Effect of ultrasonic treatment on the stability and release of selenium-containing peptide TSeMMM-encapsulated nanoparticles in vitro and in vivo

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ABSTRACT

Rice selenium-containing peptide TSeMMM (T) with immunomodulatory functions was isolated from selenium-enriched rice protein hydrolysates. However, its biological activity is difficult to be protected in complex digestive environments. In this study, T was encapsulated within zein and gum arabian (GA) through ultrasound treatment to improve its bioactivity and bioavailability. The zein@T/GA nanoparticles were formed using ultrasonic treatment at 360 W for 5 min with a 59.9% T-encapsulation efficiency. In vitro digestion showed that the cumulative release rate of zein@T/GA nanoparticles reached a maximum of 80.69% after 6 h. In addition, short-term animal studies revealed that the nanoparticles had an effect on the levels of tissue glutathione and improved peptides’ oral bioavailability. Conclusively, these findings suggest that the ultrasound-treated polysaccharide/protein system is suitable for encapsulating active small molecular peptides. Furthermore, it provides a novel foundation for studying the bioavailability of active substances in functional foods.

1. Introduction

Selenium (Se), an essential trace element, has been widely used as a supplement in rice for our daily diet [1,2]. According to our previous studies, Se is present in the form of selenomethionine for rice proteins [1]. Previous results showed that Se-enriched rice protein hydrolysates (SPHs) protect PC12 cells from Pb²⁺-induced cytotoxicity and apoptosis [3]. Furthermore, the Se-containing peptides TSeMMM and SeMDPGQQ showing immunomodulatory functions were isolated from SPHs using a RAW264.7 cell model. The synthesized rice Se-containing peptides demonstrated neuroprotective effects against Pb²⁺-induced oxidative stress in mice hippocampal HT22 cells [4]. Additionally, experimental results showed that TSeMMM has stronger immunomodulatory activity [5]. TSeMMM and SeMDPGQQ are hydrophilic and hydrophilic peptides, respectively. There are fewer studies on hydrophilic peptides, so it is more urgent to study the embedding of hydrophilic peptides. Like most food-derived peptides, Se-containing rice peptides are susceptible to degradation by digestive enzymes in the complex environment of the gastrointestinal tract after oral ingestion. This reduces the bioactivity of peptides and inhibits them from reaching target tissues or exerting their biological effects, thus limiting their practical application in the food and pharmaceutical industries [6].

Nanotechnology is an emerging development in the food industry [7]. Protein-based nanoparticles are receiving increased attention due to their excellent functional properties of biodegradability, compatibility, and high nutritional value [8,9]. Zein is often used as a drug carrier due to its nontoxicity, biodegradability, good biocompatibility, targeted and controlled drug release [10,11]. Zein also has a unique amino acid composition that results in poor solubility in water but good solubility in aqueous solutions of 50%–90% (v/v) ethanol [12]. Furthermore, by altering solvent polarity, zein can readily self-assemble into micro/nanoparticles that have been extensively used as potential delivery systems for bioactive compounds [13,14]. However, its application is hampered by the weak repulsion caused by uncharged amino acids on the zein surface which can easily aggregate [15]. Therefore, zein is often combined with polysaccharides to form composites, thus increasing the stability of zein-involved systems [16,17], such as carrageenan [18], carboxymethyl chitosan [19], and gum arabian (GA) [20–22]. As a natural anionic polysaccharide, GA is widely used in the food industry due to its wide pH range, high ionic strength, and long-lasting temperature stability [23,24]. GA strengthens the zein network and improves the stability of the zein involving system,
and proteins in the molecular chain make GA hydrophilic and lipophilic.

Ultrasonic treatment has been considered an effective tool for enhancing the interactions between biological macromolecules [25]. Ultrasonic treatment has been conducted to modify the structure of animal and plant proteins, i.e., peanut and bovine serum proteins, resulting in changes in the particle sizes and functional properties of proteins [26,27]. Ultrasonic treatment has also been proven to modify the protein–polysaccharide complex formation [28–30]. However, there is a lack of investigation on using ultrasonic treatment to improve the functional properties of zein and GA associated with the protection of bioactive substances.

