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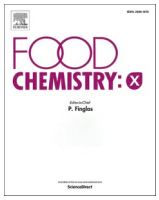

Study on the structure–activity relationship of rice immunopeptides based on molecular docking

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ABSTRACT

Research on food-derived immunoregulatory peptides has attracted increasing attention of scientists worldwide. However, the structure–activity relationship of rice immunopeptides was not clearly. Herein, 114 rice immunopeptides were obtained by simulating the enzymatic hydrolysis of rice proteins and were further analyzed by NetMHCIipan-4.0. Subsequently, the molecular docking was used to simulate the binding of immunoreactive peptides to major histocompatibility complex class II (MHC-II) molecules. Results show that S, R, D, E, and T amino acid could easily form hydrogen bonds with MHC-II molecules, thus enhancing innate and adaptive immunity. Finally, glucose-modified rice immunopeptides were to investigate the binding of the peptides with MHC-II molecules after glycosylation modification; this provided a theoretical basis for the targeted modification of the generated immunopeptides. All in all, the present study provides a theoretical foundation to further utilize rice processing byproducts and other food products to enhance immunity.

1. Introduction

Rice is the largest staple food crop in China, with an annual production of approximately 210 million tons. The protein content of rice processing byproducts accounts for approximately 8 % (Qu et al., 2022). After enzymolysis, the obtained rice proteins contain many bioactive peptides that exert multi-function of reducing blood pressure (Yang, Wang, Zhang, & Ma, 2020), showing antioxidative activity (Chen et al., 2021), and improving immunity (Zhang, Ni, Ou, Cao, Cheng, & Wen, 2023). Different from other plant proteins, such as soy protein, rice protein is a preferred food for some special people, like baby (Dupont et al., 2020). Immunopeptides are a series of biologically active peptides with an immunoregulatory function (Kote, Pirog, Bedran, Alfaro, & Dapic, 2020). Major histocompatibility complex (MHC) class II molecules present peptides derived from exogenous or membrane-binding proteins to $CD4^+$ T cells. These antigens are internalized through endocytosis or pinocytosis and are processed by a resident protease to produce peptides with 10–25 amino acids (aa), which are loaded onto MHC-II molecules. The resulting peptide–MHC complex is then exported to the cell surface, where it interacts with the T cell receptor of $CD4^+$ T cells and initiates their effects and regulatory functions. Activated $CD4^+$ T helper cells also interact with B cells to stimulate the production of IgG antibodies (Roche & Furuta, 2019). Presently, because of the lack of research on the interaction between immunopeptides and MHC-II molecules, the structure–activity relationship of peptides is unclear.

The traditional method to obtain immunogenic peptides is to use proteases such as pepsin and trypsin for digestion. Then, the enzymatic hydrolysis products are identified by mass spectrometry. Their immunogenicity function is further confirmed via cell experiments. The whole process is not only time consuming and laborious, but also it has a low screening flux for desired peptides. By contrast, computer-aided screening not only save time and financial costs, but also have higher throughput (Sayd et al., 2018). Presently, protein sequences are published in the National Center for Biotechnology Information (NCBI), Universal Protein, and other databases. Enzymolysis simulation tools include ExPASy PeptideCutter (https://web.expasy.org/peptide_cutter/), BIOPEP (Nongonierma & FitzGerald, 2018) (https://biochemia.uwm.edu.pl/biopep-uwm/), and Rapid Peptides Generator software (Cox & Mann, 2008).

Molecular docking involves the study of the optimal binding conformation and interaction mode between molecules based on the principle of geometric shape and energy matching of ligands and

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receptors (Śledź & Caflisch, 2018). Molecular docking can be classified into rigid docking (Desta, Porter, Xia, Kozakov, & Vajda, 2020), semi-flexible docking (Kurcinski, Badaczewska-Dawid, Kolinski, Kolinski, & Kmiecik, 2020), and flexible docking (Ravindranath, Forli, Goodsell, Olson, & Sanner, 2015) according to whether the receptor and ligand can rotate to change their conformation. Presently, researchers have developed several docking tools and programs, among which Autodock Vina is widely used because of its fast docking speed and ease of operation (Pozzi, Di Pisa, Benvenuti, & Mangani, 2018). Molecular docking and molecular dynamics simulation can be used to screen and optimize peptide structures and predict interactions between various molecules (Skariyachan, Khangwal, Niranjan, Kango, & Shukla, 2021). The application of computational and network pharmacological analyses can reduce the time and cost of screening bioactive peptides and reveal their potential mechanisms of action.

The ε-amino group is a reduced sugar carbonyl group in food that undergoes dehydration and condensation reaction to produce *N*-substituted glycosylamine compounds. Glycosylation can occur spontaneously, and its modification can improve the functional properties of food proteins/peptides (Yang et al., 2022). For example, glycosylation modification can improve the immunogenic activity of peptides from protein enzymolysis (Chun & Lee, 2022). However, because of inadequate studies on the structure–activity relationship of rice immunopeptides, the immunogenic activity of glycosylated rice peptides is not well elucidated.

