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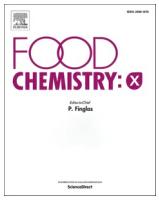

Identification of novel saltiness/saltiness enhancing peptides in egg proteins: Molecular docking, action mechanism and *in vitro* activity validation

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ABSTRACT

In this study, novel saltiness/saltiness enhancing peptides VESQTNGIIR, NQITKPNDVY, and DEDTQAMP were identified from the hen egg proteins *via* virtual enzymatic, molecular docking, and electronic tongue analysis. Their saltiness enhancement effect was analyzed by e-tongue analysis. Saltiness enhancement rates of saltiness peptide VESQTNGIIR were 47.24 %, 95.28 % and 105.94 %, and those of the saltiness peptide NQITKPNDVY were 32.40 %, 70.16 %, and 71.25 % at the salt reduction concentrations of 25 %, 35 % and 45 %, respectively. Saltiness enhancement rates of saltiness enhancing peptide DEDTQAMP were 5.83 % and 11.24 % at 25 % and 35 % salt reduction concentrations, respectively. Molecular docking demonstrated that Glu286, Arg330, Arg424, and Arg583 may be the key amino acids interacting with TMC4, whereas that carbon hydrogen bond, conventional hydrogen bond, and attractive charge interactions were important forces in peptides-TMC interactions. The study indicated that the peptides VESQTNGIIR and NQITKPNDVY may be ideal saltiness/saltiness enhancing peptides.

1. Introduction

Salt, the most fundamental and widely used salt preparation, can enhance the taste and quality of foods, and its most important role is to increase saltiness(Albarracín, Sánchez, Grau, & Barat, 2011; Gao et al., 2024). Saltiness is one of the five essential flavors, alongside sour, sweet, bitter and umami, of which "salty" is a necessary taste feeling(D. Y. Shen et al., 2022; Y. Zhang, Venkitasamy, Pan, Liu, & Zhao, 2017). However, excessive salt intake will damage organs such as the heart, brain and kidney, as well as elevate the chances of developing hypertension and depression(Penz, Joffres, & Campbell, 2008; Tiyasatkulkovit et al., 2021). In addition, a high-salt diet can make people more susceptible to obesity, asthma, kidney disease and stomach cancer, among other diseases(Cappuccio, D'Elia, Kandala, & Strazzullo, 2009). Asian countries, especially China, daily salt intake far exceeds WHO guidelines(X. L. Chen, Wu, & Huang, 2022; F. J. He & MacGregor, 2010; Hosseini-Esfahani et al., 2017; Webster, Trieu, Dunford, & Hawkes, 2014). Therefore, the challenge of salt reduction while maintaining acceptability of the products is major for the food industry.

Two common types of salt reduction in the food industry are employed. One way is to directly decrease the quantity of salt and alter its composition(Hurst, Ayed, Derbenev, Hewson, & Fisk, 2021). However, reducing the amount of salt added to food can seriously affect the taste and flavor of the food(Hoppu et al., 2017). The altered structure of salts requires advanced processing technology(Hurst et al., 2021). The other approach is to use salt substitutes(Barnett, Diako, & Ross, 2019; Pateiro, Munekata, Cittadini, Domínguez, & Lorenzo, 2021). Presently, other mineral salts, *i.e.,* potassium chloride and calcium chloride, were utilized as substitutes for sodium chloride in the preparation of food products (Greer et al., 2020; Pateiro et al., 2021). However, salt substitutes exceeding a certain amount (≥40 %) can result in a notable bitter and metallic taste. Therefore, under the premise of ensuring the taste, a safe strategy of salt reduction without saltiness reduction is proposed. Saltiness/saltiness-enhancing peptides can be used as potential research objects for salt reduction due to their safety and non-toxicity. Some saltiness peptide/saltiness enhancing peptide DD, ED, DDD, SPE, FI, IQ, PK, IE, TF, LQ, KER, EDEGEQPRPF, ADHDLPF, DIQPEER, DEPLIVW, and LPDEPSR have been identified from food proteins

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Table 1
Water solubility, toxicity prediction of peptides and docking with TMC4.

Peptide	Peptide length	Solubility	Toxicity			CDOCKER ENERGY(kcal/mol)
			Ames Mutagenicity	Developmental Toxicity Potential	Skin Sensitization	
VESQTNGIIR	10	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−155.158
NQITKPNDVY	10	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−151.167
DEDTQAMP	8	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−147.081
AEINEAGR	8	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−129.412
QTAADQAR	8	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−127.315
SGISSAES	8	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−116.245
ESIIN	5	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−103.202
ANENI	5	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−103.119
AEER	4	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−101.849
TQINK	5	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−98.3175
ASEK	4	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−97.1836
EEK	3	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−95.3995
GITDV	5	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−92.3939
DSTR	4	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−92.0566
EER	3	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−91.6503
QCVK	4	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−84.0818
TEW	3	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−72.1805
ADHP	4	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−70.4193
CIK	3	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−70.1065
DIL	3	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−69.7089
EPIN	4	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−68.7204
VVR	3	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−64.3232
IK	2	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−61.1331
SM	2	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−48.6819
SA	2	GOOD	Non-Mutagen	Non-Toxic	Non-Sensitizer	−47.4506

(D. Chen et al., 2023; Y. P. Chen, Wang, Blank, Xu, & Chung, 2021; Wang et al., 2024; Yamamoto et al., 2014; Zheng et al., 2021). Egg white protein is a high quality raw material for bioactive peptides(Ji, Zhao, Yu, & Wu, 2022). Especially, of the protein contained in egg whites, ovalbumin is 54 %(Mine, 1995). And a variety of bioactive peptides with different physiological effects, including antimicrobial, antioxidant and immunological activities, have been identified from ovalbumin(Bhullar & Wu, 2020; Davalos, Miguel, Bartolome, & Lopez-Fandino, 2004; Holen, Bolann, & Elsayed, 2001; Yu et al., 2020; Zhao, Zhang, Yu, Ding, & Liu, 2020). Thus, ovalbumin as a high quality source of active peptides can be utilized to screen potential saltiness/saltiness enhancing peptides. The advantages of peptides as a food additive include: (i) the ability for occupying the specific chemical spaces in protein targets that are challenging by small molecules, (ii) chemical modification to improve their stability, (iii) higher degree of specificity(Taghizadeh, Taherishirazi, Niazi, Afsharifar, & Moghadam, 2024).

The perception of saltiness is mediated by a variety of ion channels and receptors, such as the transmembrane channel-like 4 (TMC4), epithelial sodium channels and transient receptor potential vanillic acid (Shigemura et al., 2013; Son & Park, 2018). TMC4 is the first anion/chloride channel identified that belongs to the TMC family(Kasahara et al., 2022), and can be employed as a novel target for the identification of saltiness to facilitate the virtual screening of saltiness/saltiness enhancing peptides. Virtual screening, as a new method, can replace traditional enzymatic hydrolysis and be used effectively for screening and identification the active peptides.

