Carboxymethyl modification of konjac glucomannan affects water binding properties

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A B S T R A C T

The water binding properties of konjac glucomannan (KGM) and carboxymethyl konjac glucomannan (CMKGM) are important for their application in food, pharmaceutical, and chemical engineering fields. The equilibrium moisture content of CMKGM was lower than that of KGM at the relative humidity in the range 30–95% at 25 °C. The water absorption and solubility of CMKGM in water solution were lower than that of KGM at 25 °C. Carboxymethyl modification of KGM reduces the water adsorption, absorption, and solubility. Both carboxymethylation and deacetylation could confer hydrophobicity for CMKGM. These data provide the basis for expanding CMKGM application.

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1. Introduction

Konjac glucomannan (KGM) is a high molecular weight, water-soluble, and non-ionic natural polysaccharide derived from roots and tubers of \textit{Amorphophallus konjac} K. Koch (Chua et al., 2012; Williams et al., 2000). KGM is a linear polysaccharide, consisting of \( \beta \)-D-glucose and \( \beta \)-D-mannose residues in a molar ratio of 1:1.6 linked by \( \beta \)-1,4-glycosidic bonds, the acetyl groups along the KGM backbone are located, on average, every 9–19 sugar units at the C-6 position (Katsuraya et al., 2003).

KGM are widely used in food, pharmaceuticals, and chemical engineering due to their specific physical and chemical properties (Alonso-Sande, Teijeiro-Orsorio, Remuñán-López, & Alonso, 2009). However, some potential applications of KGM are limited by its high water absorption index, as high as 100 g water/g sample (Koroskenyi & McCarthy, 2001). To reduce hydration of KGM in aqueous solution, chemical modification of KGM through acetylation has been done, and acetylated konjac glucomannan exhibits lower water absorption comparing to KGM (Enomoto-Rogers, Ohmomo, & Iwata, 2013; Huang, Takahashi, Kobayashi, Kawase, & Nishinari, 2002; Koroskenyi & McCarthy, 2001).

Carboxymethyl konjac glucomannan (CMKGM) is the carboxymethyl modification of KGM. CMKGM and CMKGM derivatives have proven to be a promising new biodegradable material for the preparation of biodegradable films (Tang, Du, Zheng, & Fan, 2003; Wang et al., 2014), a biomaterial in the drug delivery systems (Du et al., 2005; Ha et al., 2011), for enzyme encapsulation (Li et al., 2011), and adsorption of heavy metal ions from aqueous solution (Niu, Wu, Wang, Li, & Wang, 2007). When these biomaterials are exposed to water solution, interactions between CMKGM-based materials and water will influence the properties of materials, e.g., moisture resistance of film, controlled release of drug, and enzyme activity. CMKGM (degree of carboxymethylation = 0.29) and soy protein isolate blend films were reported in our previous work (Wang et al., 2014), the results showed that the water adsorption of the CMKGM/SPI films progressively decreased with increasing CMKGM level, and the surface wettability of the blended films was improved with increasing CMKGM content, but the author did not give explanation for moisture resistant of films. CMKGM, as one of film components, its water binding properties inevitably influence moisture sorption of films. Hence, CMKGM-water binding properties are fundamental information for understanding material function and application.
Therefore, the objective of this work was to evaluate the water binding properties of CMKGM. Moisture adsorption isotherms, water absorption and solubility in water solution of KGM and CMKGM were determined. Mechanism of carboxymethyl modification of KGM reducing the water binding was proposed.

2. Materials and methods

2.1. Purification of native konjac glucomannan

The native konjac glucomannan was a gift from Wuhan Li Cheng Industry Co. Ltd. (Wuhan, China) and further purified by the method of Chua et al. (2012). Briefly, KGM (1.00 g) was stirred in 50% (v/v) ethanol (100 mL) for 90 min at room temperature, followed by centrifugation (5000 × g; 30 min, 25 °C) to remove the aqueous ethanol. Centrifugal sediment was added to deionized water (200 mL) and stirred for 3 h at room temperature, followed by KGM precipitation with absolute ethyl alcohol (800 mL), centrifugation, dehydoration, and oven-drying. The purified KGM was referred to as KGM-0.