In the present paper, the interaction between peptides and MHC-II molecules was analyzed based on the data obtained from *in silico* peptide screening and molecular docking, and the structure–activity relationship of rice immunopeptides was preliminarily described; these findings can lay the foundation for more efficient screening of immunopeptides in the future.

2. Materials and methods

The amino acid sequence of the rice protein was obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/). The critical enzymes pepsin (EC 3.4.23.1) and trypsin (EC 3.4.21.4) were selected for the simulation of protein hydrolysis (Sensoy, 2021). *In silico* digestion of rice protein was performed using the R script, which can hydrolyze many proteins within a short time (Supplementary material 1).

The MHC-II molecule has an open binding cleft for interacting with peptides of a vast length range, most commonly of 13–25 aa (Chicz et al., 1992). In the present study, peptides with a length of 10–30 aa were selected. NetMHCIIpan 4.0 was used to screen the peptide sequences obtained by *in silico* digestion, and the predicted binding value of the peptide sequence was expressed as %Rank values by referring to the predicted affinity ranking of the peptides. The prediction conditions were as follows: 677 types of MHC-II molecule loci, including 672 from humans (i.e., 607 HLA-DRB1, 32 HLA-DRB3, 6 HLA-DRB4, 15 HLA-DRB5, 6 HLA-DP, and 6 HLA-DQ epitopes) and five from mice (i.e., epitopes H-2-IAb, H-2-IAd, H-2-IEd, H-2-IAu, and H-2-IEk), were chosen for prediction. A %Rank value of $\leq$1 indicated strong binding (SB) to MHC-II molecules, >1 and $\leq$5 weak binding (WB); and >10 no binding; the last category of peptides was filtered out. Peptides showing SB with multiple ($\geq$3) MHC-II molecules were then screened for further investigation.

To determine the mechanism of structural–functional association between MHC-II molecules and peptides, a molecular docking study was performed using AutoDock Vina (AutoDock 1.1.2 Win32). The software ChemDraw19.1 (CambridgeSoft Corp., Cambridge, MA, USA) was used to draw the 2D structure of immunoreactive peptides. Next, Chem3D 19.1 was used to open this file. Because the structure of the ligand is in a "tense" state and does not conform to the actual conformation of the polypeptide, it is necessary to calculate the minimum energy state of this structure that conforms to the actual state and then save the file in ". pdb" format. Finally, the ligand was converted into ". pdbqt" format file by using the Open Bable GUI 2.4.1 software. The crystal structure of MHC-II was obtained from the PDB database (PDB ID: 6BIY). PyMOL 1.3 was used to calibrate the HLA structure. AutoDock Tools 1.5.4 were used to remove water and other ions from the ligands and HLAs and add Gasteiger charges. The entire binding slot of HLA was selected as the binding capsule. We then used AutoDock Vina 1.1.2 to cross-pair the ligands to HLA-A * 02:01. These ".pdbqt" files and the corresponding protein ".pdb" files were input into PyMOL to draw graphics in the ". png" format. To improve efficiency, shell script was used to achieve batch molecular docking function. The binding sites of the maximum binding energy were chosen for comparison and visualization.

The ε-amino group is a reduced sugar carbonyl group in food that can undergo dehydration and condensation reaction to produce *N*-substituted glycosylamine compounds. In the present study, 10 peptides with the highest docking energy and K terminal were selected to dock with MHC-II molecules after binding with glucose, and the changes in docking energy and docking site before and after modification were compared.

3. Results

3.1. Simulated hydrolysis of rice proteins

The rice protein sequences in the NCBI database were digested by pepsin and trypsin, and 24,544 and 15,266 peptides were obtained, respectively (Supplementary material 2, Fig. S1). After removing the repeated peptides, 2832 and 2180 specific peptides were obtained. Among them, peptides with 2–10 aa accounted for the highest proportion (82.93 % and 60.44 %, respectively). The proportion of peptides containing 10–30 aa was approximately 25.64 % and 41.70 %, respectively, and the proportion of peptides with other aa lengths was < 10 %. Compared to traditional methods, computer simulation screening has the advantages of high efficiency, economy, and environmental protection. This screening does not produce any chemical compounds harmful to the environment and meets the requirements of green development.

3.2. Prediction of immunoreactive peptide activity

The number of peptides with 10–30 aa length obtained from pepsin and trypsin hydrolysis was 987 and 885, respectively. By using the NetMHCIIpan-4.0 prediction tool, 54 and 60 immunoreactive peptides were screened from simulated enzymolysis peptides obtained following pepsin and trypsin digestion, thus accounting for only 6.2 % of the enzymolysis peptides. Some immunoreactive peptides that strongly bind to MHC-II molecules were listed in Supplementary material 2 (Tables S1 and S2).

