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Identification of tuna protein-derived peptides as potent SARS-CoV-2 inhibitors via molecular docking and molecular dynamic simulation

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A B S T R A C T

The present study aimed to identify potential SARS-CoV-2 inhibitory peptides from tuna protein by virtual screening. The molecular docking was performed to elicit the interaction mechanism between targets (M\textsuperscript{pro} and ACE2) and peptides. As a result, a potential antiviral peptide EEAGGATAAQIEM (E-M) was identified. Molecular docking analysis revealed that E-M could interact with residues Thr190, Thr25, Thr26, Ala191, Leu50, Met165, Gln189, Glu166, His164, His41, Cys145, Gly143, and Asn119 of M\textsuperscript{pro} via 11 conventional hydrogen bonds, 9 carbon hydrogen bonds, and one alkyl interaction. The formation of hydrogen bonds between peptide E-M and the residues Gly143 and Gln189 of M\textsuperscript{pro} may play important roles in inhibiting the activity of M\textsuperscript{pro}. Besides, E-M could bind with the residues His34, Phe28, Thr27, Ala36, Asp355, Glu37, Gln24, Ser19, Tyr83, and Tyr41 of ACE2. Hydrogen bonds and electrostatic interactions may play vital roles in blocking the receptor ACE2 binding with SARS-CoV-2.

1. Introduction

The rapid spread of the novel coronavirus (SARS-CoV-2) as a serious threat to the world public health is in dire need of finding nutritional supplements with potential SARS-CoV-2 inhibition effect (Munster, Koopmans, van Doremalen, van Riel, & de Wit, 2020). The main protease (M\textsuperscript{pro}, also called 3CLpro) in SARS-CoV-2 virus is a necessary therapeutic target, which together with papain-like proteases is required to process polyprotein translated from viral RNA and recognize specific cleavage sites (Dai & Zhang, 2020; Gurung et al., 2020). The structure of SARS-CoV-2 M\textsuperscript{pro} complex contains the natural inhibitor N3 (Yang et al., 2003). Therefore, inhibiting the activity of the SARS-CoV-2 M\textsuperscript{pro} enzyme would help to block viral replication (Anand, Palm, Mesters, Siddell, Ziebuhr, & Hilgenfeld, 2002). Since no human protease with the similar cleavage specificity are known, inhibitors are unlikely to be toxic (Zhang & Lin, 2020). It also has been confirmed that SARS-CoV-2 infects human host cells by an initial isolate of its spike glycoprotein (S) and the receptor angiotensin-converting enzyme 2 (ACE2) on human cells (Hoffmann et al., 2020; Tan & Aboulhosn, 2020). SARS-CoV-2 was supposed to use the ACE2 as receptor for virus entry into host cells (Kozhikhova, Shilovskiy, & Shatilov, 2020). Blocking the interaction between the S protein of SARS-CoV-2 and receptor-binding domain (RBD) of cellular receptors ACE2 can prevent virus entry. Therefore, ACE2 is also an attractive target for the treatment of SARS-CoV-2.

