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Probing Starch–Iodine Interaction by Atomic Force Microscopy

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Summary: We explored the interaction of iodine with three crystalline type starches, corn, potato, and sweet potato starches using atomic force microscopy. Results revealed that starch molecules aggregated through interaction with iodine solution as well as iodine vapor. Detailed fine structures such as networks, chains, and super-helical structures were found in iodide solution tests. The nanostructures formed due to iodine adsorption could help to understand the formation and properties of the starch–iodine complex. SCANNING 36:394–400, 2014. © 2013 Wiley Periodicals, Inc.

Key words: atomic force microscopy (AFM), starch–iodine complex, iodine vapor, iodine adsorption

Introduction

The starch–iodine interaction has been known to all of us from elementary chemistry course in qualitative and quantitative analysis. Such interaction has been widely used by chemist to define amylose and amylopectin compounds due to their different ability to bind iodine, where amylose gives a bluish and amylopectin gives a reddish brown color (Wang *et al*., '98). The starch–iodine interaction has a number of applications in determining apple fruit maturity and then the first acceptable harvest date of fruits (Smith *et al*., '79; Fan *et al*., '95), and in evaluation of the gene engineering and plant development (Nelson and Pan, '95; Rahman *et al*., 2007), in curing a female disease (Ghent *et al*., '93) and iodine deficiency disorders (Mottiar and Altosaar, 2011), and in enhancing the solubility of single-wall carbon nanotubes in aqueous solution (Star *et al*., 2002).

The nature of the starch–iodine interaction is extremely complex and remains a longstanding challenge, though there is a great promise in applications of starch–iodine complex. The structure of the starch–iodine complex has been claimed as single left-handed helix accommodates with polyiodide chain by X-ray and titration investigations (Bates *et al*., '43; Rundle and French, '43), which contributes to the blue color and the change of color with the change in chain length of the amylose (Saenger, '84; Putseys *et al*., 2010). Characterization of starch–iodine complex has also been carried out by Raman spectroscopy and UV/Vis spectroscopy (Yu *et al*., '96). All the methods mentioned above are average over a large ensemble of molecules, and claimed the ordered structure of starch–iodine complex exists not only in crystalline state but also in solution. The measurements on the scale of single molecules are also important and needed to provide information about spectrum of configurations and impart presently unpredictable properties. Although atomic force microscopy (AFM) has been used to test interactions between iodine and starch granules (Waduge *et al*., 2010), little information at molecular scale is known. With the intention to establish a basis for a broader

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evaluation of the mechanism of starch–iodine complex formation, we applied AFM to this system to seek a better understanding of the color formation at single-molecule level. Sujka and Jamroz (2009) have reported that native potato starches have higher iodine binding capacity than native corn starches. The difference in iodine binding capacity may arise from the difference in composition and crystalline structure of cereal starches and tuber and root starches. Herein we chose native corn starches (A-type polymorph cereal starches), and native potato starches (B-type polymorph tuber and root starches). Sweet potato starches are tuber and root starches but have a crystalline structure very close to A-type polymorph starches (Hizukuri, '69; Zobel, '88; Noda *et al.*, '97), and were also investigated.

Materials and Methods

Materials

Corn starch (amylose content 20.5 mass%; ash 0.04%; N 0.20%; lipid 0.12%), potato starch (amylose content 24.2 mass%; ash 0.25%; N 0.10%; lipid 0.12%), and sweet potato starch (amylose content 17.4 mass%; ash 0.44%; N 0.09%; lipid 0.25%) were from Dingxin Starch Plant (Tianjin, China). Pure amylose, iodine (I_2), and potassium iodide (KI) were purchased from Sigma–Aldrich (Saint Louis, MO). I_2/KI solution was the solution of 0.2% (w/w) molecular iodine in 2% (w/w) potassium iodide.

Starch Sample Preparation for AFM Imaging

Commercially available corn, potato, and sweet potato starches were mixed with double distilled water (0.1 mg/1 ml) in a covered vial and stirred for 2 min. The mixture was gelatinized by incubation in boiling water bath for 30 min. The solution of the gelatinized starches (10 μl) was dropped hot onto a newly cleaved mica surface (1 cm × 1 cm) by a pipette. The starch samples were dried by nitrogen, for AFM imaging.