As shown in Fig. 1, the length of immunopeptides was 11–20 aa. In the pepsin and trypsin simulated hydrolysates, the proportion of immunoreactive peptides with a length of 13–16 aa was 61.11 % and 60.00 %, respectively. Therefore, this study combined immunoinformatics methods with experimental methods to explore new methods for batch searching and controllable preparation of active peptides; this approach opens up a new avenue for screening food-derived immunoreactive peptides. For example, Wen et al. successfully screened immunoregulatory active peptides by combining NetMHCIIpan-4.0 prediction with *in vitro* cell experiments (Wen et al., 2021). Peaman et al. used the BIOPEP database and the Peptide Ranker tool to screen peptides with antioxidant activity (Pearman, Ronander, Smith, & Morris, 2020).

3.3. Study of the conformational relationship of rice immunoreactive peptides

The ligand peptides were found to bind to MHC-II molecules mainly through α-aa (9GLN, 53SER, 55GLU, 62ASN, and 69ASN) and β-aa

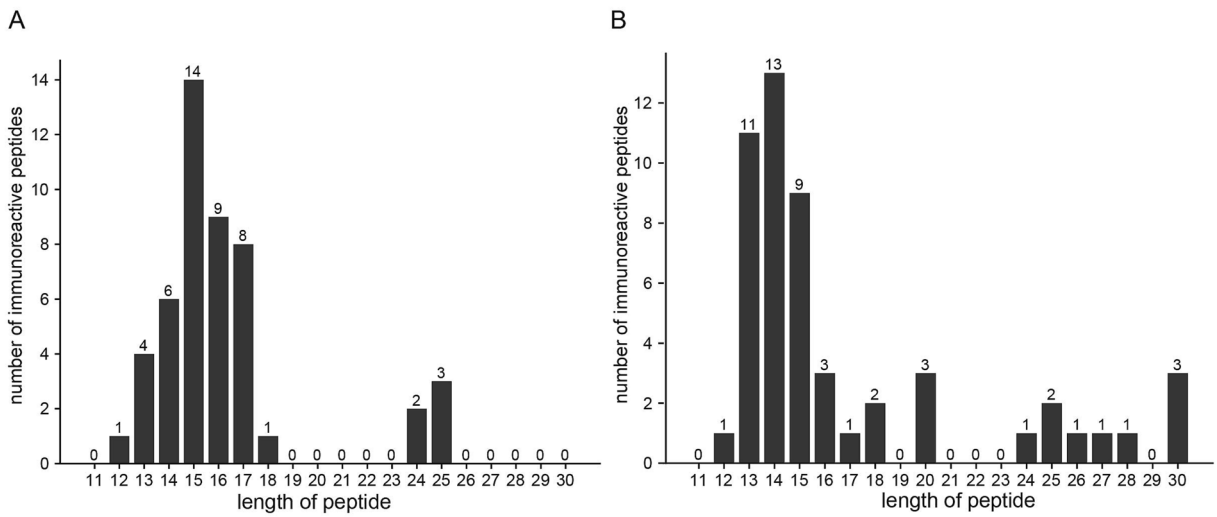

Fig. 1. Length distribution of amino acid sequences of immunoreactive peptides obtained by simulated enzymatic digestion. A: pepsin; B: trypsin.

(61TRP, 70GLN, 71ARG, 81HIS, and 82ASN) residues of the chain with the formation of a hydrogen bond. Table S3 in Supplementary material 2 shows the sequence of the binding frequency of ligand aa and MHC-II molecular aa residues. The binding frequency of S, R, D, E, and T with most amino acids was high. Cian et al. used endopeptidase and exopeptidase to hydrolyze the seaweed *Porphyra columbina* to obtain the hydrolysate (*Pc* RH), which was rich in D, A, and E aa and could promote the proliferation of rat lymphocytes (Cian, López-Posadas, Drago, Sánchez de Medina, & Martínez-Augustin, 2012). Yang et al. obtained small peptides from salmon protein that could significantly enhance lymphocyte proliferation and natural killer cell activity, and these peptides were rich in D and E aa (Yang et al., 2009). All these findings conform to the abovementioned principle.