To date, no specific antiviral drug and clinically effective vaccine are available for the prophylaxis or treatment of the highly virulent SARS-CoV-2 infections in humans. In this situation, protein as a nutritional supplementation may be a helpful approach to improve immunity against SARS-CoV-2. The protein macromolecules were degraded into amino acids and peptides by gastrointestinal enzymes (Yao, Luo, & Zhang, 2020). In addition, many previous studies reported antivirus peptides with long chain peptides, including anti-Japanese Encephalitis virus peptide P1 (TPDCTRWWCPLT) (Wei et al., 2020), anti-Respiratory syncytial virus anti-LTP (R8K4K2KAC) and SA-35 (MITH-GCYTRTRHKLKTL) (Kozhikhova et al., 2020), and the anti-West Nile Virus Envelope Protein peptide P9 (CDVIALACHLNT) (Bai et al., 2007). Currently, the peptides are the potential therapeutic agents for their selectivity, specificity, low levels of side effects, and predictable metabolism. A number of highly potent antiviral peptides, such as the anti-HIV C-peptide (SJ-2176) (Jiang, Lin, Strick, & Neurath, 1993) and enfuvirtide (Lazzarin et al., 2003), have been approved for use as antiviral drugs, indicating that antimicrobial peptides can be developed into safe and effective antiviral therapeutics and prophylactics. The
binding abilities of these peptides to M\textsuperscript{pro} and ACE2 were evaluated, and the peptides with high affinity to the two enzymes could be expected to have a little bit potential inhibition on SARS-CoV-2. Tuna is a high content of nutrition ingredients food, and is widely consumed as a part of modern human diets (He, Su, Sun-Waterhouse, Waterhouse, Zhao, & Liu, 2019). Furthermore, it has been found that tuna hydrolysates have many biological activities, especially, the inhibitory activity against angiotensin converting enzyme (ACE) (Lee, Qian, & Kim, 2010; Li, Wang, Zhang, Wang, Zhu, & Ma, 2015). The ACE inhibitors may have the potential to prevent and to treat the acute lung injury after SARS-CoV-2 infection (Pati, Mahoto, Padhi, & Panda, 2020; Zheng & Cao, 2020). So, tuna-derived peptides can be used as nutritional peptides supplementation and have potential inhibition of SARS-CoV-2 activity.

Nowadays, isolating, purifying and identifying bioactive peptides from protein hydrolysate is time-consuming, however, the process can be simplified and accelerated by multistep virtual screening method and in silico gastrointestinal (GI) digestion (Vercruysse, Smagghe, Matsui, & Van Camp, 2008). Computer analysis of bioactive peptides released after food proteolysis is useful (Gangopadhyay et al., 2016). Molecular docking refers to docking peptides with the active center of targets in DS software, which generates the CDOCKER energy in the process. The CDOCKER energy values are a standard to predict the stability of peptides-targets connection. Lower CDOCKER-energy value revealed that ligand was more likely to bind with the receptor and achieve more favorable conformation. (Nongonierma, Mooney, Shields, & Fitzgerald, 2013). Many studies have confirmed the reliability of in silico screening methods, which can be regarded as valid alternatives to classic methods (Fu, Young, Løkke, Lametsch, Aluko, & Therkildsen, 2016; Yu, Dong, et al., 2020; Yu, Ji, et al., 2020; Zhao, Xue, & Yu, 2019).

The purpose of present study was to identify novel peptides for COVID-19 patients from tuna protein as nutritional supplementation. To facilitate the rapid discovery of this peptides, a combination strategy of in silico hydrolysis and molecular docking was performed to discover novel inhibitory peptides against M\textsuperscript{pro} and the host receptor ACE2. The potential mechanism of peptides with virus targets M\textsuperscript{pro} and ACE2 was explored by Discovery Studio (DS) 2017 R2 software. And molecular dynamic simulations (MD) was performed to determine the binding affinity of peptide with the main protease and ACE2 of SARS-CoV-2 at room temperature. This study will improve the physical condition of COVID-19 patients and provide a new strategy for the treatment of SARS-CoV-2.

2. Materials and methods

2.1. GI digestion of tuna protein

Pepsin (EC 3.4.23.1), Trypsin (EC 3.4.21.4), and Chymotrypsin (EC 3.4.21.1) are three typical enzymes in the gastrointestinal tract, which were chosen for proteolysis in the present study (Yu, Dong, et al., 2020). The amino acid sequence of tuna skeletal myosin heavy chain (Accession of NCBI: BAA12730.1) was chosen from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/). The program ExPASy PeptideCutter (https://web.expasy.org/peptide-cutter/) (Zhao, Chen, Li, Xu, Shao, & Tu, 2016) could predict the cleavage sites of protease in a protein sequence (Hochstrasser, protein identification and analysis tools in the ExPAsy server), which was used to hydrolyze tuna skeletal myosin protein. Subsequently, peptides with more than 2 amino acids were selected for the following virtual screening.