Starch–Iodine Reaction Procedure

The starch–iodine interactions were carried out in two different ways. First, the starch samples were deposited on mica surface, and mounted on sample stage in AFM chamber for imaging. Then a drop of I_2/KI solutions (10 μl) was added to the surface, incubated for 2 min and removed immediately by wipers (Kimwipes) and followed by gentle nitrogen blow. The other way is using iodine vapor to treat the gelatinized starches, which were deposited on mica surface. The samples were placed into closed glass desiccators containing iodine crystals and a glass vial containing water to provide a water-saturated atmosphere, and kept for 30 days at room temperature. Gelatinized starch solutions (1 ml) were also mixed with I_2/KI solutions (20 μl), and the mixture (10 μl) was deposited on newly cleaved mica surfaces for AFM imaging.

AFM Imaging

Imaging of starch was performed with a NanoMan AFM (Nanoscope IV, Bruker Co, Santa Barbara, CA) equipped with a G scanner. Silicon cantilevers (NSG-11, NT-MDT) with a nominal force constant of 11 N/m were used. All operations were carried out in air at room temperature. Regular AFM images were collected before and after iodine reactions using tapping mode AFM. In an AFM height image, the height of the features was described in gray mode. The height, length, and angles of the features could be analyzed. The apparent height measurement was conducted by AFM offline software Version 530b4 (Bruker Co, Santa Barbara, CA).

Results

Reactions Between Starch and I_2/KI Solutions

In this study, we report that the interactions of gelatinized starches with I_2/KI solution (0.2%, w/w molecular iodine in 2%, w/w KI) and iodine vapor. Tapping mode AFM was performed to starch samples before and after treatment of iodine aqueous solutions. AFM height images of gelatinized starch revealed a relatively uniform layer of ~1 nm in height (Fig. 1A,C, E). Some fine chains about 0.6 nm in height were found in AFM images of small scan areas in all three samples as we reported previously (Liu *et al.*, 2005; An *et al.*, 2008, 2011). The AFM tip was withdrawn before adding iodine aqueous solutions, and was re-approached to the surface. Because NanoMan does tip scan and only moves in *z*-direction while tip is withdrawing and approaching, and the surface was not touched or moved during the operation process, the imaging can be performed probably at the same scan area. Significant changes of topography of the three kind starches were observed (Fig. 1B,D,F). Aggregations and networks were typical structure of starch-iodine complexes and presented a big contrast in height images. The height of networks of corn starches in Figure 1B ranges 0.7–6.9 nm. Small particles were found coupled into the networks with 17 nm in width and 6 nm in height. The networks showed in Fig. 1D,F ranges 0.6–8.5 nm in height. Similar networks and aggregations were also found in other scan areas. The structures are very stable in air and can last several hours or days.

Imaging in smaller scan area can reveal detailed structures. Figure 2 depicted chains, branches, and