The glucomannan content of KGM-0 was determined using 3, 5-DNS colorimetric assay according to the methods of Agricultural Standard of People’s Republic of China (Chinese, 2010). The total starch content in KGM-0 was quantified using the total starch assay kit (Megazyme, Bray, Ireland) according to the manufacturer’s instructions. The total nitrogen in KGM-0 was determined by Kjelldahl method (AOAC, 2000a: method 979.09) using a protein analyzer (K9860 Kjeltec Analyzer, Hanon Instruments, China), and recalculated into proteins by multiplying it by a factor of 6.25. Moisture and ash in KGM-0 were determined using AOAC methods (AOAC, 2000b: method 945.38).

2.2. Preparation of carboxymethyl konjac glucomannan (CMKGM)

Carboxymethyl konjac glucomannan (CMKGM) was prepared by the method of Zhou et al. (2006) with minor modifications. Briefly, one gram KGM-0 was added to 200 mL deionized water with stirring at 200 rpm for 2 h. Then sodium acetate dissolved in 40 mL 70% (v/v) ethanol was added. After 1 h, 0.6 g monochloracetic acid dissolved in 25 mL 70% (v/v) ethanol was added. After a few hours of chemical reaction, the product was precipitated with 80% (v/v) aqueous ethanol, then washed with 50% (v/v) aqueous ethanol until there was no residual chloridion in the filtrate, filtered off and dried. CMKGK samples with different substitution degrees were obtained by the change of the reaction temperature (50, 60, and 70 °C), the dosage of sodium acetate (2 and 4 g), and the reaction time (1, 2, and 3 h). The degree of substitution (DS) of carboxymethylated KGM samples was determined by the potentiometric titration according to a previous report (El-Sherbiny, 2003). The carboxymethylated KGM samples with different DS were referred to CMKGM-1, CMKGM-2, and CMKGM-3, respectively.

The weight percent of acetyl-substituted residues in the KGM backbone was determined by a published method (Chua et al., 2012; Chen, Zong, & Li, 2006), with minor modifications. Briefly, the solutions of Ca. 0.4 mol L⁻¹ KOH and 0.2 mol L⁻¹ HCl were prepared. Potassium hydrogen phthalate (PHP) (1000 g) was dissolved in de-ionized water and titrated with KOH solution using phenolphthalein indicator. The titration volume was recorded as Vₐ (mL) and the mass of PHP was recorded as mPHP (g). HCl solution (10.00 mL) was titrated with KOH solution using phenolphthalein indicator and the titration volume was recorded as Vₐ (mL). The sample (mₛ = 1.000 g) was stirred in de-ionized water (250 mL) for 3 h at room temperature. The solution was titrated with Ca. 0.1 mol L⁻¹ KOH using phenolphthalein indicator to a permanent pink color (pre-neutralization). KOH solution (10.00 mL) was then added and the mixture stirred for 3 h at room temperature. The excess alkali was back titrated with HCl and the titre recorded Vₐ (mL). The solution was stood for 2 h, and then any additional alkali, which may have leached from the sample, was titrated if pink color of the solution appeared again. A blank (to which no sample had been added) was titrated in parallel and the titre recorded Vₐ (mL). The content of acetyl groups in the samples was calculated by evaluation of Eq. (1).

\[
\text{Acetyl content(%) } = \frac{430 \times m_{\text{PHP}} \times (V_a - V_{\text{ca}})}{204.23 \times V_a \times m_s}
\]  

(1)

2.3. Determination of particle size, molecular weight, and apparent viscosity of KGM and CMKGM

The KGM and CMKGM samples were ground with mortar and pestle, and then passed through a 0.15 mm sieve (Cole-Parmer). The powder was suspended in 95% (v/v) ethanol, and then particle size and specific surface area of the powder were measured using BT-9300H Laser Particle Size Analyzer (Dandong BetterSize Instruments, Dandong, China) at 25 °C.