The objective of this study was to investigate the effect of ultrasonic treatment on the formation and properties of zein@T/GA nanoparticles. The properties of nanoparticles were characterized on the basis of the particle size, dispensability index, and encapsulation efficiency (EE). Scanning electron microscopy (SEM) and atomic force microscopy (AFM) were used to observe changes in the morphological appearance of the nanoparticles. Fourier-transform infrared spectroscopy (FTIR) and fluorescence spectroscopy (FS) were used to analyze the intermolecular interactions of zein@T/GA nanoparticles. Additionally, inductively coupled plasma-mass spectrometry (ICP-MS) was used to determine the cumulative release rate of nanoparticles digested in vitro and the tissue distribution in vivo. The results of this study will provide a powerful carrier system involving zein@T/GA nanoparticles that will improve the bioactivity and bioavailability of TsE MMM. This will enable a better practical application of Se-containing rice peptides in the food and pharmaceutical industries.

2. Materials and methods

2.1. Materials and chemicals

The Se-containing rice peptide TsE MMM was customized and synthesized by TP Peptide Biotechnology Co., Ltd. (Nanjing, China), and the pure was 95% identified using high-performance liquid chromatography (HPLC, 1260, Agilent Technologies Ltd., USA). Zein was obtained from Sigma-Aldrich Company (St. Louis, MO, USA). GA, disodium hydrogen phosphate, and citric acid were obtained from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Pepsin, pancreatin, and porcine bile salt were obtained from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). A total glutathione/oxidized glutathione assay kit was obtained from the Jiancheng Institute (Nanjing, China). Nitric acid (HNO3, 65%) was provided by Merck (Darmstadt, Germany). Absolute ethanol (C2H5OH, 99.8%) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Other chemical reagents were analytical grade ones that were purchased from Macleans Biochemical Technology Co., Ltd. (Shanghai, China). Deionized water (18.25 MΩ cm), obtained from a Milli-Q water system (Millipore, Bedford, MA, USA), was used throughout the whole experiment. Moreover, 60–8-weeks-old specific-pathogen-free (SPF) mice (Viton Lever Experimental Technology Co. Zhejiang, China) were found to weigh between 19 and 21 g, production license, SCXK (Zhejiang) 2019–0001. Animal Experiment Center in Nanjing Agricultural University [SYXK (Jiangsu) 2011–0036] approved this mice experiment, which was in accordance with the National Laboratory Animal Welfare Guidelines and Animal Experiment Ethics.

2.2. Preparation of zein@T/GA nanoparticles

2.2.1. Optimization of the ratio of zein to GA

GA and zein were purified to remove the insoluble impurities. To prepare the zein/GA nanocomposites, the zein powders were mixed in an ethanolic water solution (70%, v/v), whereas the GA powders (0.01, 0.02, 0.05, 0.10, and 0.20 g) were dissolved in deionized water. Subsequently, a 1.0% (w/v) reserve solution of zein and 0.1%, 0.2%, 0.5%, 1.0% and 2.0% (w/v) of GA reserve liquids were prepared. The zein/GA nanocomplex solution was obtained by mixing an equal volume of stock solution for these two above mentioned materials [31]. The zein stock solution was diluted by adding 39 mL of deionized water (adjusted to pH 8.0), and an equal volume of GA stock solution was added after 2 h. The supernatant was centrifuged after 2 h to obtain the zein/GA nanocomplex with the mass ratios of 10:1, 5:1, 2:1, 1:1, and 1:2 (indicated by zein/GA x:y).

2.2.2. Binary zein-T nanocomplexes

Prepare T stock solution at a concentration of 1000 µg/mL. Then, based on the experiments, zein was dissolved in an ethanol mixture comprising ethanol, water, and T stock solution, in a volume ratio of 7:2:1 until zein was completely dissolved to obtain the binary nanocomplex zein-T (zein@T) stock solution.