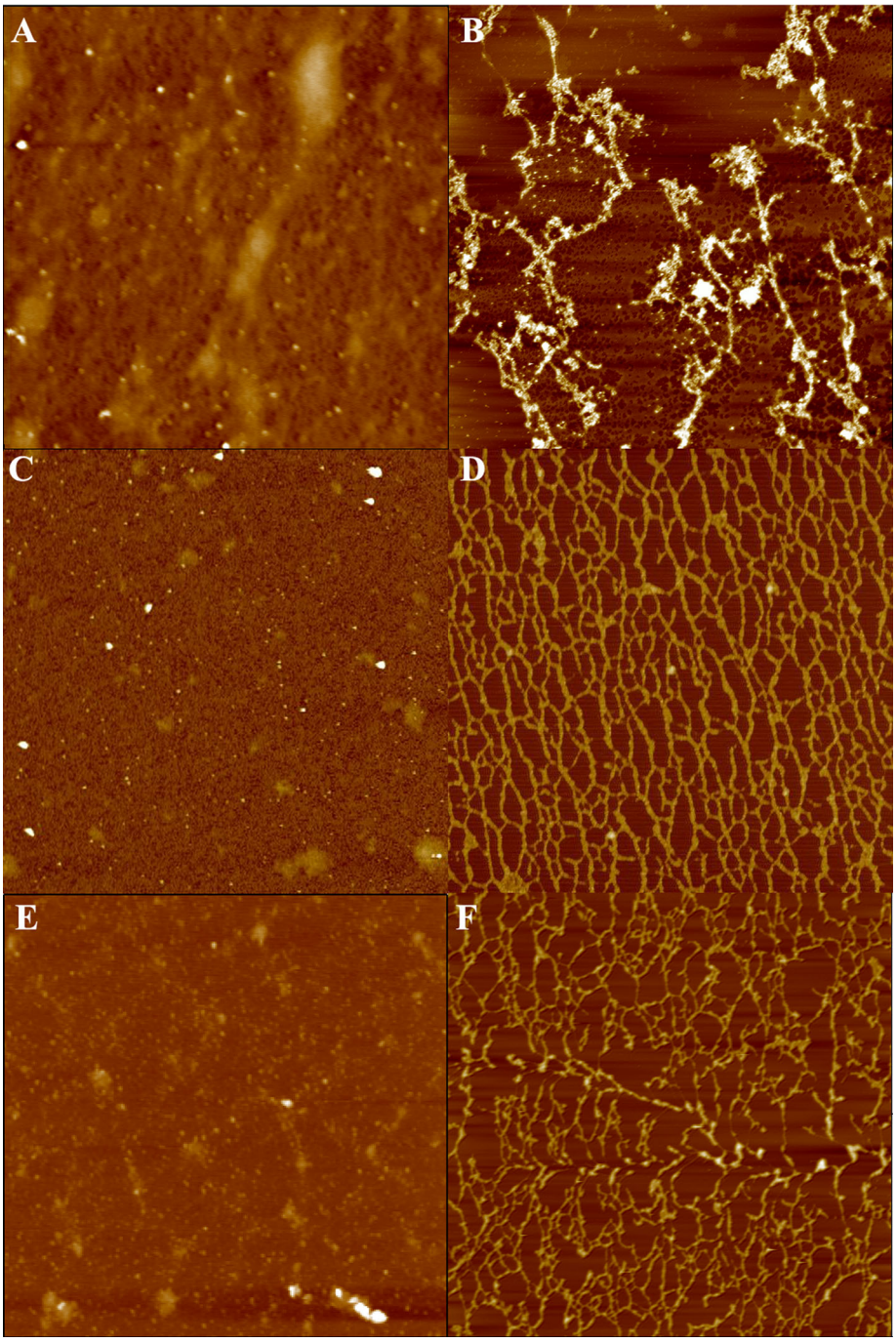

Fig 1. AFM image before (A, C, E) and after iodide treatment (B, D, F) with the gelatinized starch. (A) and (B), corn starch; (C) and (D), potato starch; (E) and (F), sweet potato starch. (A, C, E), Scan size: 5 μm × 5 μm, height range 10 nm. (B, D, F), Scan size: 5 μm × 5 μm, height range 20 nm.

helical structures in 1 μm × 1 μm scan area. Chains were highly branched. Some chains twist together and form super helices in potato starches (Fig. 2B). As white arrow indicated (Fig. 2B), most of the helices are right-handed. In sweet potato starches, rod-like chains with different apparent height were found between two higher features (as black arrows point in Fig. 2C, 5–8.5 nm in height). These linear chains were measured 0.5–1.2 nm in height (cross-section profile at position X_2, X_3, X_4, X_5 in Fig. 3) and 40–300 nm in length. Most chains are thick (~5 nm in height, at position X_1 in Fig. 3). The thick and thin chains are linking together to form networks.

Reactions Between Starch and Iodine Vapor

To further test iodine adsorption, the reactions between gelatinized starch samples and iodine vapor

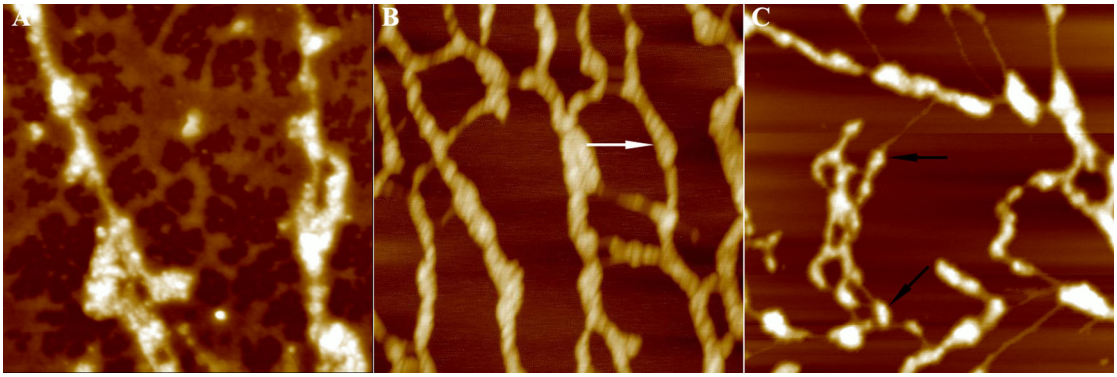

Fig 2. AFM images of small scan area after iodide treatment, enlarged from Figure 1B,D,F. (A), corn starch; (B), potato starch; (C), sweet potato starch. Scan size: 1 μm × 1 μm, height range 10 nm. White arrow (in B) points to right-handed super-helices, and black arrows (in C) point to higher features adjacent to thin chains.

