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The K\(^+\) and Mg\(^{2+}\) decreased the adsorption of soy hull polysaccharides on glycocholic acid *in vitro*

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Abstract: This study aimed to explore the effect of ion on the interaction between soy hull polysaccharides (SHP) and glycocholic acid (GCA). The determination of bile acids (BAs) binding rate, FT-IR, and zeta potential revealed that the binding rate of SHP to GCA (fell about 14 %), hydrogen bond peak area (fell about 149), and zeta potential (fell about 13 %) showed a sharp downward trend after K\(^+\) and Mg\(^{2+}\) treatment. However, the apparent viscosity increased and the chain structure became closer, as detected by shear rheology and AFM analysis. The root mean square deviation, radius of gyration, and root mean square fluctuation levels were estimated through molecular dynamic simulations, revealing that adding mixed ions decreased the stability of the SHP–GCA complex at 50 ns. Therefore, it was meaningful to study the effect of ion species in the intestinal environment on the binding of dietary fibers to BAs. The findings might guide the selection of other food types during polysaccharide intake.

Keywords: glycocholic acid; molecular dynamics simulation; soy hull polysaccharides.

1 Introduction

Several previous studies showed that the dietary fibers reduced the plasma cholesterol level in humans [1]. People interested in this phenomenon found that the key to reducing cholesterol by dietary fibers was to adsorb bile acids (BAs) [2]. However, many factors affected BA adsorption, such as the source of dietary fibers, physical-chemical properties of polysaccharides, and reaction environment [3–5]. Different bile salts have different binding affinities for pectin and cellulose with the secondary bond [6]. The secondary bond was greatly influenced by ionic strength. Ions played a key role in the intestine. The salt consumption altered gut bacterial diversity, and salt ions participated in maintaining the intestinal balance [7]. However, the effect of ions on the adsorption of BAs by dietary fibers was unclear. Also, it was difficult to study the effect of intestinal ions on the adsorption of BAs by dietary fibers *in vivo*.

The molecular dynamics simulation has proved to be an important tool to provide insights into the structural and dynamic properties of carbohydrates and protein, with the development of force fields for biological macromolecules and advances in modern computational methods in recent years [8–10]. Molecular modeling could observe explicit conformations of carbohydrates and identify the main structure of chains with desired properties [11, 12]. Previous studies also discussed the influence of ions on the simulation system. Smith et al. provided insights into the effects of Ca\(^{2+}\) and Na\(^+\) on a detailed model of the yeast plasma membrane to identify structural and dynamic changes [13]. As a result of CaCl\(_2\) and NaCl stresses, changes were seen in the surface, hydrogen bonding, transmembrane ergosterol positioning, and dynamics of the model yeast plasma membrane [13]. Gurtovenko et al. performed a molecular dynamics simulation on the effect of NaCl on dimyrystoylphosphatidylcholine and found that the change in salt-induced membrane dynamics was largely dependent on the composition of the membrane [14]. Therefore, the effect of metal ions on the adsorption of BAs by SHP could be clarified by molecular simulation technology and *in vitro* experiments.

Soy hulls have great potential to be used as a functional component due to its 59.9%–72.2% insoluble fibers and 3.9%–12.7% soluble fibers [15]. SHP is a soluble polysaccharide extracted from soy hulls, which has various biological activities [16]. We found that SHP improved the blood lipid level and gut microbiota structure of rats fed a high-fat and high-sucrose diet [17]. SHP also alleviated the symptoms of colitis in mice by delaying the absorption of BAs and preventing the development of intestinal mucosal
injury [18]. In addition, we found a hydrogen bond interaction between SHP and BAs; the force of glycocholic acid (GCA) was significantly higher than that of other BAs [19]. Therefore, we explored the effect of salt ions on the interaction between SHP and GCA.