This work aimed to identify novel saltiness peptide/saltiness enhancing peptide from ovalbumin by virtual screening and molecular docking. After virtual enzymolysis of ovalbumin using the PeptideCutter program, the obtained peptides were screened for water solubility and toxicity using Innovagen and Discovery studio software, respectively. The 3D structure of the TMC4 receptor and its usability were constructed and evaluated using AlphaFold2. Subsequently, the selected peptide was docked to the salty receptor TMC4. Finally, the saltiness of these peptides was confirmed using the electronic tongue. Moreover, the molecular mechanisms of saltiness peptide/saltiness enhancing peptide and TMC4 were also revealed. This work may contribute to the identification of new saltiness/saltiness enhancing peptides from hen egg.

2. Materials and methods

2.1. Chemical reagents

Sodium chloride, ethanol, hydrochloric acid, tartaric acid, potassium chloride, and potassium hydroxide were purchased from Sinopharm chemical reagent Beijing Co., Ltd. (Beijing, China). Human embryonic kidney cells (HEK-293 T) and human gastric mucosal epithelial cells (GES-1) were obtained from the cell bank of the Chinese Academy of Sciences. All other chemicals in the experiment were of analytical grade.

2.2. In silico hydrolysis of ovalbumin

The protein sequence of ovalbumin (Accession of NCBI: AAB59956) in hen egg was retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/)(accessed January 4th, 2024). Ovalbumin was hydrolyzed *in silico* using three main gastrointestinal tract enzymes, pepsin (pH 1.3), trypsin and chymotrypsin in the ExPASy PeptideCutter (http://web.expasy.org/peptide_cutter/) (accessed January 4th, 2024) program(Gasteiger et al., 2005). Peptide chain length affects peptide absorption efficiency, shorter peptide chains generally result in better absorption(W. L. Shen & Matsui, 2017). Therefore, peptides with a length of less than ten amino acids were collected for the following research.

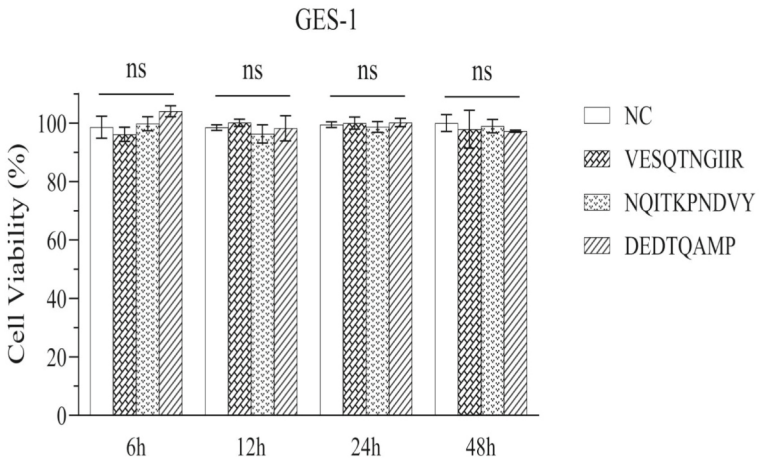
2.3. Toxicity and solubility prediction of peptides

Solubility of peptides was first predicted in peptide property calculator (http://www.innovagen.com/) (Gupta et al., 2013) (accessed January 4th, 2024). After that, the possible toxicity of the peptides including mutagenicity, developmental toxicity potential (DTP), and skin sensitization (GPMT) was predicted using Discovery Studio (DS) 2017 R2 software (Dassault Systemes Biovia, San Diego, CA, USA)(J. He et al., 2019).

2.4. Molecular docking of peptides and TMC4

The model of TMC4 was constructed and its usability was evaluated using AlphaFold2 (accessed November 6th, 2023)(shown in Fig. S1).

(a)



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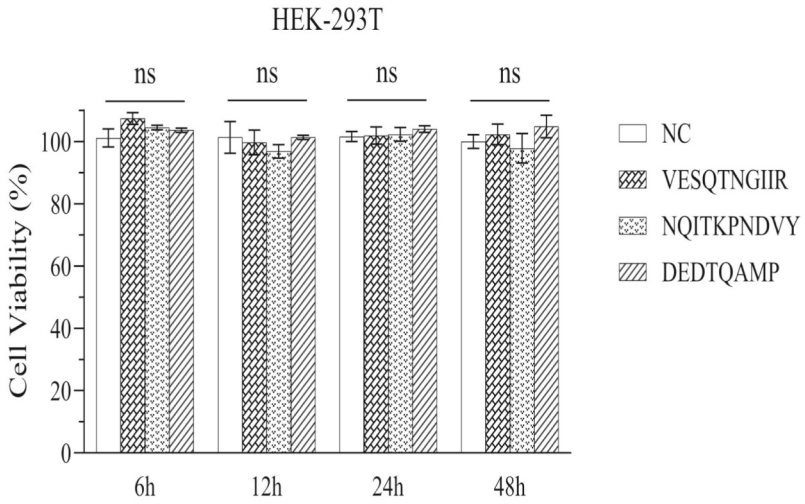

Fig. 1. The CCK8 cytotoxicity assay with three peptides VESQTNGIIR, NQITKPNDVY, and DEDTQAMP. (a) Effects of the three peptides on cell viability of GES-1 cells at 6 h, 12 h, 24 h, and 48 h, respectively, (b) Effects of the three peptides on cell viability of HEK-293 T cells at 6 h, 12 h, 24 h, and 48 h, respectively. The NC group is the blank control, cultured with complete medium without peptides treatment.

Alphafold2 utilizes deep learning algorithms to integrate protein structures, and in this way obtains structural models that are typically as accurate as empirically determined structures(Cramer, 2021). The amino acid sequence of TMC4(Accession of NCBI: NP_001138775–2) was obtained from NCBI (accessed November 26th, 2023). This was input into the monomer_ptm model in Alphafold v2.3.2 for protein structure prediction. All parameters were default values. The usability of the model was evaluated by the pLDDT score of the software. Then, the obtained TMC4 receptor model was preprocessed in DS 2017 R2 software by removing water and adding hydrogen atoms. TMC4 has two active pockets that bind well to the salty taste enhancing peptide, using the key amino acids of the 2 active pockets: Trp147, Ile152, Lys568, Phe572, Tyr565, Arg151, Thr148, and Pro144; Leu684, Thr421, Lys685, and Ala333, Thr337, Arg424, Arg330, Arg688, Ser423, Arg580, Tyr677, and Ala334. The two activity pockets provide a location and chemical environment for the binding of saltiness/saltiness enhancing peptides, and provides binding space for peptide segments.Two active centers were defined at x = 4.34, y = 4.66, z = 22.83, with a radius of 14.4; and x = −28.04, y = 6.91, z = 9.06, with a radius of 26.7, completing the receptor model(D. Y. Shen et al., 2022). Finally, molecular docking was performed using the CDOCKER program and the potential peptides were screened based on the CDOCKER-Energy value.

