Food Chemistry 271 (2019) 697–706

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Molecular docking and simulation of the synergistic effect between umami peptides, monosodium glutamate and taste receptor T1R1/T1R3

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ARTICLE INFO

*Keywords:*
Umami peptide
MSG
T1R1/T1R3
Synergism

ABSTRACT

In order to investigate the synergistic effect between umami peptides, monosodium glutamate (MSG) and the taste receptor T1R1/T1R3, a novel bivariate model was created based on our previous work. The results showed three specific changes upon the addition of MSG between umami peptides and T1R1/T1R3, in terms of energy and conformation. First, the addition of MSG enlarged the size of the binding cavity of T1R3 from 534.125 $A^3$ to 1135.75 $A^3$. Second, the addition of MSG caused small peptides to bind with T1R3, with the lowest docking energy and docking interaction energy, −77.2295 and −60.7146 kcal/mol respectively. Third, five binding residues ,including Glu-429, Gln-302, Gly-304, Try-107 and His-364, increased which play critical roles in hydrogen bonding. They are consistent with the results of electronic tongue and facilitate better understanding of the synergism. Furthermore, novel umami and umami-enhanced compounds could be discovered, based on the use of the novel bivariate model.

## 1. Introduction

Umami peptides are well-known food additives used to improve or influence the taste of foods. The umami taste of L-glutamate can be drastically enhanced by 5′-ribonucleotides, and the synergy is a hallmark of the taste quality. Umami peptides and monosodium glutamate can produce a synergistic effect (Nishimura et al., 2016). The synergistic effect arises from binding of the umami peptide to the umami receptor T1R1/T1R3. In 2000, the first umami receptor, taste-mGluR4, was discovered (Ogasawara, Yamada, & Egi, 2006). Taste-mGluR4 is a metabotropic glutamate receptor; a special dimeric G-protein-coupled receptor (GPCR) (Karangwa, Linda, Meigui, Cheserek, & Zhang, 2013) located in the membranes of the taste cells of the taste buds. T1R1/T1R3 (Uematsu, Tsurugizawa, Kitamura, Ichikawa, & Torii, 2011; Zhuang, Lin, Zhao, Dong, & Su, 2016) and a special mGlu receptor, which is related to the brain glutamate receptor mGluR1, are two other umami receptors that have been identified (Marui, Tada, Fukuoka, Wagu, & Kusumoto, 2013). Recent findings on umami taste indicate that the signal mediated by the pathway involving mGluRs may play a different role to that derived from T1R1/T1R3. The mGluRs signal occurs mainly in the posterior tongue and contributes to behavioural discrimination between umami and other taste compounds, whereas the T1R1/T1R3 signal occurs mainly in the anterior tongue, and plays a major role in preference behaviour. It is now accepted that mGluR4 can respond to glutamate and analogues, such as L-(+)-2-amino-4-phosphonobutyrate (L-AP4), and that mGluR1 is also involved in the umami taste response to glutamate (Yu, Zhang, Miao, Li, & Liu, 2017). In addition, studies on T1R1/T1R3 and umami compounds indicate that the receptor responds to glutamate and this response is enhanced by IMP and GMP. It is speculated that T1R1/T1R3 could respond to umami peptides, which act as ligands for the receptor (Dang, Gao, Xie, & Ma, 2014). Biological assays would be necessary to show tested peptides as T1R1/T1R3 ligands in the future.

Molecular docking is widely used in the fields of structural molecular biology and structure-based drug design (Bagnasco, Cosulich, Speranza, Medini, & Lanteri, 2014; Kim, Son, Kim, Misaka, & Rhyu, 2015; Zhang, Venkitasamy, Pan, Liu, & Zhao, 2017). It is a computational procedure used in the field of structure-based rational drug design to identify the correct conformations of small molecules and to estimate the strength of protein-ligand interactions (Khan, Shawon, & Halim, 2017; Zhou, Wang, Ye, Chen, & Tao, 2017). At present, the identified umami peptide receptor is a heterodimer of taste receptor (T1R1/T1R3), and it is the only umami receptor that is sensitive to both Glu and umami peptides. In 2008, Zhang, Klebansky, Fine, and Xu (2008) proposed a ubiquitous in-binding model of the uric acid receptor with glutamic acid (Glu) and inosinic acid, inosine monophosphate

