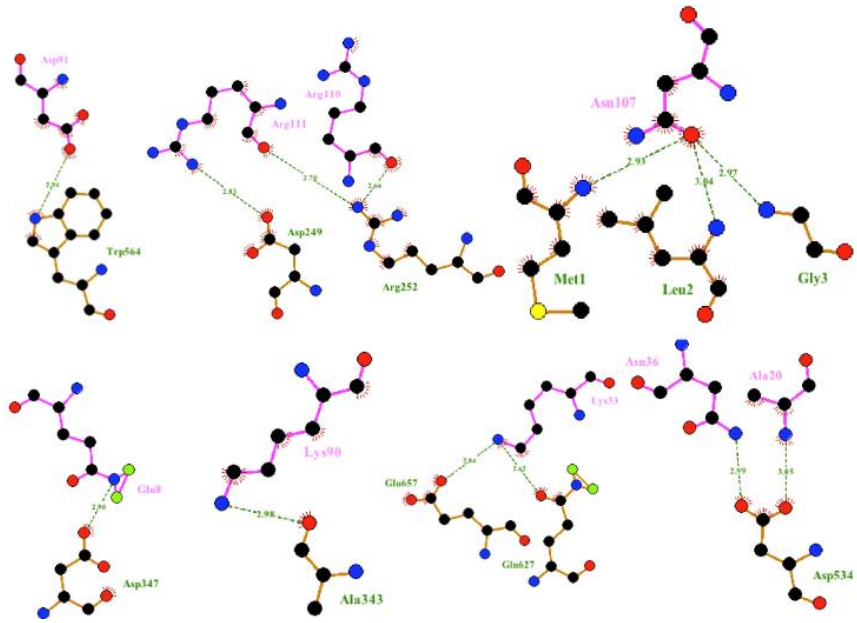

Fig. 2. *The protein-protein docking between neoculin chain D and the human sweet taste receptor T1R3.*

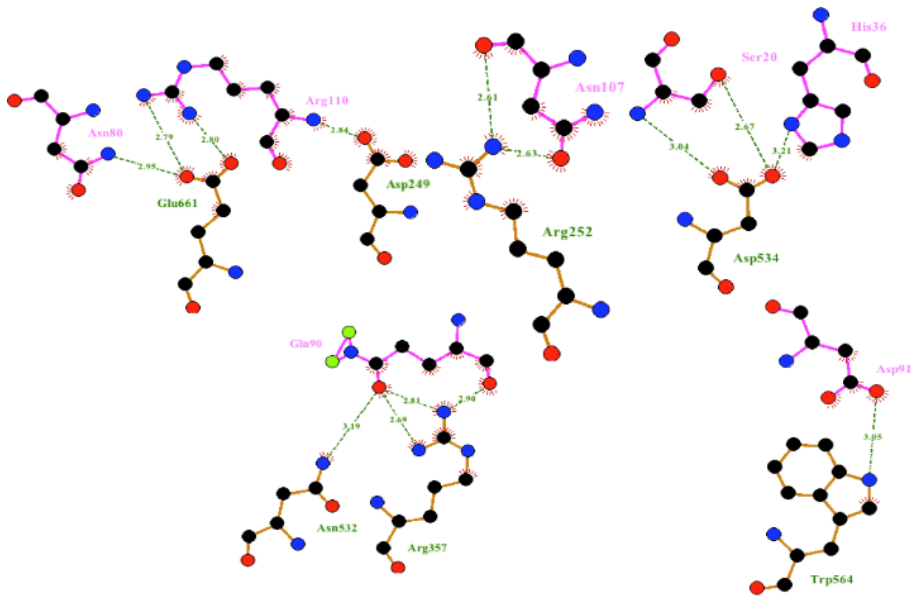

Fig. 3. *The protein-protein docking between neoculin chain G and the human sweet taste receptor T1R3.*

receptor. In addition to this, the same experimental study mentioned about the cysteine rich domain (CRD) which is located between the residues 489 and 567, may play an additional role in binding of neoculin. Likewise, the cysteine rich domain (CRD) is significant for the responding of the human sweet taste receptors to other natural sweet taste proteins such as brazzien, monellin and thaumatin, according to a previous study using heterologous expression of T1R2 and T1R3[17].

The current docking study shows the residues Asp91, Asn36, Ala20 Lys90 from neoculin chain D, and Ser20, His 36 and Asp91 from neoculin chain G are binding with the cysteine rich domain (CRD).

Beside that study, there is another research mentioned about the importance histidine involved in the taste modified ability of neoculin using cell based calcium imaging and human sensory test, for that research was needed to figure out the pH sensitive functional alters in the protein as ligand generally and its interaction with the taste receptors specifically. These histidines are 11, 14 and 36 at the neoculin basic subunit (NBS) and 14 and 36 at the neoculin acidic subunit (NAS), as well as these results mentioned about the His11 as the most critical residue affect The neoculin taste modification ability, which may not bind or interact directly to the human sweet taste receptors, but it modify the neoculin structure and activity, because its manage the equilibrium between the active and inactive site of the neoculin [5].However, the current docking study shows the His11 does not bind directly to the human sweet taste receptors, but His36 at the chain G of neoculin binds to T1R3.

CONCLUSIONS

The main objective of this study is to perform a protein-protein docking simulation, between the 3D structure of the sweet protein neoculin, which has been experimentally determined, and the human sweet taste receptors T1R2 and T1R3, which are determined by using homology modeling method, and The results of this research is very crucial to provide more insight regarding the interactions between the receptor and the ligand.

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