2.5. Peptide preparation

The peptides with potential saltiness/saltiness enhancing property were synthesized and provided by Shanghai Science Peptide Biological Technology Co.Ltd. (Shanghai, China). Its purity was evaluated using high performance liquid chromatography, while its molecular weight was determined through mass spectrometry(Zhao et al., 2020).

2.6. Peptide cytotoxicity

GES-1 cells and HEK-293 T cells in logarithmic growth phase with good growth condition were taken and inoculated into 96-well plates at a cell concentration of 2×10^4 cells/mL, 100 μL per well, and cultured

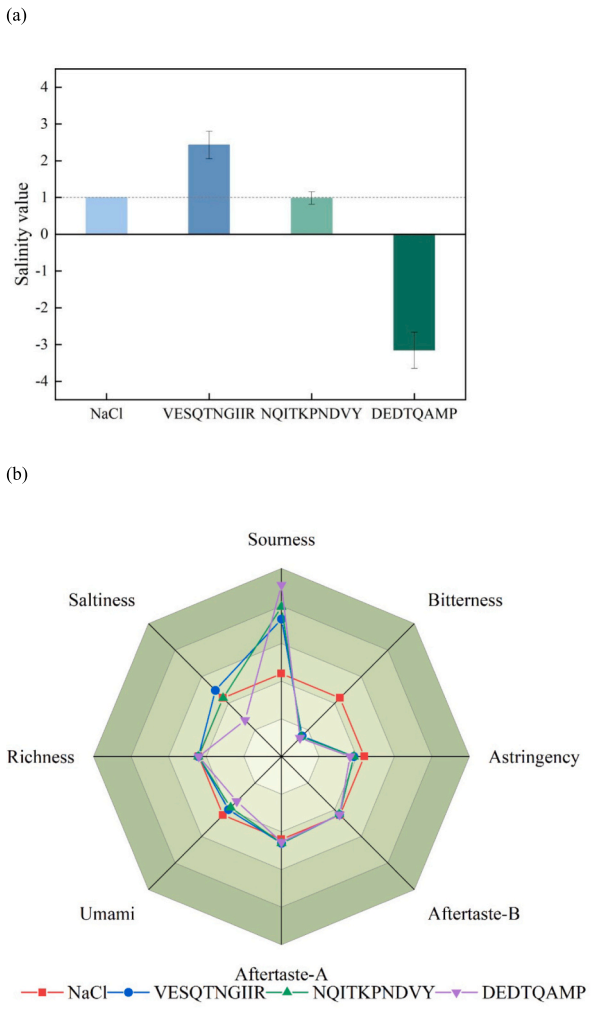

Fig. 2. Electronic tongue taste properties of synthetic peptides with 0.05 mg/ml NaCl as reference. (a) saltiness characteristics of synthetic peptides and NaCl, (b) taste characteristics of synthetic peptides and NaCl.

overnight at 37 °C with 5 % CO2. After the cells were attached to the wall, the cell culture medium was replaced with that containing peptides VESQTNGIIR, NQITKPNDVY, and DEDTQAMP at a concentration of 50 μg/mL, and a blank control group was set up with four replicate wells in each group. After 6 h, 12 h, 24 h and 48 h of culture, 10 μL of CCK-8 reagent was added, and the culture was continued for 1 h. The OD value of each well was detected at 450 nm using an enzyme marker and statistically analyzed.

$$\text{Cell viability}(\%) = \frac{\text{Experimental group OD value} - \text{Blank group OD value}}{\text{Control group OD value} - \text{Blank group OD value}} \times 100\%$$

2.7. Electronic tongue detection of taste characteristics

The SA402B electronic tongue (INSENT, Kanagawa, Japan) was employed to determine the taste characteristics of potential saltiness-enhancing peptides. The instrument has five taste probes for collecting taste information for umami, saltiness, sour, bitter and astringent tastes. A tasteless sample containing 30 mM potassium chloride and 0.3 mM tartaric acid was used as a reference and sodium chloride was chosen as a positive control for the experiment. The synthetic peptide was prepared into 0.05 mg/mL solution. The peptides solution was mixed with the appropriate proportion of NaCl solution, and three experimental groups of 25 % salt reduction, 35 % salt reduction and 45 % salt reduction were prepared respectively. The electronic tongue needs to be cleaned and calibrated prior to operation and was programmed as follows: sample collection for 30 s, solution adjustment for 30 s and cleaning for 330 s (Zhu et al., 2020). Each sample was analyzed a total of four times, deleting the first data where the measurements were unstable, and finally taking the last three data to evaluate the saltiness enhancement effect using a linear equation as shown below(D. Chen et al., 2023):

$$\text{saltiness enhancement}\ (\%) = \frac{x2 - x1}{x1} \times 100\%$$

where x_1 is the salt taste intensity of the pure NaCl solution and x_2 is the salt taste intensity of the peptide mixed with NaCl. We used a one-way ANOVA method for analysis of variance.

2.8. Statistical analysis

All experiments were conducted in triplicate and data were expressed as mean ± standard deviation. Origin 2021 and Microsoft Excel 2019 were used for charting. IBM SPSS Statistics 23 was used for one-way analysis of variance. Data were considered significantly different when the P values < 0.05.

3. Results and discussions

3.1. Virtual screening of saltiness/saltiness enhancing peptides

Ovalbumin contained 386 amino acids, were virtually digested to yield a total of 64 peptides. Besides, the water solubility of peptides is also a key factor affecting their absorption in the human body. By predicting the solubility of peptides, 41 peptides with good water solubility were selected. Toxicity is also a major issue for the development of active peptides. Mutagenicity, DTP, and GPMT are the key problems in drug development, which also apply to the development of active peptides(Kazius, McGuire, & Bursi, 2005; Sazonova, Chesnokov, Zhivotovsky, & Kopeina, 2022; Shiraishi et al., 2021; White, Mueller, Gallavan, Aaron, & Wilson, 2003; Xu et al., 2012). After prediction, 25 water-soluble and non-toxic peptides were finally screened for molecular docking. The structure model of TMC4 was successfully constructed, the PLDDT of rank1 was 78.6, and the model availability was good. The CDOCKER-Energy scores of the 25 peptides successfully docked with TMC4 are shown in Table 1. The lower CDOCKER-Energy scores, the higher the likelihood that the peptide will attach to TMC4, allowing for a more favorable conformation(H. Chen, Chen, Zheng, Xiang, & Xu, 2022). The peptides VESQTNGIIR (−155.158 kcal/mol), NQITKPNDVY (−151.167 kcal/mol), and DEDTQAMP (−147.081 kcal/mol) had lower CDOCKER-Energy scores than the other peptides. The CDOCKER-Energy scores of the published saltiness/saltiness enhancing peptides KEMQKN, DIQPEER, and RGEPNND to TMC4 were − 129.532, −124.653, and − 123.084 kcal/mol, respectively(D. Chen et al., 2023; Xie et al., 2023). The CDOCKER-Energy scores of known saltiness/saltiness enhancing peptides are all higher than VESQTNGIIR, NQITKPNDVY, and