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https://doi.org/10.1016/j.foodchem.2018.08.001
Received 26 March 2018; Received in revised form 16 July 2018; Accepted 1 August 2018
Available online 02 August 2018

(IMP), based on the analysis of intracellular $Ca^{2+}$ by means of homology modeling and template crystal structure analysis. They illustrated the synergistic effect was related to the Venus flytrap (VFT) domain of T1R1. In the model, Glu bound near the hinge region of VFT, IMP bound to the adjacent site and approached the opening of VFT to stabilize the closed conformation. Zhang et al. (2008) reported that IMP and GMP interacted with the VFT domain of T1R1, representing unique positive regulation of family C GPCRs. Bellisle (1999) clarified that the enhanced umami effect of Glu, 5′-nucleotides and umami receptor proteins was possibly due to the spatial conformational change of umami receptor proteins. Yoshida, Kawabata, Kawabata, and Nishimura (2015) reported that the mechanism of the synergistic effect of umami taste involved an allosteric modulator, and IMP stabilized the conformation of the extracellular domain of T1R1. Nishimura et al. (2016) found that the umami substances could enhance the flavour of aromatic substances, according to the Aroma Chicken Model (ACM). Flavour aftertaste was mainly evaluated by a sensory subtraction experiment with glutamate and phosphate. The addition of Glu and IMP to 0.4% NaCl in chicken broth improved the taste significantly. The addition of 0–0.3% MSG increased the aftertaste of the chicken soup by 2.5 times compared to the soup without MSG. The addition of 0.05%, 0.075% or 0.1% MSG and IMP had higher umami intensity than the addition of MSG alone. However, when the concentration of MSG exceeded 0.3%, the flavour intensity reached a stable level. Multiple ligand binding sites for several umami compounds have been characterized using molecular modeling and site-directed mutagenesis studies of human-rodent or human-squirrel monkey chimeric receptors. In contrast, the binding sites for umami peptides have not been well defined (Toda et al., 2013).

Different nucleotides, umami peptides and other umami substances have synergistic effects, which could increase the umami intensity to several times that of MSG (Kinnamon, 2009). These studies focused on the synergistic effect of nucleotides and MSG, and the authors found the synergistic effect promoted the development of nucleotide flavour, including chicken essence and mushroom essence. Compared with nucleotides, umami peptides have obvious advantages, including good processing performance, easy absorption, non-antigenicity and physiological activity. Since most of the components, such as amino acids, peptides and organic acids, contributed to umami during protein hydrolysis, umami peptides may form and promote flavours, such as the salinity of salt, sweetness of sugar, as well as counteract sour and bitter flavours. However, there are few reports on the mechanism of umami peptide and monosodium glutamate (MSG) interaction with umami receptor (T1R1/T1R3). In this paper, we report the synergistic characteristics of 36 umami peptides and MSG from molecular docking of the umami receptor. Through greater understanding of the synergistic mechanism, umami peptides could be used as a healthier alternative for MSG in food products. This study provides a theoretical basis for the development and research of nutritional flavour agents and provides a theoretical reference for the interactions of taste components in food.