2.3. Ultrasonic treatment for zein@T/GA nanoparticles

The zein@T nanocomplex was considered a unit reacting with GA to obtain zein@T/GA nanoparticles, using a protocol similar to that mentioned in section 2.2.1. The effect of zein@T nanocomplex pre-treatment on zein@T/GA nanoparticles was investigated using an ultrasonic equipment (Model SB25-12DTDN, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China). This is indicated by the EE. A range of ultrasonic power levels (i.e., 0 W, 120 W, 240 W, 360 W, 480 W) was applied to treat the zein@T nanocomplex solution samples for 5 min. The probe was immersed in the sample solution during the ultrasonic treatment. The solution sample was kept in an ice bath to ensure a low temperature. The work mode for ultrasonic treatment was set as working for 3 s and then pausing for 3 s. At a fixed power level of 360 W for the ultrasonic treatment on each sample, the ultrasonic time was set over a range of 0 min, 5 min, 10 min, 20 min, and 30 min, while the ultrasonic-assisted pH was adjusted over a range of 4.0, 6.0, 7.0, 8.0 and 10.0.

2.4. Determination of Se content using ICP-MS

The zein@T/GA solution was filtered through an ultrafiltration tube (3 kDa). The solution was centrifuged (TG16-WS, Xiangyi, China) at 10,000 rpm for 20 min to remove the zein@T/GA nanoparticles. Subsequently, the resultant solution was aspirated. The solution was mixed with 5 mL of HNO3 (65%) in a polytetrafluoroethylene (PTFE) vessel and placed in a microwave digestion system (Mars 6 Classic, CEM, USA). After the digestion, the tubes were removed to drive off the acid and be cooled. This solution was then transferred to a centrifuge tube and concentrated to 5 mL with 2% (v/v) nitric acid. The samples were analyzed using ICP-MS (7700X, Agilent Technologies, USA) to determine the Se content in the samples. Identical concentrations of T and zein/GA nanoparticle solutions were prepared according to the above procedure, and all experiments were analyzed in triplicates. The EE of nanoparticles was calculated as follows:

\[
\text{Encapsulation efficiency} \% = \frac{C_0 - C_1 - C_2}{C_0} \times 100 \%
\]  

\(C_0\): Se content of T solution  
\(C_1\): Se content of zein/GA nanoparticles  
\(C_2\): Se content of zein@T/GA nanoparticles

Mice were executed after blood collection, and the small intestine, heart, liver, spleen, lungs, kidneys, and thymus tissue were taken. Exactly 0.1 g of tissue was mixed well with 5 mL of HNO3 (65%) and subjected to microwave digestion. After cooling, the sample was fixed with 2% (v/v) nitric acid, and the concentration in the sample was determined using ICP-MS.
2.5. Characterization of zein@T/GA nanoparticles

2.5.1. Nano ZS analysis

A Nano ZS instrument (ZS-90, Malvern Instruments Ltd., UK) was used to estimate the size distribution and polymer dispersity index (PDI) of zein@T/GA nanoparticles. All tests were conducted at a scattering angle of 173° and 25 ± 0.1 °C. Each measurement was conducted in triplicates.

2.5.2. FTIR and FS analysis

Samples were measured using FTIR ( Nicolet iSS, Thermo Fisher Scientific, USA) using a potassium bromide press method. The scanning conditions were set up with a spectral range of 4000–400 cm⁻¹. The number of scans was 64 times, and the resolution was 4 cm⁻¹. Samples were assayed using FS (P-7000, Hitachi High-Technologies, Japan), following a method reported previously with slight modifications [32,33]. A solution at a 5 mg/mL concentration was obtained by diluting the sample in 70% (v/v) aqueous ethanol (adjusted to pH 8.0). Spectra were recorded at 295 and 450 nm. Parameters for scanning were set as follows: the excitation wavelength of 280 nm, scanning speed of 100 nm/min, and slit width of 5 nm for excitation and emission.

2.5.3. Micromorphological analysis using SEM and AFM

The morphological appearance of zein@T/GA, zein/GA, zein at a 1 mg/mL concentration was characterized using SEM (Quanta FEG 250, FEI, USA) and AFM (Dimension Icon, BRUKER, Germany).