Molecular weight was measured by size exclusion chromatography (Shodex SB 805HQ, Showa Denko America, New York, NY) coupled on-line to a MALS system with the detector Dawn Helos II and Optilab rEX (Wyatt Technology Corporation, Santa Barbara, CA). The samples were dissolved in 0.1 mol L⁻¹ NaCl solutions and filtered with 0.45 μm pore size filter (Millipore). The concentration of samples was from 0.1 to 0.5 mg mL⁻¹. The injected volume was 200 μL. The flow rate was 0.40 mL min⁻¹. The columns temperature was maintained at 25 °C. The data obtained with MALS detectors were analyzed using ASTRA software (version 4.90.07 for Windows, Wyatt Technology Corporation, Santa Barbara, CA).

Stock solution of samples (1%, w/w) was prepared by adding sample (0.5 g) to de-ionized water (49.5 g), the mixture was stirred for 12 h at 25 °C. The apparent viscosity was performed using a rotational viscosity meter (NDJ-8S, Shanghai Hengping Scientific Instrument, Shanghai, China) at 25 °C. The viscosities of KGM-0 and CMKGM 1–3 were measured with a No. 3 rotator set at 30 rpm and a No. 1 rotator set at 12 rpm, respectively.

2.4. Dynamic vapor adsorption of KGM and CMKGM

The water sorption isotherms of KGM-0 and CMKGM samples 1–3 were determined using a DVS Intrinsic apparatus (Surface Measurement Systems, London, UK). The sample (Ca. 6 mg) was placed in a sample pan and exposed to a relative humidity (RH) range from 0 to 95% (RH = 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 95%, respectively). Mass equilibrium at each humidity stage was reached by measuring the percentage rate of change in mass with time (dm/dt). Once the dm/dt was below a predetermined threshold value (dm/dt = 0.002%/min) over a 10 min period and equilibrium was achieved at each humidity stage. All measurements were performed at 25 °C. Experimental data points were collected and plotted as an isotherm using Microsoft Excel 2010 together DVS Standard Analysis Suite Version 6.3.

Only one measurement was carried out for each sample in the current study. Because DVS method has high data reproducibility and can provide accurate isotherms at any desired relative humidity (0–95%) with short measurement time at different preset isotherm temperatures, the highly reproducibility of the data using this DVS technique is generated (Hill, Norton, & Newman, 2009), and the water adsorption isotherms of KGM-0 gave high reproducibility in this study (data not shown).
2.5. Water adsorption isotherms analysis using GAB model

A variety of mathematical models have been established and applied to interpret the moisture adsorption isotherms of foods, most of them tend to be modified versions of the Brunauer, Emmett and Teller (BET) and Guggenheim, Andersen and de Boer (GAB) equations, the GAB sorption equation is an improvement on the BET model and successful in overcoming many of the limitations of the original BET formulation for modeling and correlating water adsorption isotherms in foods and other materials (Vasquez, Braganza, & Coronella, 2011). The GAB adsorption equation can be applied over a wider range of water activity intervals than can the BET equation (Basu, Shivhare, & Mjumdar, 2006; Timmermann, 2003; Timmermann, Chirife, & Iglesias, 2001). So the water adsorption isotherms of KGGM-0 and CMKGM samples 1–3 were analyzed using GAB model in this study. GAB model described by Eq. (2) was applied to fit the experimental data.

\[
M = \frac{M_0 \times Ck \times RH}{(1 - kRH)(1 - kRH + CkRH)} \tag{2}
\]

where, \(M\) is the equilibrium moisture content (g water/100 g dry weight basis) at the relative humidity (RH), \(M_0\) is the monolayer moisture content (g water/100 g dry weight basis), \(C\) is the energy constant related to the difference between the free enthalpy of the water molecules in the pure liquid state and in the monolayer, it is proportional to the rate between both the attachment and the escape rate constants of the primary sites, \(k\) is the ratio between the the standard vapor pressure of the liquid and the vapor pressure of the sorbate in the secondary (upper) layers, it is proportional to the rate between the attachment rate constant and the escape for all higher layers.

