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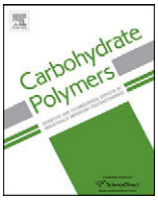

Molecular simulation of the complex of konjac glucomannan–borate in water

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ABSTRACT

The formation of konjac glucomannan–borate complex in water solution has been investigated by experimental and molecular simulation methods. The energy, radical distribution function of borate anion ($B(OH)_4^-$), and mean square displacement of the complex were studied during the molecular simulation, and the results indicate that one type of helical complex can be formed based on the hydrogen bonds between borate anion and helical chain of KGM. The hydrogen bond is formed by the interaction of borate anion and –OH groups on C (6) of mannose and glucose. Temperature has little effect on the helical conformation of the complex. It was proposed that the complex can form gel through the aggregation arrangement of helical chain.

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1. Introduction

Konjac glucomannan (KGM) is an essential polysaccharide that is the main component of the konjac flour produced from the tubers of *Amorphophallus konjac* C. Koch. It consists of â-1-4-linked glucose and mannose units, and the ratio of glucose: mannose is between 1:1.5 and 1:1.6 (Pang, Lin, & Zhang, 2003). KGM could form gel with the metal salts which could be hydrolyzed into multi-hydroxy hydrate (Li, Wang, & Sun, 2003). Borate is a good buffer agent, which could be fully hydrolyzed into borate anion ($B(OH)_4^-$) in water solution. Because of the tetrahedron conformation, borate anion could coordinate with KGM molecular chain easily to form network structure, and finally form gel (Li et al., 2003a; Li, Pang, & Liao, 2004).

In our previous work, the structure of KGM–borate complex has been studied by ^{11}B NMR, ^{1}H NMR, IR, and Raman characterizations (Li et al., 2004). The characteristic vibration of B–O bound in 1380 cm^{-1} in infrared spectrum, and B–H vibration in 2640 cm^{-1} in Roman spectrum indicate that the coordination complex is formed. The main reaction site was the hydroxyl at C (6) of mannose and glucose, and the hydroxyl at C (2), C (3) of mannose. One type of intramolecular complex can be formed when the ratio of KGM:borate anion was 1:1. Because of the diversity of helical conformation of KGM in solution, and the limit of traditional experimental methods, the conformation and formation mechanism of KGM–borate complex have not been fully understood (Li & Xie, 2003; Yui, Ogawa, & Sarko, 1992; Ogawa, Yui, & Mizuno, 1991).

So far, molecular docking and molecular dynamic simulation method have been widely used in the study of macromolecule conformation and the interaction with their complexes (Pang, Sun, & Guan, 2005; Pang, Sun, & Sun, 2006; Pang, Xu, & Bai, 2009). In the previous studies, we investigated the formation mechanism, formation sites, and microscopic conformation of KGM molecular helices by molecular dynamics and experiment (Jian, Yao, & Wang, 2010). In this work, we investigated the conformation, potential energy, and radical distribution function of the KGM–borate complex using molecular dynamic simulation in order to disclose the coordination and gelatin mechanism. This work will provide a new method and theoretical guide for the coordination mechanism between natural biological macro-molecules and inorganic molecules.

2. Method

2.1. Establishment of KGM model

Based on the primary structure of KGM (Fig. 1), the primary model of left-handed helical KGM was established by Build Biopolymer Module in Sybyl 8.1 software, using the dihedral angle of glycoside bond $\varphi = -123$, $\psi = -90$ as parameters. The primary model was optimized by molecular mechanics, followed by the molecular dynamics simulation on O2 workstation. The parameters in molecular dynamics were set as follows: isothermal system with $T = 300$ K, relaxation method utilized to adjust the tempera-

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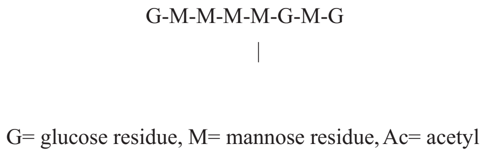


Fig. 1. Structural composition diagram of KGM.

ture, integration step of calculation to 0.001 ps. The system was run for 1000 ps.