2.6. In vitro release tests

Based on the previous methods, the digestive behavior of zein@T/GA composite nanoparticles was assessed using an in vitro simulated gastrointestinal digestion model [18]. A solution of zein@T/GA was mixed with simulated gastric fluid (SGF, comprising pepsin 3.0 mg/mL, NaCl 2.0 mg/mL, pH 1.5) at a volume ratio of 1:10. This solution was kept in a beaker, and the beaker was then placed in a constant temperature water bath shaker (CSS501-SP, Chongqing Sida Testing Instruments Co., Ltd., China) under 200 rpm at 37.0 °C for 2 h. After 2 h, the pH of the solution was adjusted to 7.0 with NaHCO₃ to stop pepsin digestion. The solution environment was modified to obtain a simulated intestinal fluid (SIF, comprising trypsin 3.0 mg/mL, bile salts 12.0 mg/mL, NaCl 8.8 mg/mL, and KH₂PO₄ 6.8 mg/mL). During incubation, samples were collected at 1h intervals. The samples were processed according to the microwave digestion method in section 2.4. ICP-MS detected the Se content of the solution. The cumulative in vitro release efficiency of nanoparticles was calculated as follows based on the change in the Se content of the sample.

\[
\text{Cumulative release efficiency} \% = \frac{C_a}{C_o} \times 100\% \tag{2}
\]

\( C_o \): Se content of zein@T/GA solution without in vitro digestion
\( C_a \): Se content of zein@T/GA solution after in vitro digestion for different times

2.7. Evaluation of reduced glutathione (GSH) content in mouse tissues

Sixty Balb/c mice were divided randomly into six groups (10 mice per group). The zein@T/GA nanoparticles (0.01 g, 0.05 g, 0.10 g, 0.20 g, and 0.50 g) were dissolved in 1 mL water and then intragastrically administered to the mice at a rate of 0.2 mL/10 g. Samples from the blank group were replaced with saline. After 4 h of gavage, mouse serum, heart, liver, kidney, spleen, stomach, small intestine, and thymus tissues were taken and stored at −80 °C until use.

Mice tissues were processed according to the kit manufacturer protocol; then, total glutathione and oxidized glutathione in mice tissues were measured using a Burroughs i-mark Enzyme Labeler (Bio-rad, USA) at 405 nm. Then, the GSH content in mouse tissues was calculated using the following equation:

\[
\text{GSH} = \frac{TGSH - 2 \times GSSH}{2} \tag{3}
\]

TGSH: total glutathione
GSSH: oxidized glutathione

2.8. Statistical analysis

All experimental data were analyzed using SPSS (Statistical Package for the Social Sciences) v.23.0 (SPSS, Inc., Chicago, IL, U.S.A.). Across all the samples, the significant difference of experimental data was compared using Duncan’s test (P < 0.05). Correlation analysis heat maps were conducted using the R language package (v.4.0.2 drawing).

3. Results and discussion

3.1. Particle size and PDI of zein/GA nanoparticles at different GA concentrations

Fig. 1A showed the distribution of particle sizes for zein and zein/GA nanocomplexes with a range of mass ratios. Proteins were self-assembled into nanoparticles when the zein solution was added drop-wise to the aqueous solution with a change in polarity. The particle size distribution of zein was multimodal. When the GA was mixed with zein at the mass ratio of zein to GA of 10:1, 5:1, and 2:1, the particle size
distribution of zein/GA shifted toward the double-shouldered peaks. With increasing GA concentration, the single peaks for zein/GA nanoparticles were located at 187 and 210 nm at the mass ratios of 1:1 and 1:2, respectively. Notably, the sharpest and narrowest peaks were observed at mass ratios of 1:1. The results of this study were consistent with those obtained in previous studies [22]. Fig. 1B showed the PDI results for different mass ratios of zein/GA nanoparticles. The presence of GA in the zein solution caused a decrease in PDI values. The lowest PDI was 0.156 $\pm$ 0.006 at a zein/GA mass ratio of 1:1. The smaller the PDI value, the more stable the solution system [34]. Based on the results, binary nanocomposites were formed at a zein/GA mass ratio of 1:1. This was effectively used for further experiments.