The data were processed using OriginPro 8.0 software (OriginLab Corporation, USA). A non-linear least squares fitting (NLSF) method was performed to compute the parameters for the GAB equation. The coefficient of determination (R²) and mean relative deviation modulus (E) were used to evaluate the goodness of fit of the GAB model. The E value was calculated by Eq. (3).

\[
E = \frac{100}{N} \sum_{i=1}^{N} \left| \frac{M_{ei} - M_{pr}}{M_{ei}} \right| \tag{3}
\]

where, \(M_{ei}\) and \(M_{pr}\) are the experimental and predicted equilibrium moisture content, respectively. \(N\) is the number of experimental data. An E modulus below 10% is considered as a valid fitting.

2.6. Water absorption and solubility of KGM and CMKGM in water solution

The water absorption was measured according to reported methods (Mahasukhonthachat, Sopade, & Gidley, 2010), with some modifications. Solubility was measured according to reported methods (Chen, Li, & Li, 2011; Han et al., 2012). Briefly, sample (0.10 g) was dispersed in distilled water (24.90 mL) with magnetic stirring overnight at 25 °C. The solution was then centrifuged at 4000 rpm for 20 min at 25 °C. The supernatant was weighed (\(m_1\)). The supernatant was dried at 105 °C and the weight (\(m_2\)) was measured. The water absorption and solubility were calculated as follows Eqs. (4) and (5).

Water absorption (g water/g dry weight basis) = \[\frac{m_1 - (m_0 - m_2)}{m_0 - m_2}\] \tag{4}

Solubility (g dry basis/100 mL water) = 4\(m_2\) \tag{5}

where, \(m_0\) was the dry basis weight of the sample.

2.7. Surface morphology

The powder sample was sprinkled onto a double-faced adhesive tape and then gold sputter coated. The sample surface was observed by scanning electron microscopy (SEM, JSM-6390LV, JEOL, Tokyo, Japan) at an accelerated voltage of 10 kV.

2.8. X-ray diffraction

The X-ray diffraction patterns were determined on KGM and CMKGM powders by an X-ray diffractometer (XRD, D8 Advance, Bruker, Karlsruhe, Germany) equipped with Cu-Kα radiation (\(λ = 0.1542\) nm). The scan data were recorded from angles of 2°–50° at 10° min⁻¹ scan rate in 2θ.

2.9. Atomic force microscopy (AFM)

The topography of the polysaccharide molecule was obtained using a Digital Instruments atomic force microscope (DI Nanoscope TV, Veeco Company, Plainview, NY) equipped with an E-scanner. Tapping mode with nominal spring constant of 5–100 N/m and nominal resonance frequencies of 10–200 kHz were employed. The instrument was operated in tapping mode with topography scan size in the range 2 × 2 μm² to 20 × 20 μm². Polysaccharide solutions were prepared by dissolving 16 mg samples in 200 mL ultrapure water with continuous stirring at 25 °C for 10 h, and then diluted to 2 μg/mL. A drop of the polysaccharide solutions was pipetted onto a mica substrate, and allowed to dry at room temperature.

2.10. Statistical analysis

All experiments except for the measurement of adsorption isotherms with one test were replicated three times. Data were analyzed with a one-way analysis of variance (ANOVA) with SPSS for Windows (version 13.0; SPSS, Inc., Chicago, IL) at the 5% significance level. Results were given as the mean value of three independent measurements ± standard deviation.