2 Materials and methods

2.1 Preparation of SHP

Soy hulls (Heihe 43 soybeans) were obtained using a dry-peeling method (Yu Wang Group, Shan Dong, China). According to the earlier study [20], the soy hulls were dried and crushed to powder for 1% ethanol decolorization. The soy hull residues were dissolved in 2.5% sodium oxalate solution and then irradiated by a microwave (PJ23C-SCL, Midea Corp., Foshan, China) at for 85 °C 35 min. The solution was centrifuged at 4500 rpm for 10 min (Avanti J–25, Beckman, California, USA), and the supernatant was concentrated in a rotary evaporator (RE–3000A, Shanghai Yarong, China). Next, a double volume of 95% ethanol (v/v) was added. After, the precipitate was dried at 65 °C to produce SHP. The SHP was dialyzed for 7 d.

2.2 Combination of SHP with GCA

We added 10 mmol/L GCA (Yuanye Biotechnology Co., Ltd, Shanghai, China) to four types of 1% SHP solution and adjusted to pH 8.0. The SHP–GCA mixed system was constantly stirred and incubated for 0, 4, 8, and 12 h at 37 °C [21]. The four types of SHP solutions contained different types and concentrations of salt ions: 0 mM ions, 10 mM KCl, 10 mM MgCl₂, and 10 mM mixed ions (KCl:MgCl₂ = 10:3) reacted with GCA.

2.3 Determination of BAs binding rate (BBR)

The SHP–GCA mixed system was centrifuged at 5000 rpm (Avanti J–25, Beckman, California, USA) for 10 min [22]. Then, 7.5 mL 60% sulfuric acid solution and 2.5 mL supernatant were mixed for 20 min at 70 °C. After, the solution was put in ice-bath for 5 min. The absorbance of mixed system was detected at 387 nm. The BBR was calculated using the equation: BBR (%) = \[\frac{A_1 - (A_2 - A_0)}{A_0} \times 100\]. \(A_0\) = absorbance value of water + BAs; \(A_1\) = absorbance value of SHP + BAs; and \(A_2\) = absorbance value of water.

2.4 Determination of fourier-transform infrared spectroscopy (FT–IR)

The dried samples were ground with a required amount of KBr and pressed into a 1–2 mm sheet for FT–IR (Scimitar 2000, Agilent, USA) analysis at a frequency range of 400–4000 cm⁻¹ resolution.

2.5 Detection of zeta potential

The NANO–ZS90 potential (Brookhaven Instruments Corporation, Holtsville, USA) analyzer was used for detection. The test temperature was 25 °C, take 1 mL of sample in the sample dish, balanced it for 2 min, and collected ten continuous light scattering readings. Each sample need to be measured for 3 times.

2.6 Detection of shear rheology

The apparent viscosity of samples at 0, 4, 8, and 12 h was performed using a rotational rheometer (Discovery HR–1, TA Instrument, USA) at shear rate 0.01–100 s⁻¹. The mixed sample was loaded on a rheometer plate for linear shear with parallel-plate geometry, diameter 40 mm and gap 50 mm. Continuous measurement 30 data points for 300 s at 37 °C.

2.7 Atomic force microscope (AFM) analysis

The dried sample was dispersed in deionized water. 20 µg/mL samples were stirred for 24 h. 10 µL drops in the 20 µg/mL sample were moved directly to the surface of the freshly cut mica, and the sample surface was dried for measurement. The scanning probe microscopy photos were collected by using an AFM equipped with silicon cantilevers, and tapping AFM.

2.8 Preparation of materials

According to a previous reports, the SHP molecular chain structure was got using Discovery Studio (DS) 2017 R2 Client software [23]. The 3D molecular structure of GCA (ID: 10140) was obtained from the PubChem molecular library (Figure S1).

2.9 Molecular dynamic simulation analysis

The sugar chains and small target molecules were added with Gasteiger–Hücke empirical charge combined with nonpolar hydrogen, and the rotatable keys were set through AutoDockTools software. The σ bonds between heavy atoms were all set as rotatable bonds, while sugar chains were all considered rigid structures. The AutoDock Vina program was used to conduct a conformation search and energy optimization in 80 × 80 × 80 Å square box set on the sugar chain during docking. The calculation was stopped after the conformational refinement was achieved. The optimal conformation was selected based on the lower the energy and the more stable the structure.