2.2. Molecular docking of peptides and M\textsuperscript{pro} of SARS-CoV-2

All peptides were docked to M\textsuperscript{pro} of SARS-CoV-2 using CDOCKER program in DS 2017 R2 software, aimed to screen the potential M\textsuperscript{pro} inhibitory peptides. The X-RAY diffraction structure of M\textsuperscript{pro} in complex with inhibitor N3 was downloaded from the RCSB Protein Data Bank (PDB ID: 6LU7) (https://www.rcsb.org/), with the structure resolution of 2.16 Å (Jin et al., 2020). The inhibitor N3 and water molecules of M\textsuperscript{pro} were removed, and hydrogen atoms were added before docking by DS 2017 R2 software (Dassault Systemes Biovia, San Diego, CA, USA). The structure of the peptides was drawn by DS 2017 R2 software (Yu et al., 2018). The natural compounds (baicalin and baicalein) have shown the inhibitory activity of SARS-CoV-2 (Su et al., 2020a), which were downloaded from (https://pubchem.ncbi.nlm.nih.gov/). The ligands were minimized with CHARMM force field. Ligands and M\textsuperscript{pro} were docked by CDOCKER protocol of DS 2017 R2. The docking was carried out with coordinates x: −10.8, y: 12.5, z: 69.0, with a radius of 13.8 Å. The parameters were default. CDOCKER energy values of in-silico docking was performed to discover peptides with low CDOCKER-energy value.

### Table 1

| Peptide     | Docking score with M\textsuperscript{pro} | Solubility |
|-------------|-------------------------------------------|------------|
| EEAGGATAAQIEM | 154.676 kcal/mol GOOD                     |
| QAEEAAEQANTH  | 150.145 kcal/mol GOOD                     |
| EEEQEAEEK     | 148.618 kcal/mol GOOD                     |
| QTRENEK       | 132.888 kcal/mol GOOD                     |
| EQDTIASH      | 132.832 kcal/mol GOOD                     |
| EEAOER        | 131.632 kcal/mol GOOD                     |
| QATESQIK      | 129.159 kcal/mol GOOD                     |
| EQTER         | 125.889 kcal/mol GOOD                     |
| IDVER         | 124.963 kcal/mol GOOD                     |
| IEEIRK        | 124.963 kcal/mol GOOD                     |
| GADAIRK       | 121.809 kcal/mol GOOD                     |
| DDAVIR        | 121.009 kcal/mol GOOD                     |
| VETEK         | 120.161 kcal/mol GOOD                     |
| EEEQKE        | 120.042 kcal/mol GOOD                     |
| TEIQTAP       | 119.848 kcal/mol GOOD                     |
|VDASEER        | 118.695 kcal/mol GOOD                     |
| EGAQK         | 117.485 kcal/mol GOOD                     |
| QQREBD        | 117.262 kcal/mol GOOD                     |
| QEREK         | 117.232 kcal/mol GOOD                     |
| QADSVAE       | 117.088 kcal/mol GOOD                     |
| AIITDAAM      | 116.956 kcal/mol POOR                     |
| EAVAK         | 116.669 kcal/mol GOOD                     |
| GEIQIDN       | 115.839 kcal/mol GOOD                     |
| NAERDK        | 114.26 kcal/mol GOOD                      |
| QTENGGE       | 112.528 kcal/mol GOOD                     |
| QGEVVED       | 111.960 kcal/mol GOOD                     |
| EQIK          | 110.023 kcal/mol GOOD                     |
| TQQHIE        | 109.476 kcal/mol GOOD                     |
| EVSRIK        | 109.195 kcal/mol GOOD                     |
| DAERV         | 108.908 kcal/mol GOOD                     |
| SEVDR         | 107.165 kcal/mol GOOD                     |
| EATISAS       | 106.675 kcal/mol GOOD                     |
| TIEDQ         | 105.871 kcal/mol GOOD                     |
| EEAR          | 105.193 kcal/mol GOOD                     |
| ETDAIQR       | 104.486 kcal/mol GOOD                     |
| VAEQE         | 103.805 kcal/mol GOOD                     |
| EQVAM         | 103.438 kcal/mol GOOD                     |
| AEEBE         | 103.407 kcal/mol GOOD                     |
| DEAEAA        | 103.111 kcal/mol GOOD                     |
| NQIK          | 102.210 kcal/mol GOOD                     |