were conducted. The starch samples were exposed to iodine vapor under conditions of 30% and 100% relative humidity for 30 days. Starch samples were imaged before and after iodine vapor reaction. Obvious changes of starch samples were not observed after 1 day iodine adsorption. No significant changes of starch samples were observed after 30 days iodine vapor adsorption at 30% relative humidity. The starch morphology changes due to iodine adsorption were apparent after 30 days at 100% relative humidity (Fig. 4B,E,H). Aggregations and networks were observed. For corn starch, holes formed in the starch films and the height of corn starch-iodine complex were 0.6–1.3 nm (Fig. 4B). A significant change was found in potato starches, and the height of the starch–iodine complex was 1.2 ± 0.1 nm ($n = 69$, Fig. 4E). The changes of sweet potato starches were between the corn starches and the potato starches, and the height of the features after iodine inclusion was 0.6–1.2 nm. As the control, gelatinized starches were stored in air at room temperature at 30% relative humidity environment for 30 days without exposure to iodine vapor to see if the aggregations were caused by drying during storage. AFM images of the control after 30-day storage in air were similar to those before exposure to iodine vapor. No significant change was found for all these three type starches.

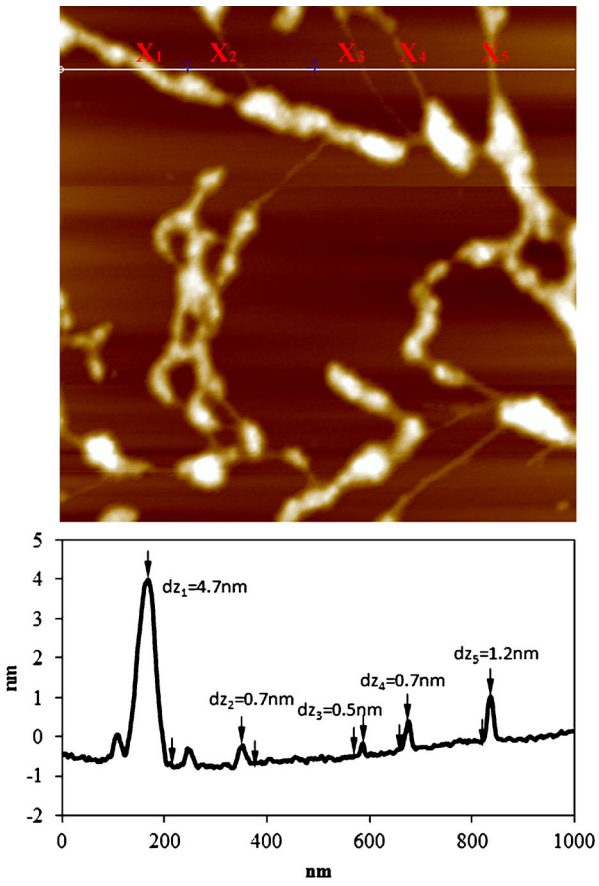

Fig 3. Cross-sectional profile of chains of sweet potato starch-iodine complex. The apparent heights of chains are 0.5–1.2 nm at position X_2–X_5.

Discussion

In this study, we investigated the structure of starch–iodine complex formed in two reactions between starch and I_2/KI solutions or between starch and iodine vapor at nanometer scale using AFM. Data from both reactions demonstrate the aggregation and the formation of networks, which are not reported before. Super-helix structures were found in the reaction between starch and I_2/KI solutions, suggesting that water plays a crucial role in the iodine adsorption. Although the fact that these complex structures associate to the formation of dark blue color still remains unclear, we are close to the answer to that question.