3.4. Glycosylation modification study of rice immunopeptides

Table S4 in Supplementary material 2 shows the changes in the binding ability of immunoreactive peptides to MHC-II molecules before and after glucose binding. Following the modification with glucose, the docking energy of the peptide and MHC-II molecule changed, with both an increase and decrease in the docking energy observed. These results indicate that glycosylation modification can alter the binding of the peptide to the MHC-II molecule. Fig. 2 shows the docking diagram between the GY-15 peptide and MHC-II molecules before and after glycosylation. Glycosylated ligands altered the configuration and spatial extension of MHC-II molecules, and the binding sites of peptides and MHC-II molecules as well as the sites for hydrogen bond formation were changed. The docking energy of the peptide and MHC-II molecules before and after modification decreased from −8.9 kcal/mol to −11.4 kcal/mol, thus indicating that they were more closely bound. The modification following glucose binding changed the binding mode of the peptide and MHC-II molecules and enhanced their binding force. In future studies, glycosylation can be used to modify immunoreactive peptides to enhance their immunogenic activity. Sandra et al. (van Vliet, García-Vallejo, & van Kooyk, 2008) reported that mannose receptor-mediated antigen uptake could effectively enhance the antigen capture ability of dendritic cells and improve the restricted antigen presentation of MHC-II molecules. A lactose-modified enzymatic peptide mixture of lactoglobulin can stimulate RAW 264.7 macrophages to produce carbon monoxide and increase the mRNA expression of immune-related cytokines, which has a potential immune enhancement effect (Chun & Lee, 2022). Thorsing et al. confirmed that glycosylated vaccines had a stronger immune response to enterotoxigenic *Escherichia coli* than nonglycosylated vaccines (Thorsing et al., 2021).

Fig. 3 shows the docking diagram of the GW-2 peptide with MHC-II molecules before and after glycosylation. The binding sites of glycosylated peptides to MHC-II molecules changed, and the number of hydrogen bonds decreased from 10 to 4 (Fig. 3). The docking energy of glycosylated-modified GW-2 with MHC-II molecules increased from −12.0 kcal/mol to −9.8 kcal/mol (Table S4 in Supplementary material 2). Glycosylation modification can also reduce the binding force between the peptide and MHC-II molecules; hence, this principle can be used to reduce the immunogenic activity of some protein antigens. Stowers et al. used the lethal parasite *Plasmodium falciparum* to infect monkeys inoculated with the MSP-1 vaccine and found that non-glycosylated MSP-1 induced a more effective immune response than glycosylated MSP-1 (Stowers et al., 2002). Another study showed that the use of sugar side chains of glycoproteins to cover some protein mutation sequences could also play a role in altering the immunogenicity of proteins by avoiding immune surveillance (Quarmby, Phung, & Lill, 2018).

In conclusion, glycosylation modification has two possible effects on the immunogenic activity of peptides. The molecular docking software can quickly determine the specific changes and effectively guide to conduct subsequent cell or animal experiments.

4. Discussion

MHC-associated peptide proteomics (MAPP) is a powerful tool to identify the immunogenicity hotspots of biological therapeutic drugs (Quarmby et al., 2018). MAPP can be used to understand the immunogenicity of proteins, compare candidate biological therapeutic drugs, guide the de-immunization process, and study the observed immunogenicity (Karle, 2020). However, this technology is expensive and time-consuming, and the sensitivity of the results is limited by sample size, the MHC library of the tested donor queue, and the experimental procedure. Our previous study confirmed that the bioinformatics prediction website (NetMHCIIpan-4.0) could be used for practical and reliable immunopeptide screening (Karle, 2020). In the present study, we screened 114 peptides by using this method.

The peptide–MHC-II complex with a high affinity can more effectively initiate the T cell response and requires fewer antigen-presenting cells (APCs) and T cells, which may induce an immune response faster than that achieved with the low-affinity peptide–MHC-II complex to combat inflammation (Ochoa, Laio, & Cossio, 2019). In the present study, 114 peptides were individually docked with MHC-II molecules through molecular simulation, and 30 peptides than the highest affinity were screened out. S, R, D, E, and T showed high binding frequencies