2.3. Toxicity and solubility prediction of peptides

The peptide property calculator was used to predict the solubility of potential peptides, available at http://www.innovagen.com/ (Lafarga, O’Connor, & Hayes, 2015). Subsequently, the tool Quantitative Structure-Toxicity Relationship (QSTR) studies in DS 2017 was used to calculate the toxicity of selected peptides according to the important physico-chemical properties. Theory-Toxicity Prediction (TOPKAT) protocol in DS 2017 was used to predict four properties in toxicity, i.e., Mutagenicity (Ames test), Developmental Toxicity Potential (DTP),
Skin Sensitization (GPMT) and Rat Oral LD50. TOPKAT protocol could accurately and rapidly evaluate the toxicity of peptides based on their 2D molecular structure.

2.4. Molecular docking of peptides and ACE2

The native crystal structure of the human ACE2 (PDB ID: 1R42) (Towler et al., 2004) was obtained from the PDB, with the resolution of 2.20 Å. Then, water molecules were removed and hydrogen atoms were added before docking. For docking simulations, a docking SBD site sphere was made to cover the entire two virus-binding hotspots of ACE2 (Wan, Shang, Graham, Baric, & Li, 2020), with coordinates x: 81.0, y: 76.5, z: 33.0, with a radius of 19.5 Å. And the CDOCKER program was used to molecular docking simulation in DS 2017. The potent binding peptides to the ACE2 was selected based on the -CDOCKER-energy score.

2.5. Molecular dynamic (MD) simulation

MD simulations were carried out using GROMCS 2018 (Abraham et al., 2015) and the CHARMM36 force field (Brooks et al., 2009) for a period of 100 ns. A cubic box was built and the complex structures were placed in the center of the cubic box. Water molecules (TIP3P) were added to the remaining volume of the box, then each system was neutralized by adding chlorine/sodium atoms. The energy of each system was minimized by steepest descent algorithm (DosSantos, Faria, Rodrigues, & Bello, 2020). To equilibrate the system, two step simulations (NVT and NPT) were carried out by leapfrog algorithm. NVT simulation was made for 1 ns using a V-rescale thermostat (Bussi, Donadio, & Parrinello, 2007) to keep the temperature at 300 K and NPT simulation was made for 1 ns using Berendsen barostat (Rogge et al., 2015) to maintain the pressure of each system at 1 bar. The simulation files were output to calculate the RMSD (Root Mean Square Deviation), RMSF (Root Mean Square Fluctuation) and Rg (radius of gyration) (Khan et al., 2020).

Table 2
Docking with the amino acid residues of MPro.

| Ligand  | Conventional hydrogen bonds | Carbon hydrogen bonds | Hydrophobic interaction |
|---------|-----------------------------|-----------------------|-------------------------|
| E-M     | 11                          | 9                     | 1                       |
| Inhibitor N3 | 8                          | 6                     | 2                       |
| Baicalin | 5                           | 2                     | 5                       |
| Baicalein | –                          | –                     | 3                       |

("--": no interaction with the key amino acid residue).