2.2. Establishment and optimization of borate anion model

The primary model of borate anion was established in Sybyl 8.1 software, and was optimized by molecular mechanics and molecular dynamics simulation. Parameters in molecular dynamics simulation on Borate anion were set as follows: isothermal system with $T = 300$ K and coupling of 0.1 ps with cutoff = 8, dielectric constant = 1.0, integration step of calculation to 0.001 ps. The system was also run for 1000 ps.

2.3. Molecular docking

Molecular docking was carried out by Surflex-dock module in Sybyl 8.1 software, using the KGM model as receptor and borate anion model as ligand, respectively. The intramolecular KGM–borate complex was achieved after docking. Parameters for docking were set as follows: surflex-dock mode with automatic protomol generation, and threshold value = 0.50 with bloat = 0.

2.4. Molecular dynamics simulation on the complex

Molecular dynamics simulation was carried out on the complex model in periodic boundary conditions in water solution with Tripos force field. The parameters for molecular dynamics simulation were set as follows: algorithm for solvating: silverware, temperature: 300 K, initial velocity: Boltzmann, step: 0.001 ps, length: 50,000 ps, dielectric constant: 1.0, the length of box was 30.99187, respectively, in three dimensions.

2.5. Preparation of KGM–borate complex solution

One gram of purified KGM was added into 1000 ml water and dissolved completely. The solution was filtrated. Then 10 ml 1% (w/v) borate solution was added into the filtrate in a stirring condition. After stirring for 1 h and then placed for 24 h, the complex solution was obtained.

2.6. Optical rotation analysis on complex solution

Optical activity of KGM–borate complex solution was measured with 1 dm polarization tube using sodium light at 293 K. Distilled water was used as blank. Optical activity was calculated by the following equation:

$$\text{specific optical rotation} = [\alpha]_{\lambda}^{t} = \frac{\alpha}{L \times c}$$

where L is the length of polarization tube(dm) and c is the concentration of sample.

3. Results and discussion

3.1. Energetic curve of complex during MD simulation

As suggested in Fig. 2, the potential energy of the complex increased sharply in the first 10 ns because of heightening tempera-

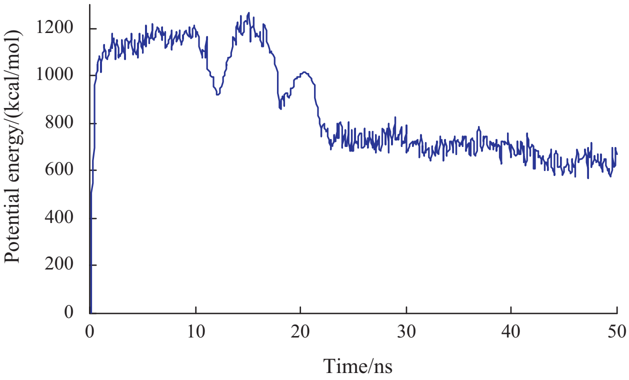


Fig. 2. Energy curve of the KGM–borate complex.

ture. An energy oscillation can be observed during 10–23 ns, which may be the result of the conformation adjustment of the complex, and the non-bounding interaction between KGM and borate anion, when borate anion began to enter the helical cavity of KGM. Finally, the energy declined, and became stable (about 620 kcal/mol) due to the formation of complex with optimized conformation between 30 ns and 50 ns.

3.2. Radius of gyration of the complex

Radius of gyration is a physical quantity which shows the molecular coil dimension during the MD simulation. The definition is shown as follows:

$$R_{gyr}(t) = \left[\frac{1}{N_{at}} \sum_{i=1}^{N_{at}} (r_i(t) - r_{CM}(t))^2 \right]^{1/2}$$

Here $r_i(t)$ means the coordinate of atom (i) at time t and $r_{CM}(t)$ means coordinate of the molecule at time t; N_{at} means the total number of atoms in the system.

As shown in Fig. 3, the radius of gyration of the complex increased in the first 10 ns, and then descended in a periodic changing mode. It kept constant between 6.4 and 6.6A after 40 ns. Its changing trend was similar to that of the potential energy. Both indicated the interacting process of KGM and borate anion. In this process, the molecular chain of KGM expanded appreciably to facilitate the entering of borate when borate approached KGM's helical cavity. The molecular chain shrinked slightly when the system reached the dominant conformation.