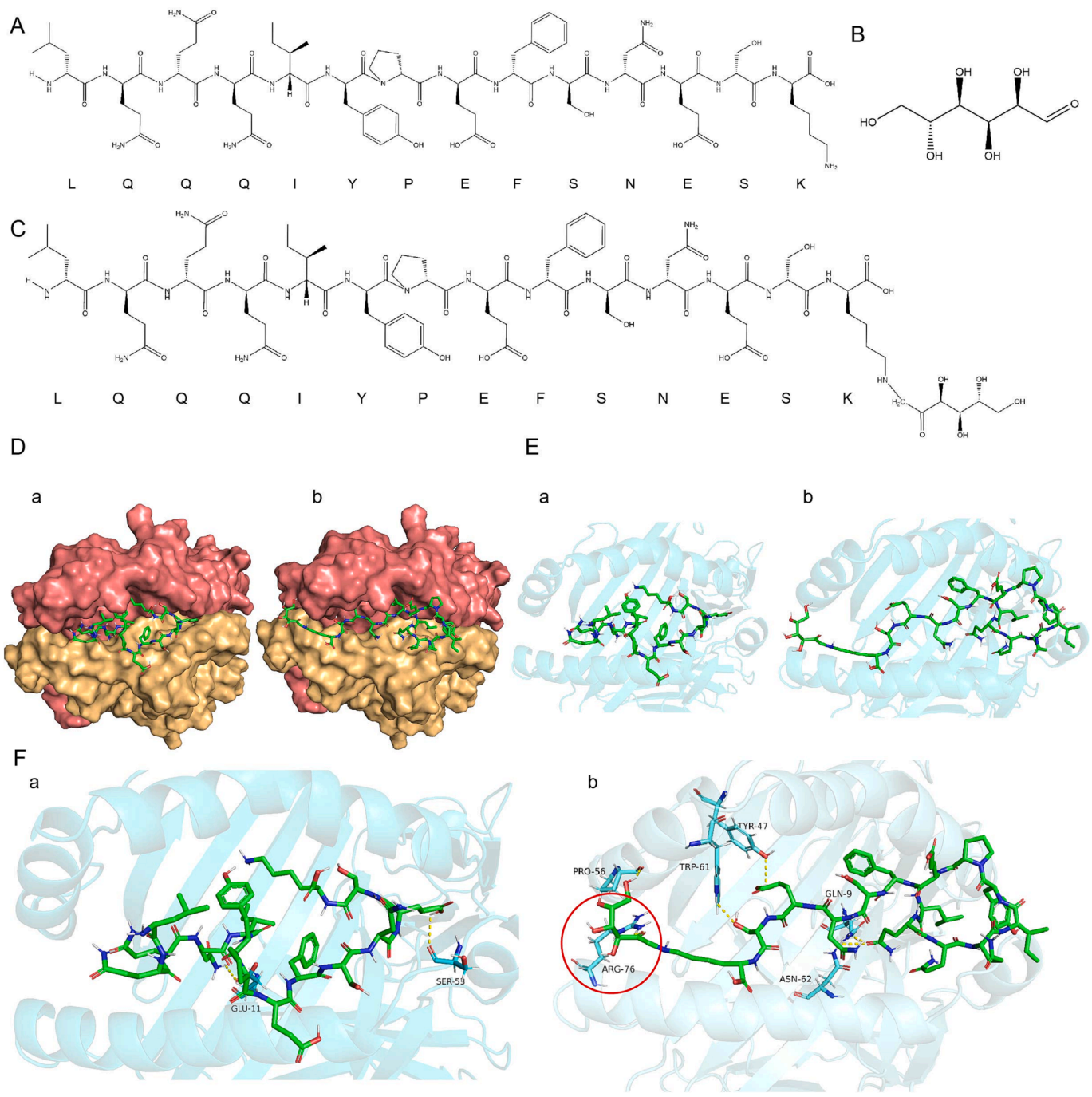

Fig. 2. Simulated docking diagram of the GY-15 peptide with MHC-II molecules before and after glucose modification. A: Chemical structure of the GY-15 peptide; B: Chemical structure of glucose; C: Chemical structure of the GY-15 peptide after glucose binding; D and E: Diagram of GY-15 peptide binding to MHC-II molecules before (a) and after (b) binding to glucose; F: Hydrogen bonds formed by the GY-15 peptide before (a) and after (b) binding to MHC-II molecules. Red circles indicate glucose molecules.

with most amino acids. The binding energy of the peptide and the MHC-II molecule changed following the modification with glucose binding. This principle can be used to enhance or decrease immunity in accordance with our practical requirements. For instance, the immunogenicity of vaccines can be improved by glycosylation modification.

To date, few MHC-II-associated naturally occurring glycopeptides have been sequenced. In 1993, Chicz et al. (1993) first reported glycopeptides naturally presented by class II human leukocyte antigen (HLA). However, because of the shortage of naturally occurring glycopeptides described in the literature, several assumptions were made regarding how protein glycosylation affected the recognition of epitopes by T cells. First, glycopeptides may affect the enzymatic cleavage of glycoproteins used for antigen processing and/or *T*-cell recognition; however, the mechanism remains unclear. Second, glycosylated residues can affect peptide–MHC binding directly or by affecting the conformation of the peptide-binding region (Harding, Kihlberg, Elofsson, Magnusson, & Unanue, 1993). Finally, the sugar fraction can directly contact TCR and affect its activity. Presently, there are relatively few research studies on glycosylated-modified peptides. The combination of immunoinformatics and mass spectrometry technology will enable to easily recognize MHC-bound peptides, which will be conducive to conduct more research on the structure, function, and immunological correlation of glycosylated-