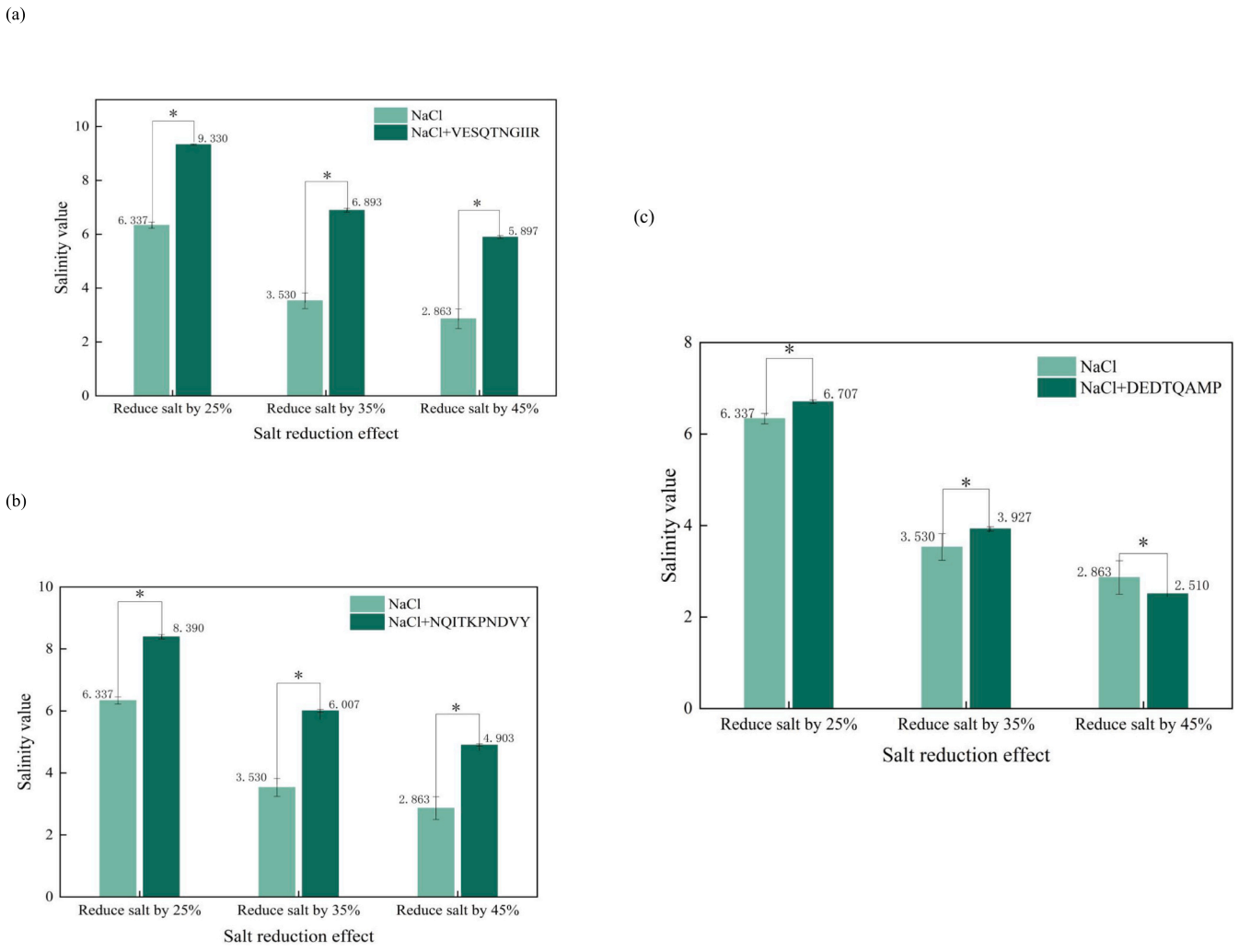

Fig. 3. Salt reduction effect of saltiness/saltiness enhancing peptides. (a) VESQTNGIIR, (b) NQITKPNDVY, and (c) DEDTQAMP. The 35 % salt reduction group has 0.14 mg/ml NaCl, the 45 % salt reduction group has 0.11 mg/ml NaCl, and the 25 % salt reduction group has 0.2 mg/ml NaCl. * ($P<0.01$).

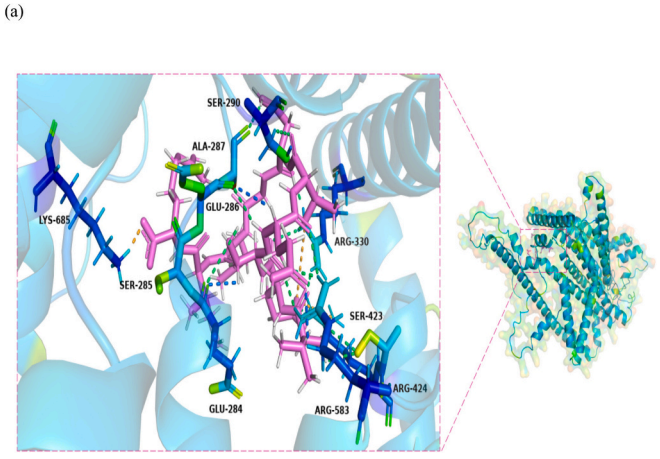

Fig. 4. The docking interactions of peptides with TMC4. (a) 3D and 2D structure of VESQTNGIIR-TMC4 complex, (b) 3D and 2D structure of NQITKPNDVY-TMC4 complex, and (c) 3D and 2D structure of the DEDTQAMP-TMC4 molecular interactions. Blue represents carbon hydrogen bond. Green represents conventional hydrogen bond. Orange represents salt bridge and electrostatic interactions. Pink represents Pi-Alkyl interaction. Light pink represents Alkyl interaction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