Reactions Between Starch and I_2/KI Solutions

Some starches were surely removed along with the removal of I_2/KI solutions after reactions. We noted that

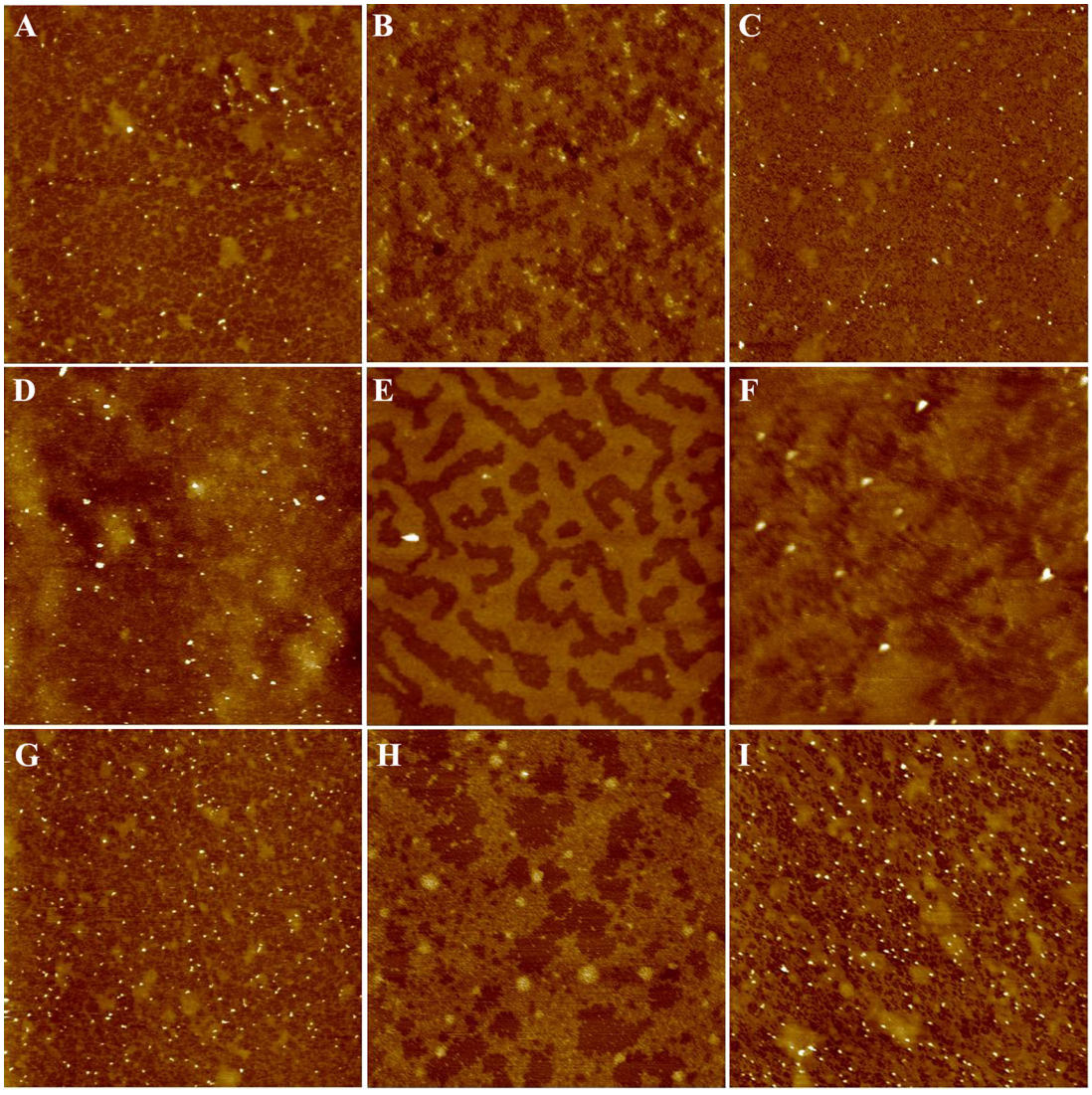

Fig 4. AFM images of starches before (A, D, G) and after iodine vapor treatment (B, E, H) and the control stored for 30 days. (A)–(C), corn starch; (D)–(F), potato starch; (G)–(I), sweet potato starch. Scan size: 5 μm × 5 μm, height range 10 nm.

the wipers became blue when we removed the I_2/KI solutions. This is because starch polysaccharide molecules are hard to adhere to mica surface, and tend to diffuse from the surface into solutions when I_2/KI solutions were added. Other molecules or contaminations may also move into solutions and flow away when the solutions were removed. As results, the images before and after reactions were inconsistent, which look like not from the same scanned area. As there is always drift during the AFM tip withdrawal and re-engagement, re-engagement cannot be exactly the same scan area as before the tip withdrawal. The drift caused relocation of the tip can be ignored in a large scan area. We did not find big differences in structures and dimensions in AFM images taken from a scan area of 10 μm × 10 μm or from other scan areas.