The Sander module of the Amber Tools16 program carried out a 50-ns molecular dynamic simulation of the complex in an explicit solvent environment, which showed that potassium and magnesium ions with a ratio of 10:3 were added to the solvent. Before simulations, the structure of sugar chains and GCA were described by GLYCAM and GAFF force fields, respectively. First, we optimized the geometry of sugar chains and GCA, performed a molecular dynamic simulation of 60 ps at 310 K, and finally conducted a molecular dynamic simulation of the NPT ensemble with 50 ns. The long-range electrostatic interactions were calculated through the particle mesh Ewald method during the simulation process. The expansion vibration of all covalent bonds in hydrogen atoms were constrained by the SHAKE algorithm so as to extend the minimum step length. The SHAKE algorithm was used to...
process hydrogen scaling vibration (the simulation step length = 2 fs, the truncation value = 10 Å).

2.10 Statistical analysis

The data were expressed as a mean ± standard deviation (SD). The statistical analysis were performed using SPSS 20.0 (IBM Corporation, USA). Differences were statistically significant using one-way ANOVA of Duncan’s multiple range tests if P < 0.05.

3 Results and discussion

3.1 K⁺ and Mg²⁺ treatment decreased BBR

Previous studies found that the intestinal reabsorption of BAs was reduced due to the presence of dietary fibers [19, 24]. The different metal ions affected the interaction between SHP and GCA. As shown in Table 1, the binding rate of SHP to GCA increased with time at pH 8 simulating the intestinal terminal conditions. The addition of ions did not destroy this trend, but the binding rate decreased significantly than in the absence of ions. The binding rate of SHP to GCA showed a sharp downward trend after Mg²⁺ and K⁺+Mg²⁺ were added. After 12 h, the binding rate of SHP to GCA was 44.01% without ions, and the binding rates were 38.62%, 33.79%, and 30.95% after adding KCl, MgCl₂, and mixed ions, respectively. This was perhaps not surprising because Mg²⁺ was associated with the formation of free carboxyl groups in SHP, which promoted the formation of a gel structure, but the ability of K⁺ was weaker than that of Mg²⁺ [25]. Other studies also proved that the gelation of polysaccharides was related to the presence of cross-linking agents, all of which affected the gelation process [26]. Therefore, the binding rate decreased because GCA could not form hydrogen bonds with the hydroxyl terminal of SHP after adding metal ions.

3.2 K⁺ and Mg²⁺ treatment changed FTIR spectra of the SHP–GCA complex

The previous research results indicated that the interaction between SHP and GCA was mainly hydrogen bonding; therefore, the binding rate was affected by hydrogen bonding [19]. The strength of the hydrogen bond was obtained by studying the hydrogen bond –OH stretching region obtained in the FTIR spectrum, and the peak value was fitted to obtain the peak area [27]. The quantitatively measured hydrogen bond peak area is shown in Figure 1a–d. The hydrogen bond peak area showed an increasing trend without ions, which was consistent with the result of BA binding. It was found that, compared with the absence of K⁺ and Mg²⁺ ions, the hydrogen bond peak area decreased significantly after 4 h and continued to display a downward trend after adding ions. The hydrogen bond peak area decreased sharply after adding Mg²⁺ after 8 h. Hence, it showed that separately adding Mg²⁺ had the greatest effect on the hydrogen bonding of SHP and GCA. Wang et al. found that the free carboxyl groups in polysaccharides were involved in the gelation process. The combination of metal ions and free carboxyl groups facilitated the formation of gels [28]. In the presence of metal ions, SHP formed a gel structure, resulting in the reduction of free carboxyl groups and the formation of hydrogen bonds in GCA. The gel-promoting ability of the Mg²⁺ ion was stronger than that of K⁺ [29]. Therefore, adding Mg²⁺ and K⁺ reduced the hydrogen bond between SHP and GCA, decreasing the hydrogen bond peak area.