3. Results and discussion

3.1. Preparation of CMKGM

The CMKGMs with different DS were prepared, and the degree of substitution of CMKGM samples, approximately 0.2, 0.3, 0.4, were selected and labeled as CMKGM-1, CMKGM-2, and CMKGM-3 for convenience, and KGM sample was labeled as KGM-0. The weight percent of acetyl-substituted residues in KGM was 1.65%. Deacetylation reaction occurs in the process of preparation of CMKGM under weak alkaline condition. However, the CMKGMs took about half the acetyl of the samples no matter what the conditions was (Table 1), indicating that the method of carboxymethylation used in this paper retained a fraction of the acetyl groups of the samples. Physical and chemical properties of KGM-0 and CMKGM samples 1–3 were determined and shown in Table 1.

3.2. Water adsorption isotherms

The water adsorption isotherms of KGM-0 and CMKGM samples 1–3 are all sigmoidal (Fig. 1), corresponding to Type II isotherms in the IUPAC classification scheme, and are consistent with the reported data for moisture adsorption isotherms of konjac glucomannan-based films at 30 °C (Cheng, Abd Karim, Norziah, & Seow, 2002; Cheng, Abd Karim, & Seow, 2007). The experimental water adsorption isotherms between KGM-0 and CMKGM samples 1–3 were similar at relative humidity (RH) lower than 30%,
Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucosamine (g/l)</th>
<th>Water content (g)</th>
<th>Ash (g/l)</th>
<th>Protein (g/l)</th>
<th>DS</th>
<th>Acetyl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KGM-0</td>
<td>90.30 ± 0.50</td>
<td>668.4 ± 0.20</td>
<td>0.13 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMKGM-1</td>
<td>91.30 ± 0.50</td>
<td>668.4 ± 0.20</td>
<td>0.13 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMKGM-2</td>
<td>91.30 ± 0.50</td>
<td>668.4 ± 0.20</td>
<td>0.13 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMKGM-3</td>
<td>91.30 ± 0.50</td>
<td>668.4 ± 0.20</td>
<td>0.13 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

While the equilibrium moisture contents of CMKGM samples 1–3 were lower than that of KGM-0 at RH > 30%, and with the higher of carboxymethyl substituted residues, the lower of the equilibrium moisture content. The introduced carboxymethyl group into the KGM-0 molecule, the hydrophobic character of CMKGM samples 1–3 is improved.

The experimental data for the adsorption curves were fitted to GAB model (Eq. (2)). The values of coefficient of determination ($R^2$) were higher than 0.999, and $E$ values were below 8% (Table 2), which reflect good fit adsorption data to the GAB model. The GAB model parameters values show that the monolayer moisture content ($M_0$) in KGM-0 was highest among the samples, while it decreased with the carboxymethyl modification (Table 2). This indicates the hydrophilicity of CMKGM samples 1–3 decreases compared to KGM-0. The energy constant $C$ increased with carboxymethylation, which suggests that there is a rise in the water-substrate interaction energy, and an increase in hydrophobicity.