3.2. Effect of ultrasonic treatments on the EE of zein@T/GA nanoparticles

Fig. 2 showed the effect of ultrasonic power (2A), ultrasonic time (2B), and pH (2C) on the EE of zein@T/GA nanoparticles. The effect of ultrasonic power on the EE of zein@T/GA is shown in Fig. 2A. The EE of the nanoparticles was 26.2% and 49.6% when the ultrasonic power was 0 and 120 W, respectively. Furthermore, at 360 W, the maximum EE of 59.9% was achieved. However, the EE decreased when the ultrasonic power was increased from 360 W to 600 W. In Fig. 2B, the ultrasonic time significantly affected the EE of zein@T/GA. The EE increased with increasing the ultrasonic time from 0 to 5 min. At 5 min, the highest EE reached 59.6%.

From 5 to 30 min, the EE decreased with increasing the ultrasonic time. This is because sonication may open up the protein structure, and more amide bonds can be bound to hydrogen bonds. The possible reason for this is that excessive ultrasound treatment can induce unfolding and aggregation of proteins, resulting in reduced EE [30]. Fig. 2C showed the effect of pH on the EE of zein@T/GA. An increase in the EE of zein@T/GA was seen due to increased pH from 2.0 to 7.0. At pH 8.0, the EE reached 57.8%. As the pH increased up to 10.0, a decrease in the EE was observed. This is probably because, under strongly basic conditions, the positively charged glutamine on zein deamidates to negatively charged glutamate residues. This weakens the glutamine electrostatic equilibrium that affects the stability of the $\alpha$-helix [35,36]. Decreased stability of the $\alpha$-helix leads to decreased stability of zein@T/GA nanoparticles and thus to decreased EE. Conclusively, the EE reaches its maximum at pH 8.0. The prepared nanoparticle solution was transparently white without flocculent precipitation. Therefore, this nanoparticle system was stable at a certain pH.

At pH 8.0 and 360 W ultrasound treatment for 5 min, the EE of zein@T/GA nanoparticles was greatly increased. Therefore, this is an optimal condition for ultrasonic treatment on the nanoparticle.

3.3. Effect of ultrasonic treatment on the stability of zein@T/GA nanoparticles

Table 1 shows the mean particle size, PDI, and zeta potential of zein, zein/GA, and zein@T/GA nanoparticles. When zein was added to the deionized water, flocculent precipitates were produced in a solution with an average particle size of 276.13 $\pm$ 5.89 nm and a PDI of 0.291 $\pm$ 0.015. Zein stock was treated in an ultrasonic cleaner (500 W, 30 s) during the dropwise addition of zein to aqueous solutions [34]. The second ultrasonic treatment serves only to quickly disperse zein or zein@T when added to the aqueous solution and prevent flocculation. Therefore, there is no need to explore the effect of sonication time and other sonication conditions. To prepare the zein/GA and zein@T/GA nanoparticles, the average particle size of 129.43 $\pm$ 2.21 nm and 98.73 $\pm$ 2.66 nm was determined, respectively, with the PDI of 0.173 $\pm$ 0.023 and 0.089 $\pm$ 0.014. This is because ultrasonic treatment mitigates the influence of polarity variation on zein solution, increasing zein solubility. It also effectively decreases nanoparticles’ average particle size and stabilizes the colloidal system. The results in this study were consistent...
The stability of the colloidal particle solution mainly depended on the size of particles and the charge on the particle surface. Thus, a solution system with high stability was observed with smaller particle sizes and a higher charge on the surface of the particles. For example, the potential of the zein solution was $-32.27$ mV, while the zeta potential of zein/GA and zein@T/GA nanoparticles was $-54.93$ mV and $-52.27$ mV. This indicates that the ultrasonic treatment causes an increase in the zeta potential of nanoparticles. When the absolute value of zeta potential is greater than $-30$ mV, the colloidal particles can stabilize the emulsion due to the electrostatic repulsion. Therefore, ultrasonic treatment has a good application in improving the stability of the aqueous system that is affected by the electrostatic repulsion between particles.