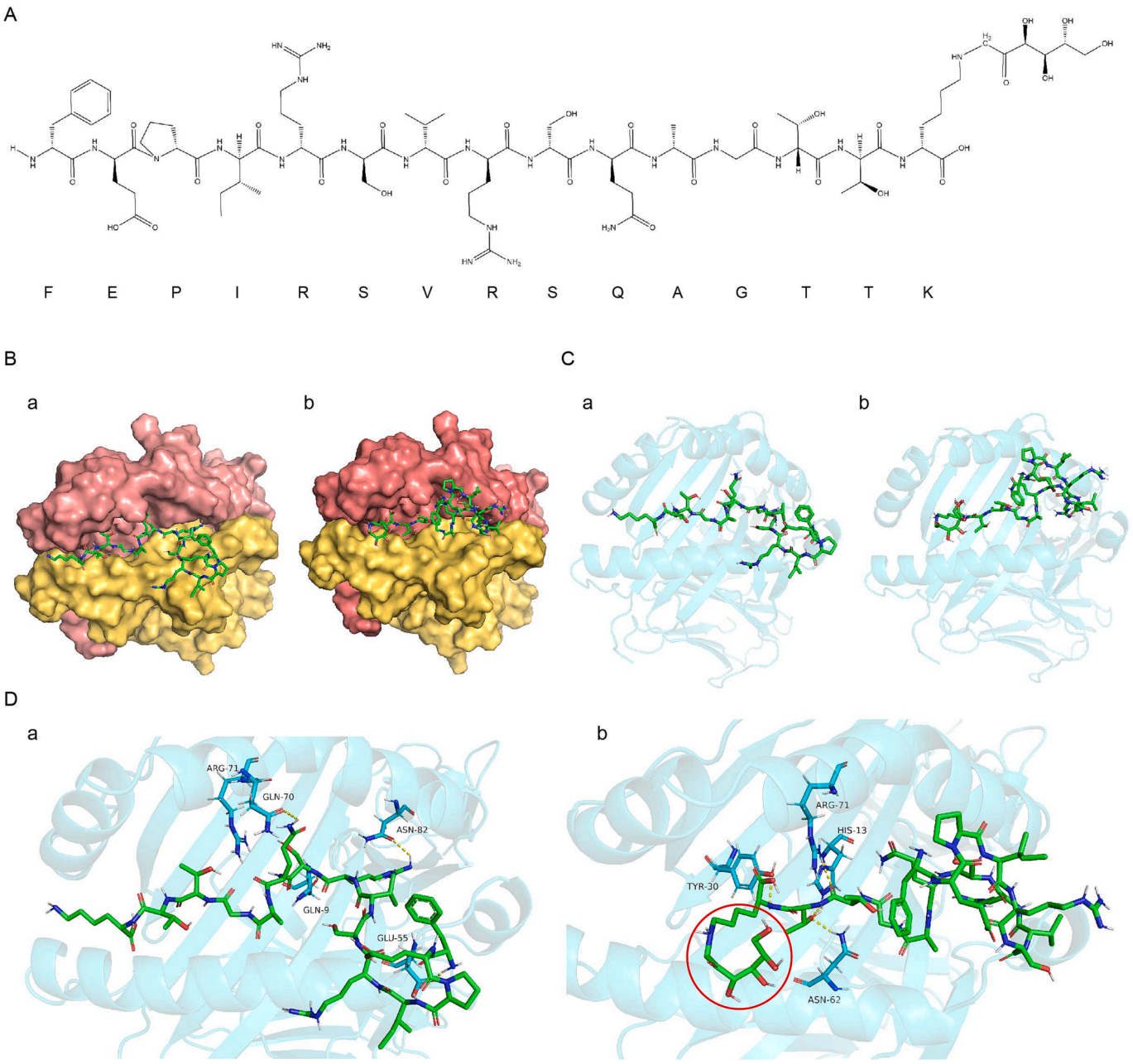

Fig. 3. Diagram of mock docking of the GW-2 peptide before and after glucose binding with MHC-II molecules. A: Chemical structure of GW-2 after glucose binding; B and C: Diagram of GW-2 binding to MHC-II molecules before (a) and after (b) glucose binding; D: hydrogen bond formed between GW-2 and MHC-II molecules before (a) and after (b) glucose binding; the bound glucose is shown by a red circle.

modified peptides and expand our understanding of these peptides.

In the future, we can combine proteomics, transcriptomics, and genomics technologies to dynamically analyze the changes in protein and gene expression of food-derived peptides involved in immune regulation and to investigate the interrelationship between these key genes by using omics tools to construct a dynamic network of cells involved in the regulation of food-derived immunopeptides.

5. Conclusions

In the present study, the rice protein sequences available in the NCBI database were enzymatically digested using R language script, and 5012 specific peptides were obtained. Next, by using NetMHCIIpan-4.0, 114 immunopeptides that could strongly bind to MHC-II molecules were screened. Autodock Vina was then used to dock the immunopeptides with MHC-II molecules. The analysis of their binding energy and binding sites revealed that S, R, D, E, and T aa could easily form hydrogen bonds with MHC-II molecules, thus improving the affinity of peptide-MHC-II and the stability of the complex and ultimately enhancing innate and adaptive immunity and improving the immune response of the body. Finally, molecular docking showed that the docking energy of glycosylated rice immunoreactive peptides and MHC-II molecules will change; moreover, the binding site also changed, thereby altering the immunogenic activity of rice immunoreactive peptides.

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CRediT authorship contribution statement

Fan Zhu: Conceptualization, Resources, Software, Validation, Visualization, Writing – original draft. **Shuwen He:** Methodology, Validation. **Ce Ni:** Formal analysis. **Ying Wu:** Validation. **Hao Wu:** Data curation, Writing – review & editing, Funding acquisition. **Li Wen:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing.