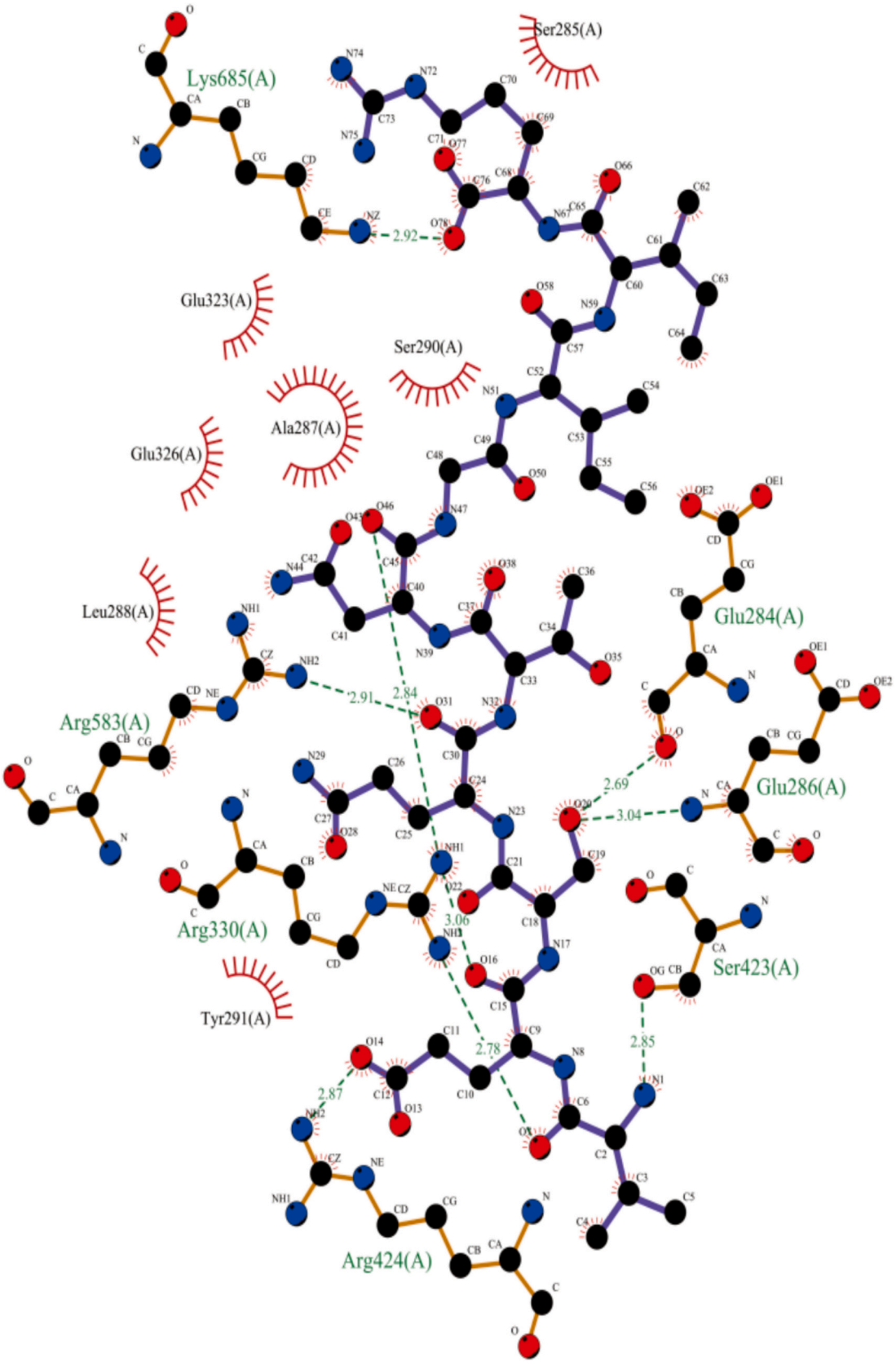

Fig. 4. (*continued*).

(b)

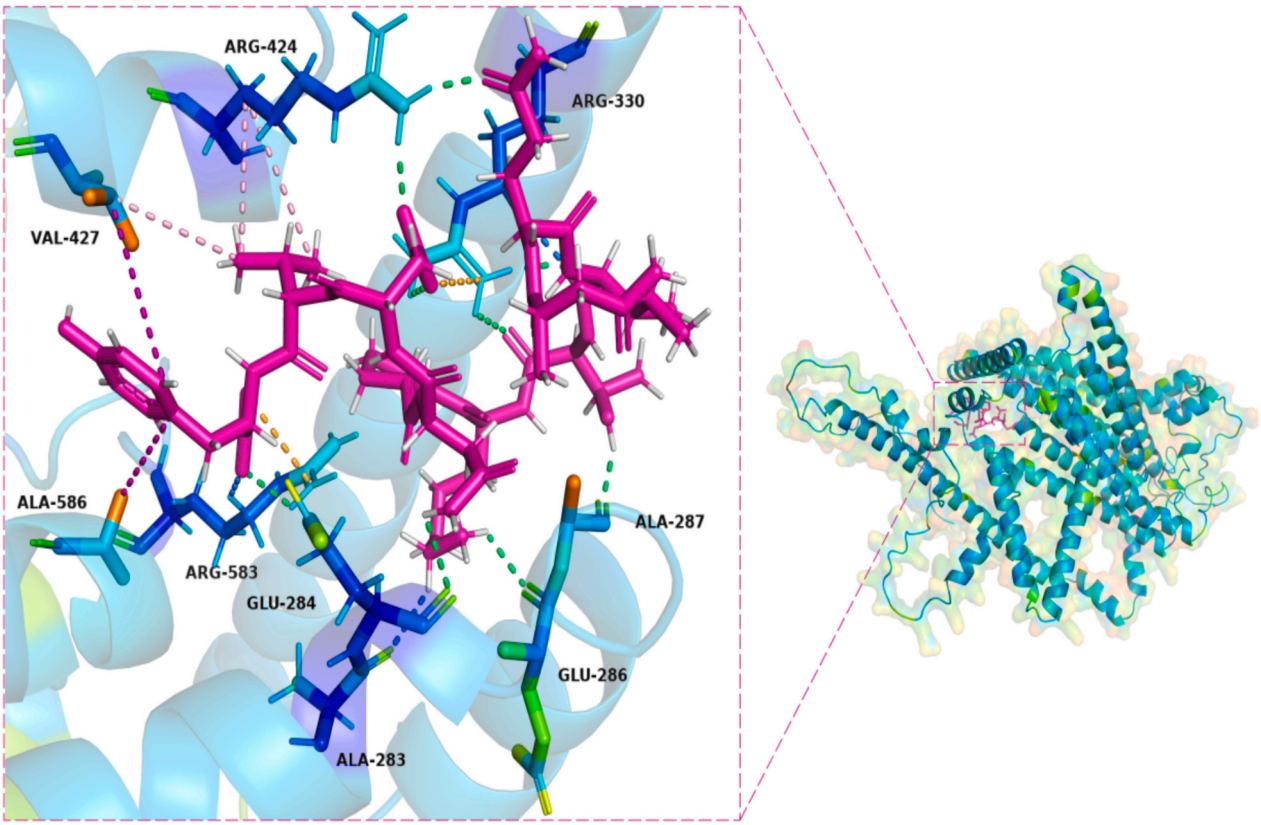

Fig. 4. (*continued*).