### 3.4. Molecular interaction of zein, T, and GA

The structural changes in zein, zein/GA, and zein@T/GA with that is shown in a study by Ren et al. [37].

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| Sample   | Particle size (d, nm) | PDI          | Zeta (mV)       |
|----------|-----------------------|--------------|-----------------|
| zein     | 276.13 ± 5.89$^a$    | 0.291 ± 0.015$^a$ | $-32.27 ± 0.72$ |
| zein/GA  | 129.43 ± 2.21$^b$    | 0.173 ± 0.023$^b$ | $-54.93 ± 1.20$ |
| zein@T/GA| 98.73 ± 2.66$^c$     | 0.089 ± 0.014$^c$ | $-52.27 ± 0.59$ |

Note: Data are means of three replicates. Values in the same column with different letters as superscripts are significantly different from each other according to Duncan’s test ($P < 0.05$).

![Fig. 3. The Fourier transform infrared spectra (A) and fluorescence spectrum (B) of T, GA, zein, zein/GA, and zein@T/GA. T: rice selenium-containing peptide TSeMMM; GA: Gum Arabian; zein@T/GA: rice selenium-containing peptide TSeMMM embedded in a zein/GA complex.](image-url)
nanoparticles are shown in Fig. 3. The characteristic peak of zein was at 3356.75 cm\(^{-1}\), while the peak of zein/GA and zein@T/GA was red-shifted to 3344.25 and 3338.05 cm\(^{-1}\), respectively. This suggests that the hydrogen bonds between the glutamine group in zein and the hydroxyl groups in GA are formed to promote the development of zein/GA and zein@T/GA nanoparticles [39,40]. The mechanism for our zein@T/GA nanoparticle development was consistent with that for zein-caseinate nanocomplex formation [41]. The amide I and amide II of zein showed the bands at 1658.34 cm\(^{-1}\) and 1540.97 cm\(^{-1}\), respectively. Compared to zein alone, the amide I band of zein/GA was red-shifted from 1658.34 to 1654.40 cm\(^{-1}\), whereas zein@T/GA was red-shifted to 1654.86 cm\(^{-1}\). The amide II bands of zein/GA and zein@T/GA were red-shifted to 1537.48 and 1536.49 cm\(^{-1}\), respectively. These results suggested hydrophobic interactions between zein, T, and GA. These hydrophobic interactions were modified by the addition of GA and T, which increased the solvent polarity [42,43]. These findings suggested that driving mechanisms for forming zein, T, and GA nanocomposites include hydrophobic interactions and hydrogen bonding.

FS has a common use for defining the conformational changes in proteins. In Fig. 3B, a typical fluorescence emission peak at 309 nm was shown for zein after the excitation at 280 nm, similar to that previously reported [22,44]. However, the fluorescence emission peak of zein/GA decreased due to the presence of GA. The possible reason was that GA combined with the hydrophobic patch of zein increased the polarity of the environment around tryptophan. This caused a fluorescence burst and then a reduction in the fluorescence intensity of zein [45,46]. Ultrasonic treatment caused the protein structure in the zein@T/GA nanoparticles to be altered, exposing more tryptophan. Furthermore, when combined with GA, it exhibited a stronger fluorescence intensity than zein/GA. The conformation of proteins was altered due to the interactions between proteins and other biopolymers [47]. Therefore, adding GA to the ethanol solution leads to a conformational change in zein.