3.3 K⁺ and Mg²⁺ treatment decreased the zeta potential of the SHP–GCA complex

Zeta potential is an important measure of repulsion or attraction between particles. Figure 2a–d shows that the zeta potential value of the sample decreased rapidly after adding ions and changed most significantly after adding Mg²⁺. After 12 h, the zeta potential of samples with Mg²⁺, K⁺, and mixed ions was found to decrease to the lowest (~20.3, ~22.0, and ~20.5 mV, respectively). This trend could be predicted since an increase in salt led to the decrease in electrostatic repulsion and the corresponding reduction in zeta potential [30]. The ions caused the reduction of steric repulsion between sugar chains, and the effect of Mg²⁺ was the strongest. Therefore, the reason for the change in potential value was
the combination of the ions and carboxyl groups of polysaccharides, which was consistent with the binding rate and FT-IR results.

### 3.4 $\text{K}^+$ and $\text{Mg}^{2+}$ treatment changed the shear rheology of the SHP–GCA complex

The effect of $\text{K}^+$ and $\text{Mg}^{2+}$ on the viscosity of samples was studied by shear rheology analysis. As shown in Figure 3a–d, SHP exhibited the same trend under the action of $\text{K}^+$, $\text{Mg}^{2+}$, and mixed ions, and the apparent viscosity increased. The four samples exhibited non-newtonian behavior (shear-thinning). The research studies also showed the apparent viscosity of system decreased with the increase in the shear rate after ions treatment [25, 30]. It was the increase in viscosity that increased the binding rate to GCA, but it was only the physical effect that confirmed the previous speculation. Compared with $\text{K}^+$ and $\text{K}^+ + \text{Mg}^{2+}$, adding $\text{Mg}^{2+}$ increased the apparent viscosity of the sample most. The research showed that inorganic salt ions affected the polyelectrolyte by blocking the electrostatic repulsion between charged groups on the polymer chain, ultimately leading to an intermolecular association and increasing viscosity [31]. The reduction of electrostatic repulsion between SHP chains was caused by the combination of metal ions and carboxyl groups, which was the same as the previous conclusion.

### 3.5 Analysis of the SHP–GCA complex structure after $\text{K}^+$ and $\text{Mg}^{2+}$ treatment

The effect of metal ions on the sample structure was observed through AFM. Figure 4a shows a relatively loose chain structure, with a large gap between the chains without the presence of metal ions.
Figure 2: Zeta potential of interaction between SHP and GCA after K\(^+\) and Mg\(^{2+}\) treatment. a: SHP–GCA; b: K\(^+\)+SHP–GCA; c: Mg\(^{2+}\)+SHP–GCA; d: K\(^+\)+Mg\(^{2+}\)+SHP–GCA. Different lowercase letters represent significant differences in different times (P < 0.05).

Figure 3: Viscosity of interaction system of SHP and GCA after K\(^+\) and Mg\(^{2+}\) treatment. a: SHP–GCA; b: K\(^+\)+SHP–GCA; c: Mg\(^{2+}\)+SHP–GCA; d: K\(^+\)+Mg\(^{2+}\)+SHP–GCA.
of metal ions. However, Figure 4b–d shows that the chain structure of the sample became closer under the influence of metal ions. Some studies proved that metal ions contributed to the aggregation between polysaccharide chains and formed network structures more efficiently [32]. As shown in Figure 5c, Mg²⁺ had the greatest impact on the sample. A very tight structure was formed between polysaccharide chains with fewer gaps, which also increased the viscosity of the sample. At the same time, this also explained the decrease in the hydrogen bond peak area shown by the infrared results. Mg²⁺ made the gap between polysaccharide chains smaller, resulting in the extrusion of GCA bound to SHP.