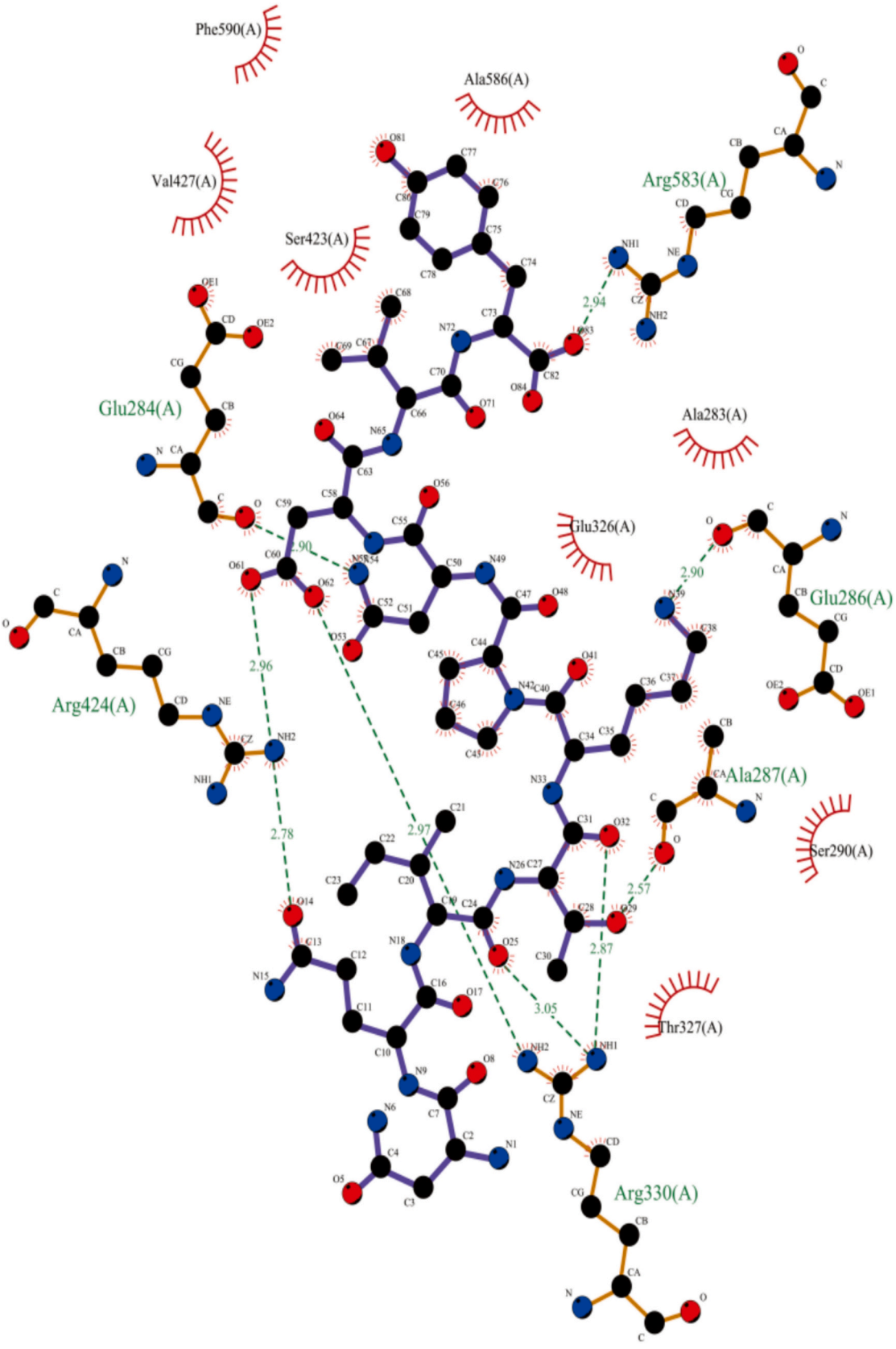

Fig. 4. (*continued*).

(c)

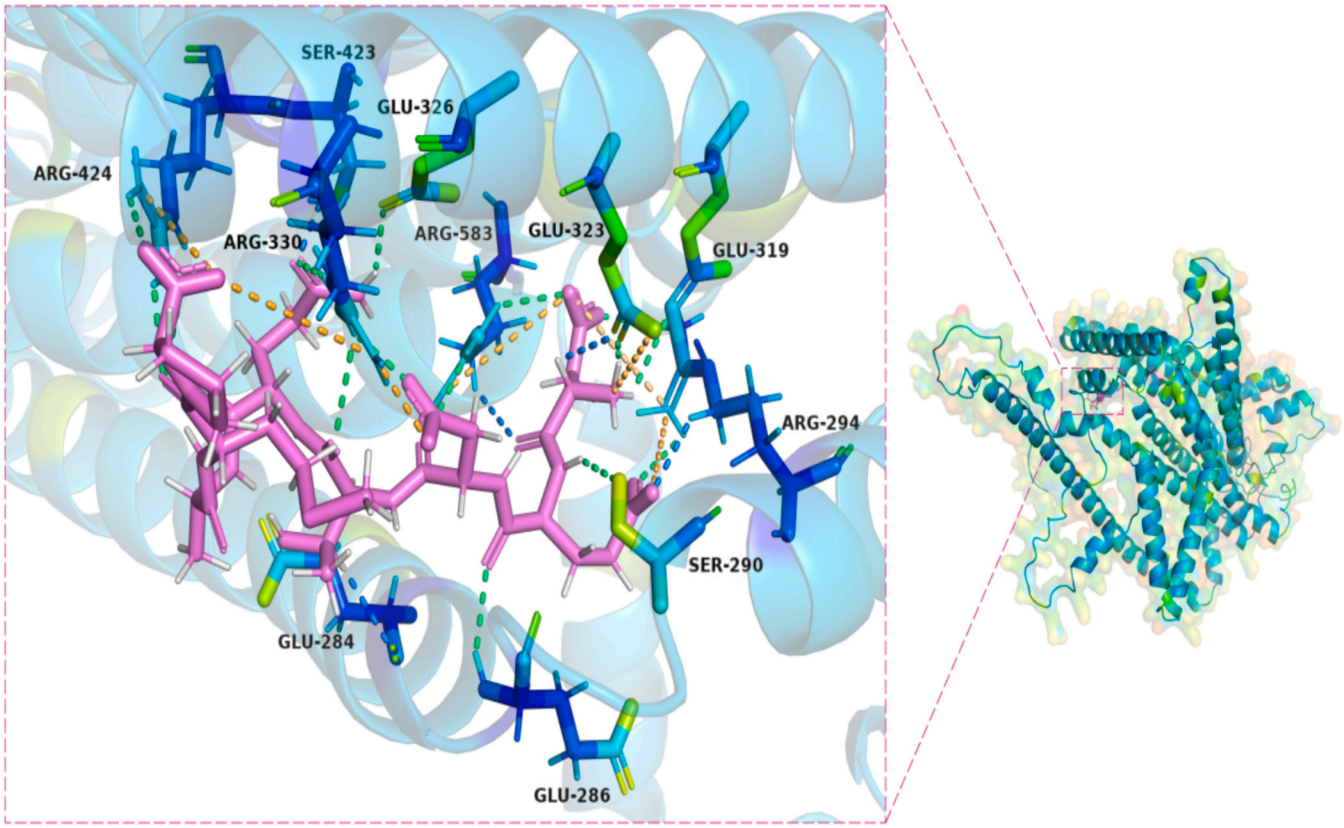

Fig. 4. (*continued*).