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.

Data availability

The authors do not have permission to share data.

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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.2024.101158.

References

Chen, H. J., Dai, F. J., Chen, C. Y., Fan, S. L., Zheng, J. H., Huang, Y. C., & Chen, C. S. (2021). Evaluating the antioxidants, whitening and antiaging properties of rice protein hydrolysates. *Molecules, 26*(12). https://doi.org/10.3390/molecules26123605. Article 3605.

Chicz, R. M., Urban, R. G., Gorga, J. C., Vignali, D. A., Lane, W. S., & Strominger, J. L. (1993). Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles. *Journal of Expermental Medicine, 178*(1), 27–47. https://doi.org/10.1084/jem.178.1.27

Chicz, R. M., Urban, R. G., Lane, W. S., Gorga, J. C., Stern, L. J., Vignali, D. A., & Strominger, J. L. (1992). Predominant naturally processed peptides bound to HLA-DR1 are derived from MHC-related molecules and are heterogeneous in size. *Nature, 358*(6389), 764–768. https://doi.org/10.1038/358764a0

Chun, S. H., & Lee, K. W. (2022). Immune-enhancing effects of β-lactoglobulin glycated with lactose following in vitro digestion on cyclophosphamide-induced immunosuppressed mice. *Journal Dairy Science, 105*(1), 623–636. https://doi.org/10.3168/jds.2021-20681

Cian, R. E., López-Posadas, R., Drago, S. R., Sánchez de Medina, F., & Martínez-Augustin, O. (2012). A porphyra columbina hydrolysate upregulates IL-10 production in rat macrophages and lymphocytes through an NF-κB, and p38 and JNK dependent mechanism. *Food Chemistry, 134*(4), 1982–1990. https://doi.org/10.1016/j.foodchem.2012.03.134

Cox, J., & Mann, M. (2008). MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nature Biotechnology, 26*(12), 1367–1372. https://doi.org/10.1038/nbt.1511

Desta, I. T., Porter, K. A., Xia, B., Kozakov, D., & Vajda, S. (2020). Performance and its limits in rigid body protein-protein docking. *Structure, 28*(9), 1071–1081. https://doi.org/10.1016/j.str.2020.06.006

Dupont, C., Bocquet, A., Tomé, D., Bernard, M., Campeotto, F., Dumond, P., & Kalach, N. (2020). Hydrolyzed rice protein-based formulas, a vegetal alternative in Cow's milk allergy. *Nutrients, 12*(9). https://doi.org/10.3390/nu12092654. Article 2654.

Harding, C. V., Kihlberg, J., Elofsson, M., Magnusson, G., & Unanue, E. R. (1993). Glycopeptides bind MHC molecules and elicit specific T cell responses. *Journal of Immunology, 151*(5), 2419–2425.

Karle, A. C. (2020). Applying MAPPs assays to assess drug immunogenicity. *Frontiers in Immunology, 11*. https://doi.org/10.3389/fimmu.2020.00698. Article 698.

Kote, S., Pirog, A., Bedran, G., Alfaro, J., & Dapic, I. (2020). Mass spectrometry-based identification of MHC-associated peptides. *Cancers (Basel), 12*(3), Article 535. 10.3390/cancers12030535.

Kurcinski, M., Badaczewska-Dawid, A., Kolinski, M., Kolinski, A., & Kmiecik, S. (2020). Flexible docking of peptides to proteins using CABS-dock. *Protein Science, 29*(1), 211–222. https://doi.org/10.1002/pro.3771

Nongonierma, A. B., & FitzGerald, R. J. (2018). Enhancing bioactive peptide release and identification using targeted enzymatic hydrolysis of milk proteins. *Analytical and Bioanalytical Chemistry, 410*(15), 3407–3423. https://doi.org/10.1007/s00216-017-0793-9

Ochoa, R., Laio, A., & Cossio, P. (2019). Predicting the affinity of peptides to major histocompatibility complex class II by scoring molecular dynamics simulations. *Journal of Chemical Information and Modeling, 59*(8), 3464–3473. https://doi.org/10.1021/acs.jcim.9b00403

Pearman, N. A., Ronander, E., Smith, A. M., & Morris, G. A. (2020). The identification and characterisation of novel bioactive peptides derived from porcine liver. *Current Research in Food Science, 3*, 314–321. https://doi.org/10.1016/j.crfs.2020.11.002

Pozzi, C., Di Pisa, F., Benvenuti, M., & Mangani, S. (2018). The structure of the human glutaminyl cyclase-SEN177 complex indicates routes for developing new potent inhibitors as possible agents for the treatment of neurological disorders. *Journal of Biological Inorganic Chemistry, 23*(8), 1219–1226. https://doi.org/10.1007/s00775-018-1605-1

Qu, T., He, S., Ni, C., Wu, Y., Xu, Z., Chen, M., … Wen, L. (2022). In vitro anti-infammatory activity of three peptides derived from the byproduct of rice processing. *Plant Foods for Human Nutrition, 77*, 172–180. https://doi.org/10.1007/s11130-022-00963-6