3.6 Analysis of the SHP–GCA docking complex conformation

Molecular dynamic simulations were also used to verify the stability of the SHP–GCA complex. The binding conformations of the SHP–GCA complexes were shown before and after K⁺ + Mg²⁺ treatment at 50 ns (Figure 5a and b). When the time is greater than 50 ns, the SHP–GCA docking complex is unstable. Root mean square deviation (RMSD), radius of gyration (Rg), and root mean square fluctuation (RMSF) levels were estimated to further determine the conformational changes in the complex.
Figure 5: Conformation and flexibility analysis of the SHP–GCA docking complex through molecular dynamic simulation. a–b: Conformational changes of the SHP–GCA docking complex before and after K⁺ and Mg²⁺ treatment at 50 ns. c–d: RMSD of SHP–GCA docking complexes before and after K⁺ and Mg²⁺ treatment at 0–50 ns. e–f: Rg of SHP–GCA docking complexes before and after K⁺ and Mg²⁺ treatment at 0–50 ns. g–h: RMSF of SHP GCA docking complexes before and after K⁺ and Mg²⁺ treatment at 0–50 ns. i–j: Energy of SHP GCA docking complexes before and after K⁺ and Mg²⁺ treatment at 0–50 ns. k–l: Hydrogen bond number of SHP GCA docking complexes before and after K⁺ and Mg²⁺ treatment at 0–50 ns.
The RMSD was used to assess the structural deviations from initial structures. It was calculated using the backbones and considering the atoms in the simulation runs [33–35]. As displayed in Figure 5c, a fluctuating trend of the RMSD value of the SHP–GCA docking complex was observed from 5 Å to 17 Å in 1–40 ns, and ΔRMSD values fluctuated within 2 Å in 40–50 ns, indicating that the complex structure achieved a steady state. However, a more violent fluctuating trend of the RMSD value of the SHP–GCA docking complex was observed in 1–40 ns after adding mixed ions, and subsequently, ΔRMSD values fluctuated within 2 Å in 40–50 ns (Figure 5d). Although both were stable in 40–50 ns, adding mixed ions improved the RMSD value of the SHP–GCA complex to about 20 Å, and the high RMSD value was not conducive to the stability of the complex. According to the calculated RMSD values, the fluctuation caused by ions decreased the structural stability of the SHP–GCA complex, which was not conducive to the combination of SHP and GCA.

Furthermore, the Rg of the SHP–GCA complex was analyzed to understand the structural compactness of conformation. Figure 5e clearly shows that the Rg values of SHP decreased from 25 Å to 20 Å in 1–50 ns, indicating that the complex structure achieved a steady state. However, a more violent fluctuating trend of the RMSD value of the SHP–GCA docking complex was observed in 1–40 ns after adding mixed ions, and subsequently, ΔRMSD values fluctuated within 2 Å in 40–50 ns (Figure 5f). Although both were stable in 40–50 ns, adding mixed ions improved the RMSD value of the SHP–GCA complex to about 20 Å and the high RMSD value was not conducive to the stability of the complex. According to the calculated RMSD values, the fluctuation caused by ions decreased the structural stability of the SHP–GCA complex, which was not conducive to the combination of SHP and GCA.

The RMSF is an important indicator for residue flexibility, indicating the flexibility of the material [37, 38]. The fluctuation trend of RMSF of the SHP–GCA complex in the range of 0–50 ns is shown in Figure 5g and h. Notably, the RMSF of the complex after K⁺ and Mg²⁺ addition showed obvious fluctuations. The higher RMSF of the residue in the sequence, the more unstable the system (Ma et al., 2020). Some residues had a higher RMSF value in the SHP–GCA complex after K⁺ and Mg²⁺ treatment. The change in the SHP–GCA complex might be due to coordination bond cross-linking.

The energy and hydrogen bond number of the SHP–GCA complex are shown in Figure 5i. K⁺ and Mg²⁺ diffusion might be around –OH of polysaccharides, resulting in a decrease in the energy and hydrogen bond number in the range of 0–50 ns. The stability of the SHP–GCA complex decreases. This is consistent with the FT-IR results.

4 Conclusions

The hydrogen bond peak area, and zeta potential value showed a sharp downward trend of the SHP–GCA complex after K⁺ and Mg²⁺ treatment. However, the apparent viscosity increased and the chain structure of the sample became closer. The calculated RMSD and Rg values showed that the mixed ions decreased the structural stability of the SHP–GCA complex. Some residues had a higher RMSF value in the SHP–GCA complex, thereby improving complex flexibility. Therefore, it was meaningful to study the SHP to BAs. The findings might guide the selection of other food types during polysaccharide intake.

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