Fig. 1. The docking interactions of EEAGGATAAQIEM (E-M) with MPro (PDB: 6LU7) and interactions with residues are shown in different colors. (a) 3D structure of peptide (E-M)-MPro complex. (b) 2D diagram of the peptide (E-M)-MPro molecular interactions. (c) The 3D hydrogen bonds surface plot at the binding site. The green color represents conventional hydrogen bond, light blue represents carbon hydrogen bond. The pink color represents alkyl interaction, and red color represents unfavorable acceptor–acceptor. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Skin Sensitization (GPMT) and Rat Oral LD50. TOPKAT protocol could accurately and rapidly evaluate the toxicity of peptides based on their 2D molecular structure.
3. Results and discussions

3.1. Prediction $M^{\text{pro}}$ inhibitory activity of peptides

A total of 142 peptides were obtained from skeletal myosin of tuna in silico digestion. In silico GI digestion, there are many challenges, such as, incomplete protein unfolding and hydrolysis of food proteins. In traditional digestion, it is hard to obtain high purity and potent active peptides with in complex mixtures of various peptides. Thus, there are differences between in silico GI digestion and real digestion. But compared with traditional digestion, in silico GI digestion is simpler, cost-effective, and faster. Subsequently, 136 peptides were successfully docked to $M^{\text{pro}}$ of SARS-CoV-2 using CDOCKER program in DS 2017 R2 software. The α-ketoamide inhibitor was a natural inhibitor similar to N3 inhibitor, which existed in the crystal structure of SARS-CoV-2 Mpro enzyme (PDB: 6Y2F) (Gurung, Ali, Lee, Farah, & Al-Anazi, 2020). The -CDOCKER-energy values of N3 inhibitor and α-ketoamide inhibitor were 102 and 50.3 kcal/mol, respectively. Compared with α-ketoamide inhibitor, N3 inhibitor had the lowest CDOCKER-energy. Therefore, 42 peptides with CDOCKER-energy values less than −102 kcal/mol were selected for following studies (shown in Table 1). Among the 42 peptides, peptide E-M had the lowest CDOCKER-energy, and it might have stronger binding affinity with the target $M^{\text{pro}}$. The -CDOCKER-energy values of peptide E-M, baicalin and baicalein were 155, 19.5 and 30.0 kcal/mol, respectively. Compared with baicalin and baicalein which have been reported to have $M^{\text{pro}}$ inhibitory activity (Su et al., 2020b), peptide E-M might have a better $M^{\text{pro}}$ inhibitory activity.

3.2. Solubility, mutagenicity, toxicity properties predictions and docking with ACE2 of unknown peptides

The solubility results indicated that all these peptides were good water solubility except peptide AITDAAM (shown in Table 1). Water solubility of bioactive peptides plays a key role in the performance of physiological functions (Lee, Hong, Kim, & Lee, 2017). Peptides with good water solubility may have the potential of high biological availability. Thus, 41 peptides were selected to toxicity predictions. The evaluation of toxicity has an influence on the safety assessment of unidentified bioactive peptides, which directly related to the people’s health. The TOPKAT mutagenicity and skin sensitization results showed that all peptides were Non-Mutagen and Non-Sensitizer (shown in Table 1). The Developmental Toxicity Potential results showed that peptides EEAQER, EEGQSE, QADSVAE, QGEVED, SEVDR and AVQSAR were toxic, others were Non-Toxic (shown in Table 1). The Rat oral LD50 value of all peptides were higher than that of etravirine (an antiviral drug with LD50: 182.2 mg/Kg) (Singh et al., 2020), indicated that...
the toxicity of all peptides may have a good safety index (shown in Table 1). In summary, 35 peptides with good water solubility, no toxicity were subjected to the molecular dock to ACE2. Eventually, only one peptide EAGGATAAQIEM (E-M) successfully docked with the virus host receptor ACE2. The -CDOCKER-energy value of the peptide E-M with ACE2 was 144 kcal/mol. Thus, the active site of ACE docked with SARS-CoV-2 spike was strongly occupied by peptide E-M, which could affect SARS-CoV-2 activity.