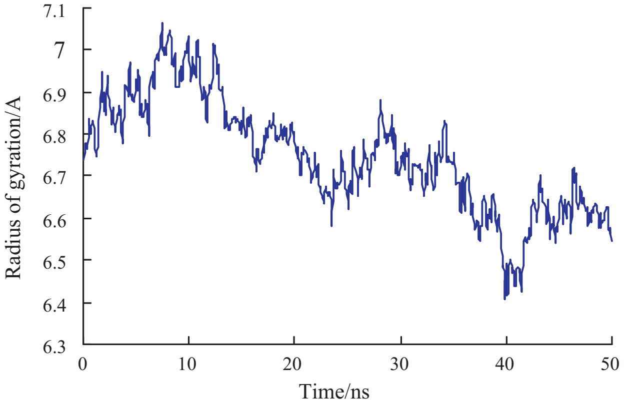


Fig. 3. The change of radius of gyration with time.

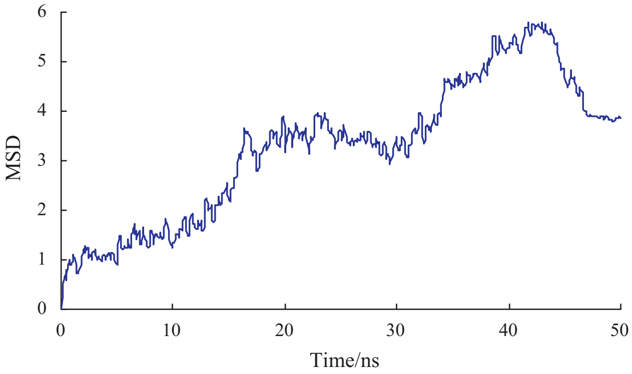


Fig. 4. Mean square displacement of borate anion.

3.3. *Mean square displacement of borate anion*

Molecular trajectory could be described by mean square displacement (MSD) with the following formula:

$$\left\langle X^2(t) \right\rangle = \frac{1}{N_m N_{t0}} \sum_m \sum_{t0} [X_i(t+t_0) - X_i(t_0)]^2$$

In this case, N_m is the number of absorbed molecules; N_{t0} is the benchmark time for calculation of average time; X_i is the mass abundance centric coordinates of molecule *i*.

MSD could be used to judge the freedom of molecular motion, whether the ligand was restricted by other factors or not. Fig. 4 shows the MSD of borate anion calculated in the MD simulation. The value increased gradually with the process, and reached maximum at 42 ns. Afterward, it descended significantly, and the complex approached the dominant conformation. It could be found from Fig. 4 that the movement of borate was restricted within the helical cavity of KGM when the systems became stable.