Our AFM data showed chain structures of starch–iodine complex with height of 0.5–1.2 nm. Early X-ray study demonstrated that iodine molecules arranged linearly within the amylose helix with an outer diameter of 13 Å (Rundle and French, '43). Herein, the chains of 1.2 nm in height are comparable to the complex with an outer diameter of 13 Å as mentioned in the literatures. We believe the thinner linear chains of 0.5 nm in height could be the α-1,4-D-glucan chains without containing iodine. As we note that the previous X-ray results were from crystalline state starches, our data could be different because the starches were not in crystalline state. The amylose–iodine complex has been claimed as left-handed helixes in literatures (Sarko and Wu, '78; Saenger, '84; Murdoch, '92; Yu *et al*., '96; Immel and Lichtenthaler, 2000; Putseys *et al*., 2010). We found right-handed helical features of potato starch–iodine complexes, which seems to conflict to previous reports. Previously, right-handed parallel-stranded double helix model was proposed for amylopectin crystalline on the

basis of the presence of antiparallel packing (Sarko and Wu, '78; Imberty *et al.*, '88). In that model, the right-handed super-helix structures are most energetically favorable in organizing left-handed helices. In fact, right-handed super-helix will never be formed, if left-handed helices were parallel packed. The evidence of right-handed features in gelatinized starch may not be enough to prove the right-handed parallel double helix model of amylopectin crystalline, but it adds new wrinkle to research of starches.

There are no literature reports of the aggregation as revealed by AFM investigations in this study. The formation of aggregated structures may result from inter-chain interactions, which need the involvement of branches of amylopectin in starch–iodine complex. Our AFM images reveal that the rod-like thin chains were between two higher features, which could be caused amylopectin side-chains wrapping around another chain. The fact that amylopectin complex with iodine was supported by previous reports (Bates *et al.*, '43; Davis *et al.*, '94).

Some imaging scientists would argue what we observed is due to artificial effect during drying. We did the test using a drop of water instead of I_2/KI solution. The collected AFM images (Fig. S1) were not similar to those showed in Figure 1B,E,H. It seems that some starches were removed away when we removed the water. Therefore, the effect of drying on AFM imaging is excluded here. AFM images of starch-iodine complex prepared from starch solutions containing I_2/KI were also collected (Fig. S2). Aggregations were found for all the three starch samples.

Reactions Between Starch and Iodine Vapor

The observed differences in AFM images could be derived from different scan area and different imaging AFM tips. We note that the environment is quite dry and the control samples have been drying during the storage, but the topography was almost not changed. Moreover, reactions between iodine vapor and starches were conducted under water saturated atmosphere, so that we can exclude the artificial drying effect.

The structures of starch-iodine complexes in Figure 3 were different from those showed in Figure 1. It is likely that, in the reactions with iodine vapor, the starch is confined to the surface and the movement of its components was limited. Potato starch performed a bigger conformation change, suggesting a higher ability to bind iodine than corn starch and sweet potato starch. Sujka and Jamroz (2009) explained their high iodine binding capacity of potato starches that B-type starches have large portion of amylopectin long branch- chains and these long amylopectin side-chains contribute to large amount of binding iodine (Jane and Shen, '93). However, this was not supported by Manion, who found that corn starch granule have better iodine binding ability (Manion *et al.*, 2011). The authors explained that corn starches, known to have starch granules with extensive surface pores, bound higher levels of iodine suggesting pores and channels may be an important factor giving iodine vapor greater access to bind within the granules. We did not find obvious changes in the case under 30% relative humidity, therefore, we would explain that is because B-type starch is more heavily hydrated than A-type starch (Imberty *et al.*, '91). Because properties are always associated with structures (Yang and Wang, 2009; Jiang *et al.*, 2013; Liu *et al.*, 2013), we believe that the observed aggregations and networks contributed to the color formation of starch-iodine complexes.

Conclusions

In summary, iodine inclusion in gelatinized starch molecules was studied by AFM. We have found two types of structure in starch–iodine complex: loose fibrous networks and aggregates. As far as we are aware, the network structure has not previously been observed or predicted for starch-iodine complexes. The aggregates indicated that the amylopectin was involved in the reaction. We also found the super-helix structures. The research outcomes help to understand the formation and properties of starch–iodine complexes.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.