3.3. Molecular mechanism of potent SARS-CoV-2 inhibitory peptide E-M

In order to clarify the action mechanism of potential SARS-CoV-2 inhibitory peptides E-M with novel virus target Mpro and ACE2, molecular docking was performed. The best interaction posture of E-M with Mpro was stabilized by 11 conventional hydrogen bonds, 9 carbon hydrogen bonds, and 1 alkyl interaction (shown in Fig. 1). The residue Thr26 (HG1 and HN) of Mpro formed conventional hydrogen bonds with the atom O14 and O49 of E-M generating lengths 2.01 Å and 2.01 Å, respectively. The atoms H68 and H74 of E-M also formed two conventional hydrogen bonds with the residue His161 (H) of Mpro at distances 3.02 Å and 2.16 Å, respectively. The O80 and H114 of E-M formed conventional hydrogen bonds with HN and O of the residue Gly143 of Mpro at distances of 2.50 Å and 2.08 Å, respectively. Moreover, Asn119 (HN), Gly143 (HN) Cys145 (SG), Met165 (SD), and Ala191 (HN) of Mpro also formed conventional hydrogen bonds with atoms O15, O56, H68, H115 and O169 of E-M, at distances of 2.24 Å, 2.19 Å, 2.62 Å, 2.72 Å, and 2.02 Å, respectively. In the docked complex, Asn119 (HA), Thr26 (O), Thr26 (O), Thr25 (HA), His41 (NE2), Met165 (HA), Gin189 (OE1), Thr190 (HB), and Gin189 (HA) formed carbon hydrogen bonds with O14, H54, H53, O49, H60, O80, H72, O169, and O112 of E-M at distances of 3.00 Å, 2.54 Å, 2.70 Å, 2.53 Å, 2.84 Å, 2.48 Å, 2.96 Å, 2.80 Å and 2.68 Å, respectively. Additionally, the residue Leu50 formed an alkyl interaction with E-M (C131) with a distance at 5.41 Å. Moreover, inhibitor N3 bound with residues Thr26, Gly143, Phe140, Gin189, His164, His41, Thr190, and Met165 of Mpro, which were crucial residues for Mpro activity (shown in Fig. 3a). E-M formed interactions with Mpro by residues Thr190, Thr25, Thr26, Ala191, Leu50, Met165, Gin189, Glu166, His164, His41, Cys145, Gly143, and Asn119, part of which overlapped with the residues of inhibitor N3 action. As shown in Table 2, the total number of conventional hydrogen bonds and carbon hydrogen bonds in E-M was obviously more than N3 inhibitor, baicalin, and baicalein, indicating that the main interaction types of the complex were hydrogen bonds. The best docking postures of baicalein and baicalin with Mpro were shown in Fig. 3b, 3c. In the complex of baikalin-Mpro, the residues Met165, His41, Gly143, and Gin189 overlapped with the residues acted by the peptide E-M. Met165 and His41 mainly participate in hydrophobic interaction, and Gly143, and Gin189 mainly participate in the formation of hydrogen bonds. Compared with E-M, N3 inhibitor, and baikalein, this result was also confirmed. Therefore, hydrogen bonds between peptide and residues Gly143 and Gin189 of Mpro may be an important screening indicator.

The best posture of E-M binding with ACE2 (shown in Fig. 2) was stabilized by 6 conventional hydrogen bonds, 6 carbon hydrogen bonds,
1 pi-donor hydrogen bond, 1 salt bridge and 2 attractive charge interactions. The residues Ser19 (HT2), Gln24 (OE1), Tyr83 (HH), Phe28 (O) Thr27 (O) and Ala36 (HN2) of ACE2 formed conventional hydrogen bonds with the atoms O168, H153, O149, H114, H114 and O56 of E-M at distances of 2.98 Å, 2.01 Å, and 2.02 Å, 2.04 Å, 3.05 Å and 2.70 Å respectively. Glu37 (O), Glu37 (HC), His34 (O), His34 (HC), Gln24 (OE1), and Ser19 (HB1) formed carbon hydrogen bonds with the atoms H46, O56, H54, O73, H140 and O168 of E-M at distances of 2.41 Å, and 2.43 Å, 2.59 Å, 2.40 Å, 3.00 Å and 2.88 Å respectively. Tyr41 of ACE2 formed a pi-donor hydrogen bond with E-M (H34) generating a length of 2.85 Å. Asp355 (OD1) of ACE2 formed a salt bridge with E-M (H4) generating a length of 2.10 Å. Two attractive charge interactions were observed in the complex (E-M)-ACE2, one involved the oxygen atom (OD1) of residue Asp355 with the hydrogen atom (H4) of E-M at a