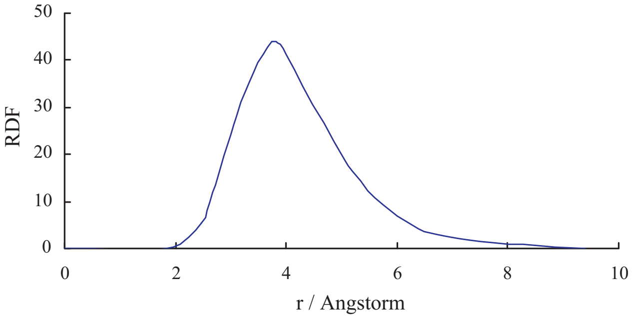


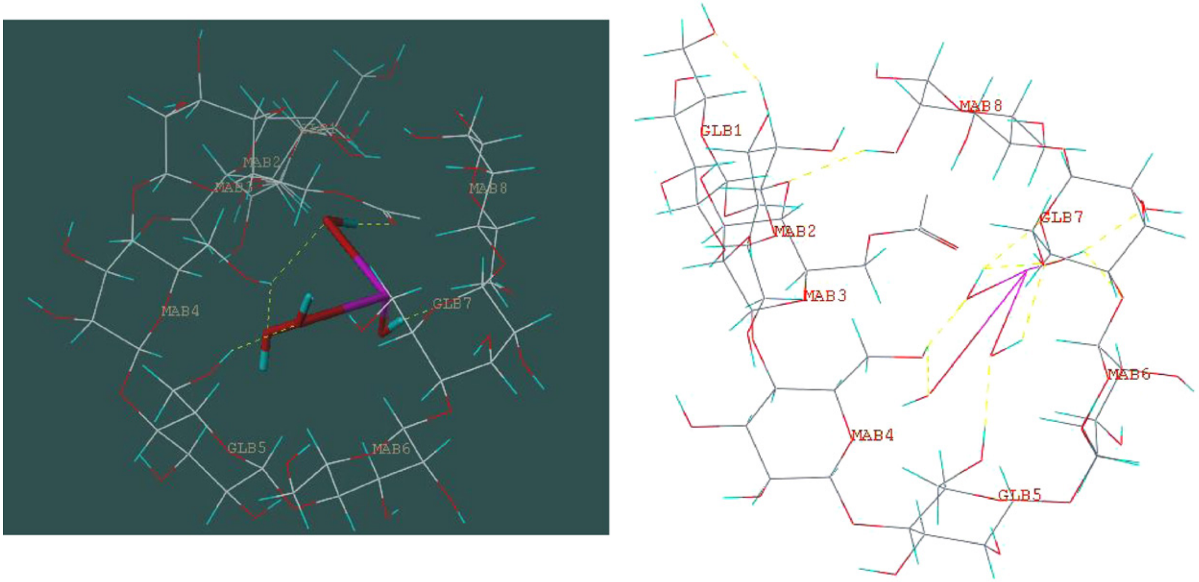
Fig. 5. Radical distribution function of borax ion and KGM.

3.4. *Radical distribution function of borate anion and KGM*

The population probability of Borate anion around KGM molecule could be determined via the calculation of Radical distribution function. As shown in Fig. 5, there was a strong peak at around 3.9 Å, which was smaller than the helix diameter (about 8.3 Å) of KGM molecule. So it was proposed that borate anion was distributed within the helical cavity of KGM to form the stable KGM–borate complex.

3.5. *Microscopic structure and the non-bonding interaction of the complex*

Fig. 6A shows the microscopic structure of the KGM–borate complex after the MD simulation. It could be seen from Fig. 6A that borate anion entered the helical cavity of KGM, and embedded in the KGM chains. The hydroxyl of $B(OH)_4{}^-$ formed hydrogen bond with the acetyl, hydroxyl of C6 of mannose and glucose, and O5 of mannose. It was also found that the molecular chain of KGM preserved helical conformation after MD simulation. So as to investigate the effect of the non-bonding interactions including van der waals force, hydrogen bond and electrostatic potential on the complex, the non-bonding interactions at different sim-



A
at 300 K

B
at 343K

Fig. 6. Microscopic structure of the complex after MD simulation at 300 K and 343 K, respectively.

Table 1
Comparison of non-bonded function of complex in solution.

Time	Vdw	H-bond	Electrostatic
$t = 10$ ns	48.328	2221.28	359.26
$t = 30$ ns	57.435	2376.91	366.56
$t = 50$ ns	55.671	2198.34	426.87

ulation period were calculated, and shown in Table 1. Among three kinds of nonbonding interactions, hydrogen bond exhibited the most significant effect on its configuration, and the electrostatic force played a less significant, and the van der waals came last.

3.6. Effect of temperature on the complex conformation

In order to investigate the rule of temperature on the chain structure and helical conformation, molecular dynamic simulation at different temperatures and optical rotation analysis were carried out on KGM–borate complex in water solution under periodic boundary condition. As shown in Fig. 6, at low temperature, KGM chain exhibited helical structure, and with the increase of temperature, the helical structure was preserved. Temperature had little effect on the helical conformation of the complex. However, the hydrogen bond site changed as temperature increased. The hydrogen bond site between acetyl of KGM and hydroxyl of borate anion disappeared, while the site between hydroxyl of C6 at mannose, glucose and hydroxyl of borate anion maintained. Furthermore, the total number of hydrogen bond changed slightly.