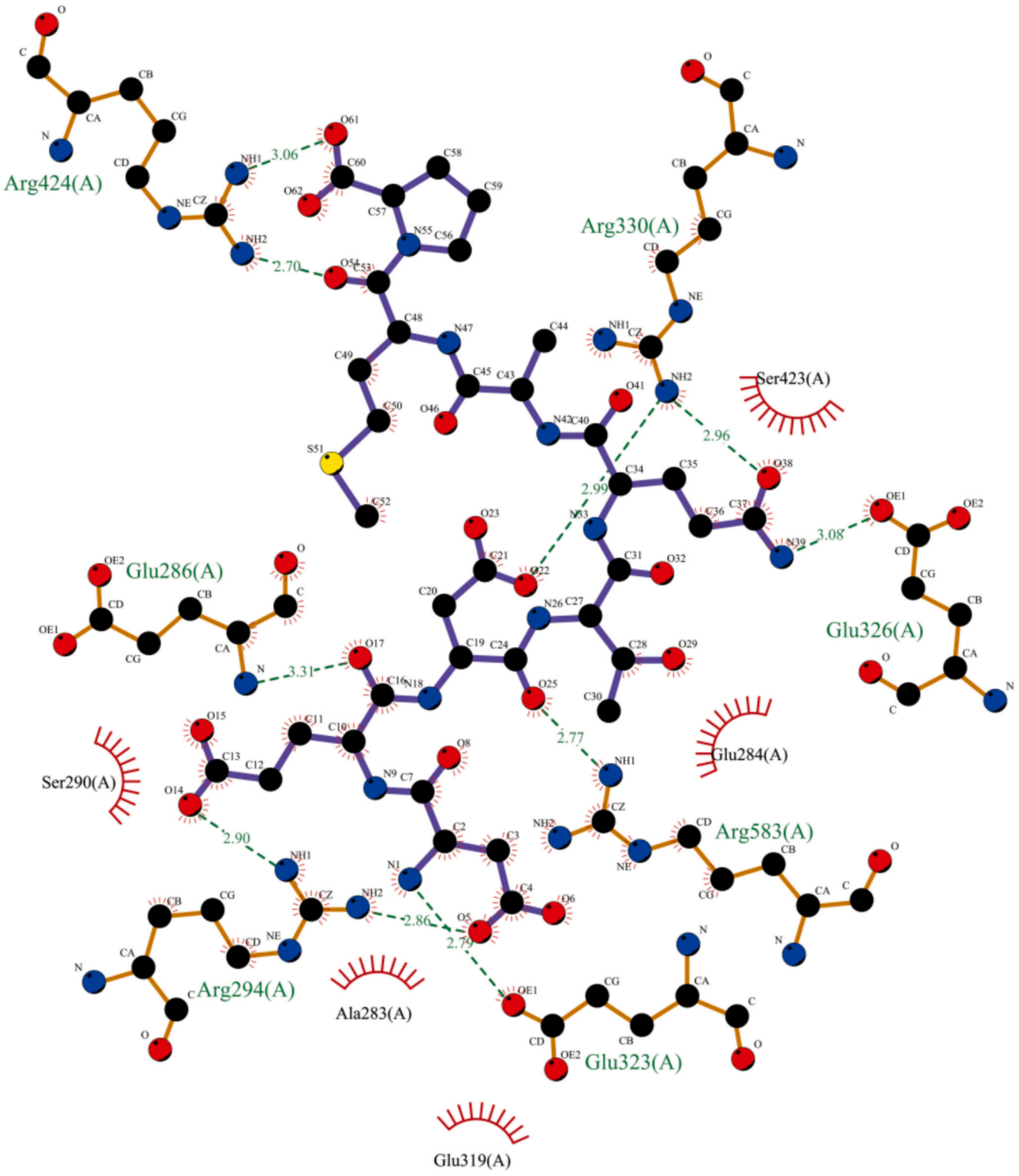

Fig. 4. (*continued*).

DEDTQAMP, suggesting a strong affinity for docking of these three peptides with TMC4. Therefore, peptides VESQTNGIIR, NQITKPNDVY, and DEDTQAMP were synthesized for further electron tongue analysis and their mass spectral results were shown in Fig. S2. Cell viability of peptides VESQTNGIIR, NQITKPNDVY, and DEDTQAMP were performed using the CCK8 method (Fig. 1). The effects of the three peptides on the viability of the two types of cells were not significantly different from those of the blank control group within 48 h, indicating that all three peptides were not cytotoxic and could be used for the further study.

3.2. *Electronic tongue evaluation of saltiness/saltiness enhancing peptides characteristics*

NaCl (0.05 mg/mL) was used as a reference (set to 1.00), with the remaining positive values indicating higher saltiness and *vice versa*. The saltiness values of Potential saltiness/saltiness enhancing peptides were

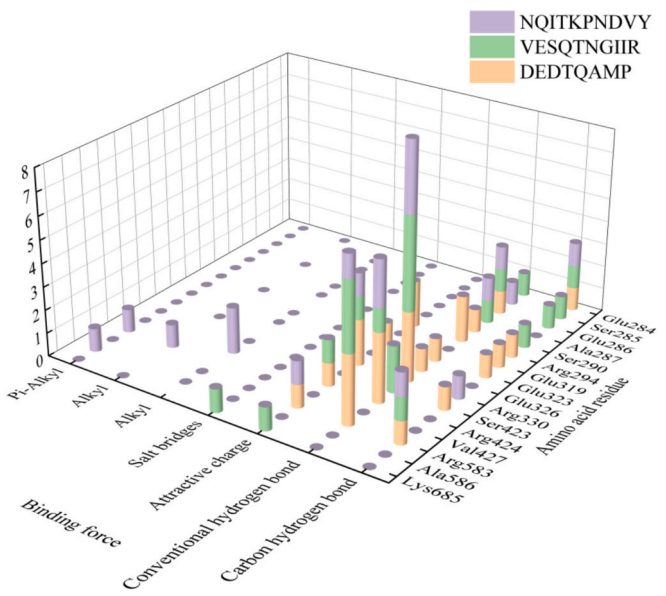

Fig. 5. The number of interactions between ligands and the amino acid residues of TMC4.