3.5. Microstructural characterization of zein@T/GA nanoparticles

The microstructures of zein, zein/GA, and zein@T/GA nanoparticles were characterized using SEM (Fig. 4A) and AFM (Fig. 4B). The zein nanoparticles were spherical with a smooth surface and an average particle size of 119 nm, shown in Fig. 4A. However, the particles were observed to be aggregated and adherent, in contrast to previous reports that individual particles were observed. Such differences were probably because the high-energy method did not obtain the experimental zein particles using a high-pressure homogenizer [11,48]. However, the individual particles were still not visible when the zein was coated with GA. This is related to the remarkable adhesion between zein and GA induced by the high hydrophilicity of GA. Interestingly, the addition of T resulted in a clearer distribution of individual nanoparticles. This is because ultrasonic treatment enhances the interactions between substances, resulting in a more homogeneous and stable system for the smaller particles.

Additionally, 3D and 2D morphology images from AFM (Fig. 4B) further indicated that the particle size of zein@T/GA nanoparticles was smaller and less aggregated. The ultrasonic treatment causes structural changes in zein, reducing the particle size of nanoparticles and enhancing the stability of the particle system. This was consistent with GA’s ability to bind the zein through electrostatic interactions to form nanoparticles with higher stability [22].

Fig. 4. Surface of SEM (A), 3D and 2D topographic AFM image (B) of zein, zein/GA, and zein@T/GA.
3.6. Protective action of zein@T/GA on T in simulated gastrointestinal digestion

Fig. 5 shows the release profile of nanoparticles. The cumulative release of zein@T/GA nanoparticles was relatively small (28.49%) during the first 120 min. This is probably caused by the small amount of T present on the surface of the nanoparticles being released first. During 120–240 min, the nanoparticles showed an abrupt release of 76.43%. This is due to the degradation of zein@T/GA nanoparticles in the small intestinal environment and the release of T encapsulated within nanoparticles. The cumulative release rate increases smoothly from 240 to 360 min, reaching 80.69% for T at 360 min. The explanation for this could be that the hydrophilic shell (GA) prevented protein (zein) from enzymatic degradation in the gastrointestinal environment. Thus, zein/GA nanoparticles provide better protection for the encapsulated T, achieving slow-release effects. The results agreed with previous studies that zein/GA particles could be effective delivery vehicles for hydrophobic active compounds [22,31].

The inset indicated the results of Se content in different tissues of mice after 4 h of zein@T/GA nanoparticles infusion. This showed that T entered the mice and was rapidly distributed to the major issues in the body after digestion. The Se content of each major tissue was in the following order from highest to lowest: liver > kidney > cecum > spleen > stomach > colon > small intestine. Our research has shown that zein@T/GA potentially improves the bioavailability of Se-containing peptide T in mice. Compared with the long-term animal experiments, our study provides a fast and accurate animal experimental method for examining Se content across all the tissues in mice. However, further research is needed on how the exact form of Se enters each tissue.

3.7. Glutathione content in mice after oral administration of zein@T/GA

The effects of zein@T/GA nanoparticle masses on the GSH content in mice tissues are presented in Table 2. It was found that the Se content (Support Table 1) and GSH content in the tissues changed to different degrees with increasing the mass of zein@T/GA nanoparticles in different tissues. The GSH content in serum, liver, kidney, spleen, and thymus varied according to the mass of zein@T/GA nanoparticles. When the mass of zein@T/GA nanoparticles was 0, the GSH content in serum, liver, kidney, spleen, and thymus tissues was 51.26 ± 2.82, 50.52 ± 1.40, 26.32 ± 1.37, 34.81 ± 1.27, and 27.75 ± 1.91 μmol/L, respectively. As the nanoparticle mass was increased, the GSH in tissues increased dose-dependently. When the nanoparticle mass increased up to 0.5 g, GSH levels in serum, liver, kidney, spleen, and thymus tissues were 74.00 ± 1.01, 70.38 ± 1.87, 35.74 ± 1.26, 49.81 ± 0.47, and 35.28 ± 0.42 μmol/L, respectively. This is because Se exerts its antioxidant function mainly through glutathione peroxidase (GPX) and catalyzes the conversion of reduced glutathione to oxidized glutathione [49,50]. Therefore, as Se levels increase, glutathione levels also increase. In contrast, no significant change in GSH content was observed for heart, lung, and small intestine tissues due to increased nanoparticle mass. This is due to the different levels of GPX in different tissues.