Optical rotation activity is necessary for the survival of helical structure; the measurement of optical rotation can give the information of molecular conformation conversion (Jian, Yao, & Wang, 2010). As shown in Fig. 7, in water solution KGM–borate complex exhibited optical rotation activity, which was in accordance with the results of KGM solution. With the heightening of temperature, the optical rotation activity descended slightly, going against the results of KGM solution (Jian, Yao, & Wang, 2010). When the temperature reached 341 K, the optical rotation activity of KGM in water disappeared completely. The results suggested that high temperature could not destroy the helical conformation of complex, and the gel mechanism of the complex may be different from that of KGM under alkaline condition. When the acetyl was removed from KGM, the helical conformation of molecular chain changed into random coil, and then the KGM molecular clew twisted and formed gel (Li & Xie, 2002). In this work, it was speculated that the complex may assemble side by side and form gel at high temperature.

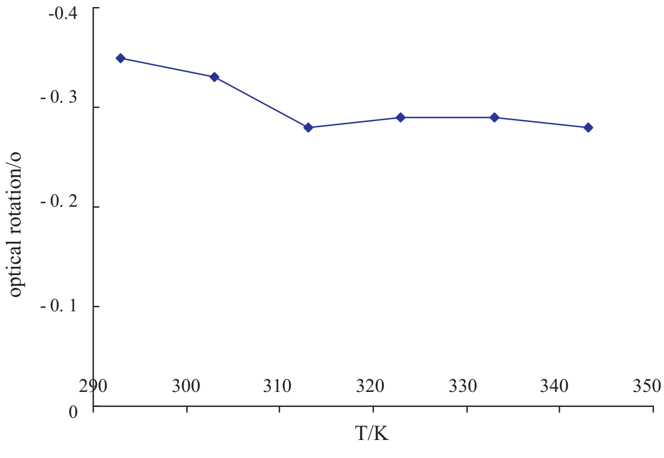


Fig. 7. Effect of temperature on the optical rotation of the complex.

4. Discussion

Much attention has been paid to the chain structure of KGM in water, but the description of its real secondary helical structure, especially the complex conformation with inorganic molecules has not been deeply studied (Pang et al., 2003). So far, the properties of the KGM–borate complex have been fully studied. For example, Li B. prepared KGM–borate gel, and investigated the textural properties, rheological properties, and viscoelasticity, etc. (Li, Wang, & Wu, 2003). The chemical structural information of KGM–borate complex was also obtained by means of infrared spectrum, nuclear magnetic resonance, mass spectrometry, etc. However, the complexity of KGM conformation in water and limitation of traditional spectrum means led to the infancy studying stage of the field.

In this work, a combination study of molecular dynamic simulation and optical rotation experiment was carried out to investigate the effect of temperature on KGM–borate complex helical structure. To our interest, we not only invalidated the previous study results (Li et al., 2004), but also found that KGM could conform stable helical complex with borate anion, and temperature had little effect on the stability of the complex, which had not been reported before. Based on these results, it was speculated that the gel mechanism of the complex may be different from that of KGM under alkaline condition. The previous studies showed that the helical conformation of molecular chain changed into random coil, once the acetyl was removed from KGM, and then the KGM molecular clew twisted and formed gel (Li & Xie, 2002). According to the thermal stability of the complex, and the other polysaccharide gel mechanism, we proposed that the KGM–borate complex may assemble side by side in helical conformation and form gel in high temperature.

Sun also studied the interaction of KGM and borate by molecular dynamics simulation. However, the initial helical model of KGM has not been established and the model is arbitrary to some extent. Furthermore, the run time was not long enough, so it could not draw conclusions that system had achieved equilibrium. In this work, MSD was used to determinate the equilibrium of the complex. This process made the results persuadable. Meanwhile, the dynamics process and energy change during the combination of KGM and borate anion were also fully studied.

In summary, our work may provide a theoretical guide for the study of KGM–borate helical structure and its gel mechanism. Moreover, it might pave a new way for the study of interaction between polysaccharide and inorganic molecules.

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