3.3. Water absorption and solubility

The water absorption of KGM-0 and CMKGM samples 1–3 was 38.8 ± 0.32, 18.6 ± 0.51, 16.7 ± 0.26, and 15.1 ± 0.49 g water/g dry basis successively (Table 3). Values of water absorption decreased with CMKGM samples 1–3, and were lower than that in KGM-0. Values of solubility (g dry basis/100 mL water) were 0.380 ± 0.003, 0.364 ± 0.002, 0.280 ± 0.001, and 0.228 ± 0.001 for KGM-0 and CMKGM samples 1–3 successively (Table 3). Values for water solubility decreased with CMKGM samples 1–3, and were lower than that in KGM-0. In previous reports, water absorption and solubility of KGM and CMKGM were measured. Value of solubility for KGM (92.6% purity, $M_w = 1.2 \times 10^6$ Da, sample solution = 0.5% (w/w)) was 0.71 ± 0.02 g dry basis in water solution/g.
Table 3
Water absorption and solubility of konjac glucomannan and carboxymethyl konjac glucomannan in water solution at 25 °C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water absorption (g water/g dry basis)</th>
<th>Solubility (g dry basis/100 mL water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KGM-0</td>
<td>38.8 ± 0.32a</td>
<td>0.380 ± 0.003a</td>
</tr>
<tr>
<td>CMKGM-1</td>
<td>18.6 ± 0.51b</td>
<td>0.364 ± 0.002ac</td>
</tr>
<tr>
<td>CMKGM-2</td>
<td>16.7 ± 0.26bc</td>
<td>0.280 ± 0.001bc</td>
</tr>
<tr>
<td>CMKGM-3</td>
<td>15.1 ± 0.49bd</td>
<td>0.228 ± 0.001b</td>
</tr>
</tbody>
</table>

Values (mean ± standard deviation, n = 3) in a column with different letters are significantly different (p < 0.05).

* Sample solution = 0.4% (w/w).

Fig. 2. X-ray patterns of KGM-0 (a), CMKGM-1 (b), CMKGM-2 (c), and CMKGM-3 (d).

Fig. 3. Scanning electron micrographs of KGM-0 (a), CMKGM-1 (b), CMKGM-2 (c), and CMKGM-3 (d) particles.

total dry basis (Tatirat, Charoenrein, & Kerr, 2012). The solubility of KGM (M_w = 7.47 × 10^5 g mol⁻¹, sample solution = 0.4% (w/w)) was 0.829 ± 0.00185 g dry basis in water solution/g total sample (Chen et al., 2011). The solubility of CMKGM (degree of substitution = 0.6, sample solution = 25% (w/w)) was 11.70 ± 0.36 g dry basis in water solution/100 mL water (Xia et al., 2010). The water absorption of KGM (92.6% purity, M_w = 1.2 × 10^6 Da, sample solution = 0.5% (w/w)) was 153.64 ± 4.70 g water/g dry basis (Tatirat, Charoenrein, & Kerr, 2012). Water absorbency of KGM (sample solution = 0.33% (w/w)) was 105.4 and 100 g water/g dry sample. (Koroskenyi & McCarthy, 2001; Liu, Luo, & Lin, 2010). Comparing results of this study with other previous studies of KGM and CMKGM, there are differences due to different experimental conditions and biological variation in samples.

Comparing our results with previous studies of starch and cellulose, the presence of carboxylate groups (COO⁻ Na⁺) in them enhances their hydrophilicity, partially because carboxylate groups are water soluble (Heinze & Koschella, 2005). It is well known that native starch is low in water solubility and cellulose is insoluble in water, while carboxymethyl starch and cellulose are easily water soluble compared to the native forms. These changes of water binding properties of starch and cellulose are due to the reduction and/or loss of crystalline structure inside the starch granules and cellulose after carboxymethylation, which are consistent with surface damage observed in SEM, thus making the granules largely amorphous (Adinugraha & Marseno, 2005; Bhandari, Jones, & Hanna, 2012; Kittipongpatana, Sirithunyalug, & Laenger, 2006; Liu, Ming, Li, & Zhao, 2012; Tatongjai & Lumdubwong, 2010). Amorphous materials absorb more water than semicrystalline material because of the destruction of the crystalline structure, which lead to the increase of exposed absorption sites (Wootton & Bamunuarachchi, 1978). In addition, amorphous materials are more hygroscopic than their crystalline counterparts due to the
ability to absorb moisture into their bulk structure in addition to surface adsorption (Ahleneck & Zografi, 1990; Zografi, 1988). So carboxymethyl starch and cellulose are soluble in water.