The data were analyzed using the corrplot package in R to explore the relationship between the zein@T/GA nanoparticle mass and GSH content (Fig. 6). We found a positive correlation between GSH and Se contents for all tissues, excluding the heart. The zein@T/GA nanoparticles were digested and absorbed into the blood for mice, and then Se was delivered by the blood across the rest of the tissues to improve Se bioavailability. A significantly positive correlation of serum GSH, liver, spleen, and thymus were observed in the study. A reduction for glutathione protects the sulfhydryl-containing proteins and sulfhydryl-containing enzymes in cell membranes, improving the body’s immunity and scavenging the oxygen free radicals [51]. In our study, with increasing the Se content in mice tissues, the content of GSH in mice tissues also increased at different degrees. Therefore, the variation in the Se content for mice tissues can potentially affect the immunity of organisms in mice.

4. Conclusions

Ultrasound-assisted methods were considered potent for preparing stable and homogeneous zein@T/GA nanoparticles. Hydrogen bonding, hydrophobic interactions, and electrostatic repulsion were the main forces that affected the formation of zein@T/GA nanoparticles. Ultrasound treatment caused an increase in the EE of nanoparticles from 26.2% to 59.9%. The nanoparticles exhibited excellent properties after ultrasonic treatment, such as smaller particle sizes and higher stability. Additionally, it was also observed that the nanoparticles were homogeneous spheres with smooth surfaces. In the in vitro digestion experiments, the cumulative release rate of zein@T/GA nanoparticles was 28.49% in the first 2 h. This leveled off at the fourth hour and finally reached a maximum of 80.69% at the sixth hour. This gradient pattern for the cumulative release of zein@T/GA nanoparticles proves that our encapsulation method has a good application for improving the bioavailability of Se. Animal experiments revealed that the glutathione content in mouse tissues varied according to the Se content, indicating an improvement in the bioavailability of rice Se-containing peptide T. Additionally, this research demonstrates that the ultrasonicated polysaccharide/protein nanoparticle system has a good application for protecting the biological activity of small molecules. Furthermore, the development of this novel system provides a solid theoretical foundation for improving the bioavailability of active peptides in functional foods and pharmaceuticals.

CRedit authorship contribution statement

Xieqi Luo: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft. Fengjiao Fan: Conceptualization, Formal analysis, Writing – review & editing. Xinyang Sun: Writing – review & editing. Peng Li: Validation. Tong Xu: Validation. Jian Ding: Validation. Yong Fang: Supervision, Writing – review & editing, Project administration.
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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Table 2
GSH content in different mice tissues.

| zein@T/GA mass (g) | GSH content (μmol/L) |
|-------------------|----------------------|
|                   | Intestine | Serum | Heart | Liver | Spleen | Lungs | Kidneys | Thymus |
| 0                 | 0.21      | 0.63  | -0.38 | 0.69  | 0.65   | 0.47  | 0.35    | 0.55   |
| 0.01              |           | 0.70  | 0.98  |       | -0.31  | 0.10  | 0.96    | 0.74   |
| 0.05              |           | 0.98  |       | 0.08  | 0.98   | 0.08  | 0.98    | 0.97   |
| 0.1               |           |       | -0.30 | 0.98  | 0.98   | 0.13  | 0.98    | 0.88   |
| 0.2               |           |       |       | 0.13  | 0.88   |       |         |        |
| 0.5               |           |       |       |       |        |       |         |        |

Note: Data are means of six replicates. Values in the same column with different letters as superscripts are significantly different from each other according to Duncan’s test (P < 0.05).

Fig. 6. Correlation analysis of GSH and selenium content in mouse tissues. GSH: glutathione. The bubble size in the top right corner indicated the degree of correlation. The color red to blue indicated the correlation is good to poor. The lower-left corner showed the correlation coefficient value, ranging from [−1, 1], with larger absolute values indicating a stronger correlation. Negative values indicate negative correlations, while positive values indicate positive correlations.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2022.105923.

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