evaluated using electronic tongue analysis(shown in Fig. 2a). The saltiness values of VESQTNGIIR, NQITKPNDVY, and DEDTQAMP are 2.43 ± 0.37, 0.98 ± 0.17, and − 3.15 ± 0.49 at a concentration of 0.05 mg/mL, respectively. Peptide VESQTNGIIR has saltiness and its saltiness value is significantly higher than that of NaCl, peptide NQITKPNDVY also has saltiness and its saltiness value is similar to NaCl, peptide DEDTQAMP has no saltiness and its saltiness value is significantly lower than that of NaCl. The bitterness of the three peptides is significantly lower than that of NaCl (Fig. 2b), which can avoid the bitter characteristics of metal salt. The three peptides also exhibited a certain sour taste, which may be due to the introduction of impurities or residual sodium acetate during the peptides synthesis process(J. C. Zhang, Zhang, Liang, Sun, & Zhang, 2023). In the salt reduction experiment, Saltiness enhancement rates of peptide VESQTNGIIR were 47.24 %, 95.28 % and 105.94 % when the salt reduction concentration was 25 %, 35 % and 45 %, respectively, which indicated that it had significant saltiness enhancement effect (Fig. 3a). The saltiness enhancement rates of peptide NQITKPNDVY were 32.40 %, 70.16 % and 71.25 % when the salt reduction concentration was 25 %, 35 % and 45 %, respectively, which indicated that NQITKPNDVY also had a significant saltiness enhancement effect (Fig. 3b). However, because peptide NQITKPNDVY itself is not as salty as peptide VESQTNGIIR, the saltiness enhancement effect of peptide NQITKPNDVY is weaker than that of peptide VESQTNGIIR at the same salt reduction concentration. The peptides VESQTNGIIR and NQITKPNDVY can be defined as saltiness peptides. The saltiness enhancement rates of peptide DEDTQAMP were 5.83 % and 11.24 % at the reduced salt concentration of 25 % and 35 %, indicating that peptide DEDTQAMP had a saltiness enhancement effect at 25 % and 35 % salt reduction (Fig. 3c). However, peptide DEDTQAMP did not show a salt-reducing effect in the experiment at a salt reduction concentration of 45 %. Therefore, peptides VESQTNGIIR and NQITKPNDVY can be considered as novel saltiness/saltiness enhancing peptides in ovalbumin. The saltiness enhancement rates of the three peptides were higher than those of the known peptides KDINNRF, KEMQKN, DIQPEER, and RGEPNND(Xie et al., 2023).

3.3. Mechanism of interaction of enhancing peptides with TMC4

Peptides VESQTNGIIR, NQITKPNDVY, and DEDTQAMP were docked with TMC4 in DS 2017 R2 software and the results were shown in Fig. 4. Their docking scores were 155.158, 151.167, and 147.081 kcal/mol, respectively. The interacting forces between peptides and residues around the active center of TMC4 were conventional hydrogen bond, carbon-hydrogen bond, salt bridges, attractive charge, Alkyl and Pi-Alkyl. The interaction between VESQTNGIIR and TMC4. VESQTNGIIR-TMC4 formed 13 conventional hydrogen bond, 5 carbon hydrogen bonds, 3 attractive charges, and 1 salt bridge(Fig. 4a). Peptide VESQTNGIIR interacts with amino acid residues Glu284, Glu286, Ala287, Arg330, Ser423, Arg424, and Arg583 of TMC4 *via* the conventional hydrogen bond; with residues Glu284, Ser285, Glu286, Ser290, and Arg583 *via* carbon hydrogen bonds; and with residues Arg330, Arg424, Lys685 *via* the attractive charge. In addition, peptide VESQTNGIIR also interacts with residues Lys685 of TMC4 *via* salt bridge. NQITKPNDVY-TMC4 formed 9 conventional hydrogen bonds, 3 carbon hydrogen bonds, 2 attractive charges, 3 Alkyl interactions, and 2 Pi-Alkyl interactions (Fig. 4b). Peptides NQITKPNDVY binds to amino acid residues Ser285, Glu286, Ala287, Arg330, Arg424, and Arg583 of TMC4 to form conventional hydrogen bonds; and to amino acid residues Glu284, Arg330, and Arg583 of TMC4 to form carbon hydrogen bonds and attract charges. In addition, peptide NQITKPNDVY also forms alkyl interactions with residues Arg424 and Val427 and Pi-Alkyl interactions with residues Val427 and Ala586 of TMC4. DEDTQAMP-TMC4 formed 15 conventional hydrogen bonds, 6 carbon hydrogen bonds, and 7 attractive charges (Fig. 4c). The saltiness enhancing peptide DEDTQAMP forms carbon hydrogen bonds with the amino acid residues Glu284, Arg294, Glu323, Ser423, and Arg583 of TMC4, respectively. The amino acid residues of TMC4, Glu286, Ser290, Arg294, Glu319, Glu323, Glu326, Arg330, Arg424, and Arg583 formed conventional hydrogen bonds with the salt-enhancing peptide DEDTQAMP, amino acid residues Arg330, Arg424 and Arg583 conventional hydrogen bonds with peptide DEDTQAMP, respectively. The peptide DEDTQAMP also forms attractive charge interaction with the residues Arg294, Arg330, Glu323, Arg424, and Arg583 of TMC4.

Conventional hydrogen bonds, carbon hydrogen bonds, and attractive charges were formed during the binding of the three peptides to TMC4, with conventional hydrogen bonds. Thus, carbon hydrogen bonds, conventional hydrogen bonds, and attractive charges are important interaction forces for peptide binding to TMC4. Amino acid residues Glu286, Arg330, Arg424 and Arg583 in TMC4 are critical for the binding of saltiness/saltiness enhancing peptide-TMC4 (Fig. 5), which may greatly affect the production of savory taste.

4. Conclusion

Three novel saltiness/saltiness enhancing peptides without cytotoxic, *i.e.,* VESQTNGIIR, NQITKPNDVY, and DEDTQAMP, were identified from the ovalbumin. The results of the electronic tongue verified that saltiness values of VESQTNGIIR, NQITKPNDVY, and DEDTQAMP are 2.43 ± 0.37, 0.98 ± 0.17, and − 3.15 ± 0.49, respectively, at the concentration of 0.05 mg/mL. The saltiness enhancement rates of peptide VESQTNGIIR were 47.24 %, 95.28 % and 105.94 % when the salt reduction concentration was 25 %, 35 % and 45 %, respectively. The saltiness enhancement rates of peptide NQITKPNDVY were 32.40 %, 70.16 % and 71.25 % when the salt reduction concentration was 25 %, 35 % and 45 %, respectively. The saltiness enhancement rates of peptide DEDTQAMP were 5.83 % and 11.24 % at the reduced salt concentration of 25 % and 35 %, respectively. Peptides VESQTNGIIR and NQITKPNDVY are saltiness peptides, and peptide DEDTQAMP is saltiness enhancing peptide. The peptides VESQTNGIIR, NQITKPNDVY, and DEDTQAMP could bind tightly to TMC4, amino acid residues Glu286, Arg330, Arg424, and Arg583 might play a key role. In addition, conventional hydrogen bonds, carbon hydrogen bonds, and attractive charges are key forces in peptides-TMC interactions. These results contribute to a better understanding of the saltiness properties of peptides VESQTNGIIR, NQITKPNDVY, and DEDTQAMP and the responding taste enhancement mechanism. This study also provides a feasible and efficient way to develop reduced-salt flavoring products using egg-based

ingredients.

CRediT authorship contribution statement

Yaxin Cao: Writing – original draft, Methodology, Formal analysis, Data curation. **Linyuezhi Yan:** Writing – original draft, Validation. **Di Liu:** Validation, Software. **Qian Zhang:** Validation, Software. **Wenzhu Zhao:** Validation, Supervision, Software. **Lin Yuan:** Validation, Software. **Yiding Yu:** Writing – review & editing, Validation, Investigation, Conceptualization. **Zhipeng Yu:** Writing – review & editing, Validation, Supervision, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102261.

Data availability

Data will be made available on request.

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