Fig. 4. The Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF) and radius of gyration (Rg) curves of the protein backbone (Ca) atoms during MD-simulation. RSMD (a), RMSF (c), and Rg (e) of Mpro in complex with peptide E-M. RSMD (b), RMSF (d), and Rg (f) of ACE2 in complex with peptide E-M. The complexes exhibited stable RMSDs, RMSFs and Rgs during a 100 ns MD simulation period.
distance of 2.10 Å, and the other one involved the nitrogen atom (N) of the residue His34, which connects with virus receptor ACE2 via hydrogen bonds and electrostatic interactions. Overall, peptide E-M has good safety due to their connecting with virus receptor ACE2 via hydrogen bonds and electrostatic interactions. In order to get an idea about the structural stability, conformational fluctuations, compactness and folding behavior of M\textsuperscript{pro} complexed with peptide E-M and ACE2 complexes with peptide E-M, we performed MD simulations for 100 ns. The analysis of RMSD and RMSF usually provides important information about the stability and flexibility of the receptor-ligand complex. High deviation and fluctuation of proteins during a simulation may show weak stability (Ghosh & Chakraborthy, 2020). SARS-CoV-2 M\textsuperscript{pro} in complexed with peptide E-M exhibited a stable RMSD between 0.25 nm and 0.4 nm (Fig. 4a) and the initial and final RMSDs during the whole simulation period were not found in the significance difference (0.2 nm and 0.3 nm). ACE2 in complexed with peptide E-M exhibited a stable RMSD between 0.15 nm and 0.25 nm (Fig. 4b) and the initial and final RMSDs during the whole simulation period were not found in the significance difference (0.1 nm and 0.2 nm). This showed a stable binding of peptide E-M with M\textsuperscript{pro} and ACE2. Moreover, residues fluctuations were also observed, not too flexible in motion. The residues fluctuation range in peptide E-M – M\textsuperscript{pro} complex is 0.053 nm-0.36 nm (Fig. 4c). The residues fluctuation range in peptide E-M – ACE2 complex is 0.075 nm-0.4 nm (Fig. 4d). Both, RMSD and RMSF stabilities were essential to infer good binding affinities (Doniach & Eastman, 1999; Dubey, Tiwari, & Ojha, 2013). Rg parameter was used to infer the degree of compactness and folding stability. A long range of variations in proteins show their weak folding. A steady value of Rg shows compactness and stable folding, which requires for proper function (Smigljes & Foita-Stogniew, 2015). On the contrary, in case of misfolding, the Rg will show a long range of variation over time (Lobanov & Bogatyreva, 2008). The Rg values for peptide E-M-M\textsuperscript{pro} complexes were found to remain almost constant (2.27–2.29 nm) from 25 ns to 100 ns with some marginal fluctuations (Fig. 4e). The Rg values for peptide E-M-ACE2 complexes were found to remain almost constant (2.52–2.58 nm) (Fig. 4f). The peptide E-M had good folding stability and high compactness with M\textsuperscript{pro} and ACE2. Thus, peptide E-M might be an effective inhibitor.

4. Conclusions

In this study, peptide E-M was identified from the skeletal myosin of tuna. Molecular docking simulation demonstrated that Gly143, and Gln189 played important roles in the interactions of peptide E-M and M\textsuperscript{pro}. Peptide E-M could block SARS-CoV-2 attachment to host cells by connecting with virus receptor ACE2 via hydrogen bonds and electrostatic interactions. Overall, peptide E-M has good safety due to their source of diet, and provide a good nutritional supplementation for COVID-19 patients. However, ex vivo and in vivo experiment will be required for further verify this conclusion. We want to share our results to anti-SARS-CoV-2 researchers as soon as possible, so we do not any further in vivo and in vitro experiments.
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