Quarmby, V., Phung, Q. T., & Lill, J. R. (2018). MAPPs for the identification of immunogenic hotspots of biotherapeutics; an overview of the technology and its application to the biopharmaceutical arena. *Expert Reviews of Proteomics, 15*(9), 733–748. https://doi.org/10.1080/14789450.2018.1521279

Ravindranath, P. A., Forli, S., Goodsell, D. S., Olson, A. J., & Sanner, M. F. (2015). AutoDockFR: Advances in protein-ligand docking with explicitly specified binding site flexibility. *PLoS Computational Biology, 11*(12). https://doi.org/10.1371/journal.pcbi.1004586. Article e1004586.

Roche, P. A., & Furuta, K. (2019). The ins and outs of MHC class II-mediated antigen processing and presentation. *Nature Reviews Immunology, 15*(4), 203–216. https://doi.org/10.1038/nri3818

Sayd, T., Dufour, C., Chambon, C., Buffière, C., Remond, D., & Santé-Lhoutellier, V. (2018). Combined in vivo and in silico approaches for predicting the release of bioactive peptides from meat digestion. *Food Chemistry, 249*, 111–118. https://doi.org/10.1016/j.foodchem.2018.01.013

Sensoy, I. (2021). A review on the food digestion in the digestive tract and the used in vitro models. *Current Research in Food Science, 4*, 308–319. https://doi.org/10.1016/j.crfs.2021.04.004

Skariyachan, S., Khangwal, I., Niranjan, V., Kango, N., & Shukla, P. (2021). Deciphering effectual binding potential of xylo-substrates towards xylose isomerase and xylokinase through molecular docking and molecular dynamic simulation. *Journal of Biomolecular Structure & Dynamics, 39*(11), 3948–3957. https://doi.org/10.1080/07391102.2020.1772882

Śledź, P., & Caflisch, A. (2018). Protein structure-based drug design: From docking to molecular dynamics. *Current Opinion in Structural Biology, 48*, 93–102. https://doi.org/10.1016/j.sbi.2017.10.010

Stowers, A. W., Chen, L. H., Zhang, Y., Kennedy, M. C., Zou, L., Lambert, L., & Miller, L. H. (2002). A recombinant vaccine expressed in the milk of transgenic mice protects Aotus monkeys from a lethal challenge with *Plasmodium falciparum*. *Proceeding of the National Academy of Sciences of the United States of America, 99*(1), 339–344. https://doi.org/10.1073/pnas.012590199

Thorsing, M., Krogh, T. J., Vitved, L., Nawrocki, A., Jakobsen, R., Larsen, M. R., & Boysen, A. (2021). Linking inherent O-linked protein glycosylation of YghJ to increased antigen potential. *Frontiers in Cellular and Infection Microbiology, 11*, Article 705468. https://doi.org/10.3389/fcimb.2021.705468

van Vliet, S. J., García-Vallejo, J. J., & van Kooyk, Y. (2008). Dendritic cells and C-type lectin receptors: Coupling innate to adaptive immune responses. *Immunology & Cell Biology, 86*(7), 580–587. https://doi.org/10.1038/icb.2008.55

Wen, L., Huang, L., Li, Y., Feng, Y., Zhang, Z., Xu, Z., & Cheng, Y. (2021). New peptides with immunomodulatory activity identified from rice proteins through peptidomic and in silico analysis. *Food Chemistry, 364*, Article 130357. https://doi.org/10.1016/j.foodchem.2021.130357

Yang, Q., Wang, Y., Yang, M., Liu, X., Lyu, S., Liu, B., & Zhang, T. (2022). Effect of glycation degree on the structure and digestion properties of ovalbumin: A study of amino acids and peptides release after in vitro gastrointestinal simulated digestion. *Food Chemistry, 373*, Article 131331. https://doi.org/10.1016/j.foodchem.2021.131331

Yang, X., Wang, L., Zhang, F., & Ma, H. (2020). Effects of multi-mode S-type ultrasound pretreatment on the preparation of ACE inhibitory peptide from rice protein. *Food Chemistry, 331*, Article 127216. https://doi.org/10.1016/j.foodchem.2020.127216

Yang, R., Zhang, Z., Pei, X., Han, X., Wang, J., Wang, L., & Li, Y. (2009). Immunomodulatory effects of marine oligopeptide preparation from Chum Salmon (*Oncorhynchus keta*) in mice. *Food Chemistry, 113*(2), 464–470. https://doi.org/10.1016/j.foodchem.2008.07.086

Zhang, Z., Ni, C., Ou, X., Cao, T., Cheng, Y., Wen, L. (2023). Research progress on immune function of food-derived bioactive peptides. Food & Mechinery 2023, 39(5), 193–202 (in Chinese). doi:10.13652/j.spjx.1003.5788.2022.60156.