In this study, unlike the crystalline structure of starch and cellulose, the particles of KGM-0 and CMKGM samples 1–3 were all amorphous (Fig. 2). The particle morphology was changed with carboxymethylation. KGM-0 showed non-regular granular structure, white oval-shaped sacs, and sharper edges; CMKGM samples 1–3 showed polygonal-shaped granules, and the surfaces showed indentations, roughness, and wrinkles (Fig. 3). Carboxymethylated modifications alter the granular surface structure of the KGM, which may affect its moisture adsorption. Our results are similar to the published data for carboxymethyl starch, the carboxymethyl modification of starch lead to changes of molecular structures and water adsorption (Cova, Sandoval, Balsamo, & Müller, 2010).

3.4. Molecular conformation

From AFM micrographs (Fig. 4), all the molecules appeared like flexible coils with extending chain structure. With the increase of degree of substitution, molecular chain went gradually from free soft linear molecule chain shape (KGM-0), to a chain conformation with a certain acute angle (CMKGM-1), multi-angle folding type (CMKGM-2), and star branching shape (CMKGM-3). KGM and CMKGM-1 could be single molecular chains in solution. For CMKGM-2, some molecular chains intertwined together. The CMKGM-3 molecular chain showed branching, just like a three-arm star polymer. In previous studies, the molecular chains of deacetylated KGM transformed from semi-flexible linear conformation to strong self-crimping elastic microspheres (Chen et al., 2011). In this research, the results of the molecular chain conformation showed that the polymer chains of CMKGMs were flexible coils.

Fig. 4. AFM micrographs of KGM-0 (a), CMKGM-1 (b), CMKGM-2 (c), and CMKGM 3 (d) with the concentration of 2 μg.mL⁻¹.
with extending chain structure just as the KGM molecular chain but more folding and branching. The changes of molecular conformation may result from the introduction of carboxymethyl group and loss of a fraction of the acetyl groups.

3.5. Mechanism of decreasing water binding

In this study, CMKGM were prepared by the introduction of carboxymethyl group, but loss of about a half of acetyl group in the molecular structure. Acetyl group is important in the molecular structure of KGM and CMKGM. It was reported that the addition of higher amount of alkali is favorable for higher degree of deacetylation, the degree of deacetylation value could be as high as 98.3%, moreover, the solubility of KGM decreased with increasing degree of deacetylation (Chen et al., 2011). When losses of partly acetyl group in KGM backbone, the molecules easily entangle each other and aggregate to form multi-branch structures (Fig. 4c and d), which was consistent with a previous report (Chen et al., 2011). In this case, there exists steric hindrance when water molecules close to CMKGM molecular chains (Fig. 5). But the presence of acetyl groups decides solubility of KGM in water. The introduction of carboxymethyl group may reduce association between polymer chains and there increase water binding. However, this hydrophilic groups introduced could also affect the hydrophobicity of polysaccharide as well, the carboxymethyl groups introduced are mainly in the C-6 position of the carboxymethylated derivative, which may break the capacity to form the micelles and mildly affect the hydrogen bonds at other positions (Jin, Zhang, Yin, & Nishinari, 2006). The rotation and vibration of the bulky carboxymethyl group prevent water molecules approaching to the hydrophilic group and made it difficult to form hydrogen bonds (Fig. 5), thereby weakening the hydration ability and contributing to the hydrophobicity. On the one hand, carboxymethyl group of CMKGM can increase water binding; on the other hand, it can decrease water binding. Both carboxymethylation and deacetylation could confer hydrophobicity for these derivatives.

4. Conclusion

The incorporation of carboxymethyl group in the KGM structure, the water adsorption, absorption, and solubility of CMKGM samples 1–3 were decreased. KGM carboxymethylation (DS = 0.2, 0.3, 0.4) decreased the hydrophilicity. Both carboxymethylation and deacetylation could make the contribution to water binding reducing effect of CMKGM.

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