## 2. Materials and methods

### 2.1. Materials

A total of 36 umami peptides were used to investigate the synergistic effect of umami peptides, MSG and the umami receptor. Among them, twelve umami peptides were synthesized by solid phase at the Jier Biochemistry Corporation (Shanghai), with a purity of over 98 g/100 g, and identified by reversed-phase high performance liquid chromatography and mass spectrum. The solid phase synthesis was of using Fmoc-based strategy. Firstly, the Fmoc-Tyr(tBu)–OH is connected to the Wang resin, and then the peptide chain is extended. Finally, the peptide resin is cut and the product is analyzed and purified and identified by the mass spectrometry. (peptide 1: Cys-Cys-Asn-Lys-Ser-Val (CCNKSV); peptide 2: Ala-His-Ser-Val-Arg-Phe-Tyr (AHSVRFY); peptide 3: Asn-Arg-Thr-Phe (NRTF); peptide 4: His-Cys-His-Thr-Asn (HCHTN); peptide 5: Pro-Asp-Leu-Pro-Asn-Thr (PDLPNT); peptide 6: Leu-Ser-Glu-Arg-Tyr-Pro (LSERYP); peptide 7: Asn-Gly-Lys-Glu-Thr (NGKET); peptide 8: Glu-Ser-Val (ESV); peptide 9: Arg-Leu (RL), peptide 10: Glu-Val (EV), peptide 11: Glu-Glu-Leu (EEL) ; peptide 12: Glu-Leu (EL). (Kim et al., 2015). Twenty-four other umami peptides (13–36) were sourced from the Bioactive peptides database (Anonymous, 2018). The twenty-four umami peptides were also synthesized by solid phase at the Jier Biochemistry Corporation (Shanghai), with a purity of over 98 g/100 g (peptide 13: Asp-Asp (DD), peptide 14: Glu-Asp (ED); peptide 15: Asp-Glu (DE); peptide 16: Glu-Glu (EE); peptide 17: Val-Gly (VG); peptide 18: Val-Val (VV); peptide 19: Val-Gly-Gly (VGG); peptide 20: Val-Glu (VE); peptide 21: Val-Asp (VD); peptide 22: Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala (KGDEESLA); peptide 23: Asp-Asp-Asp (DDD); peptide 24: Asp-Asp-Glu (DDE); peptide 25: Asp-Glu-Asp (DED); peptide 26: Glu-Asp-Asp (EDD); peptide 27: Asp-Glu-Glu (DEE); peptide 28: Glu-Asp-Glu (EDE); peptide 29: Glu-Glu-Asp (EED); peptide 30: Glu-Glu-Glu (EEE); peptide 31: Ser-Leu-Ala-Asp-Glu-Glu-Lys-Gly (SLADEEKG); peptide 32: Ser-Leu-Ala-Lys-Gly-Asp-Glu-Glu(SLAKGDEE); peptide 33: Lys-Gly-Ser-Leu-Ala-Asp-Glu-Glu (KGSLADEE); peptide 34: Glu-Glu-Asp-Gly-Lys (EEDGK); peptide 35: Glu-Tyr (EV); peptide 36: Asp-Glu-Leu (DEL)).

Solid phase peptide synthesis has evolved over three decades into a tremendously powerful method for preparing peptides and small proteins. An absolute prerequisite for successful syntheses, in all solid phase schemes, is that the reactions must proceed cleanly and efficiently because the very nature of the technique contaminates the products (Dang, Gao, Ma, & Wu, 2015).

### 2.2. Molecular docking of the umami peptide and umami receptor T1R1/T1R3

#### 2.2.1. Thirty-six umami peptides were molecularly docked using Discovery Studio software package

The homology modeling of T1R1/T1R3 was carried out by Discovery Studio (DS, version 2.1, Neo Trident Technology LTD). Homology modeling of the ligand binding region of the umami receptor was carried out using metabotropic glutamate receptor as the template (PDB ID: 1EWK) (Dang et al., 2014). Based on the 3D structure of metabotropic glutamate receptor and sequence blast result, the homology model of T1R1/T1R3 was established from the DS/Modeler protocol. The molecular dynamic simulation was performed by the DS/Standard Dynamics Cascade protocol, which especially settled the steps of the entire dynamic simulation into a parameter setting interface in DS, including force field settings, CHARMm force field and Harmond Design. The molecule was solvated with periodic boundary condition and minimized using a standard dynamics cascade protocol.

In order to choose the docking method and evaluation function, Glu was docked into T1R1/T1R3 by the DS/CDOCKER protocol. Thirty six umami peptides were used for virtual screening, based on the T1R1 receptor and T1R3 structures using Discovery Studio 4.0/CDOCKER. In addition, the metabotropic glutamate receptor was used as the template (PDB ID: 1EWK). Specific methods of the homology modeling of T1R1/T1R3 are presented in our previous work (Zhou et al., 2017). Each ligand score was compared and the dominant conformations were analyzed. The preferred conformational analysis was selected based on the score of each agonist for representing the binding of peptides 2, 6, 8, and 9 to T1R3, and 12 to T1R1 and T1R3. The binding mode of the preferential conformation and receptor was analyzed in order to search for the potential combination mode by comparative analysis.

#### 2.2.2. Kinetic simulation of umami peptide and umami receptor protein upon the addition of MSG

In order to study the conformational change, Glu was used as a model amino acid in the molecular docking experiments, based on the T1R1 receptor structure. Discovery Studio 4.0/CDOCKER was used for