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The technology of molecular manipulation and modification assisted by microwaves as applied to starch granules

Liu Zhongdong[a,b,*], Liu Peng[b], John F. Kennedy[c,d]

[a]Department of Chemistry, Xiamen University, Fujian 361005, People's Republic of China
[b]Food Science and Technology Department, Henan University of Technology, Songshan Road 140#, Zhengzhou Henan 450052, People's Republic of China
[c]Birmingham Carbohydrate and Protein Technology Group, School of Chemical Sciences, The University of Birmingham, Birmingham BI5 2TT, UK
[d]Chembiotech Laboratories, University of Birmingham Research Park, Vincent Drive, Birmingham BI5 2SQ, UK

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Abstract

Microwaves can be used to modify exactly parts of macromolecular complexes. So under this microwave condition of molecular manipulation, the nano-structural units of starch granules can dissociate from the granules, respectively, when gelatinization occurs. Using atomic force microscopy (AFM) to observe this process, it can be seen that some nano-structural chains are just flowing out of the granule and this proves that there are substantive nano-structural units in the starch granules, a phenomenon not previously observed.

Keywords: Nano-structure; Starch; Molecular manipulation; Microwave; Starch granules

1. Introduction

1.1. Microwave

Microwaves are a kind of radio waves in high frequency that lie in the range from 300 million to 300,000 million Hertz. Therefore, microwaves can probe the inside of materials easily (Wujie, 2003). Microwaves can be used as the carrier of information in radar and communication macrotechnology, and also can be used as a new kind of energy to heat and dry materials (Hamrick, 1973; Zhongdong, 2003a), and to catalyze chemical reactions (Zhongdong, 2003b) in the production of industrial and agricultural materials.

1.2. The technology of molecular manipulation

The technology of molecular manipulation is a new technology in nano-science. It is a kind of fixed-point operation to change the uniformity of the whole macromolecules. Its main aim is to stretch the bio- and poly-macromolecules from the clew or the leptospira to the straight chains in order to directly observe their structure by some special instruments such as the atomic force microscope (AFM) (Lijuan & Yingge, 2004; Kechun, 2001). One kind of this new technology is molecular combing that was first used in 1994 by Bensimon. In that examination, the chains of DNA were drawn from the helixes to the parallels in the slide by the effect of water evaporation. As a result, the DNA macromolecules were combed like the hairs (Bensimon & Simon, 1994).

1.3. Starch granules

In the microcosm, the natural starch granules are composed of (1→4)-linked and (1→6)-linked α-D-glucopyranosyl units, and in the macrocosm, the diameter of granules is mainly in the micron size range (Zobel, 1998; Robin, Mercier, Charbonniere, & Guibot, 1974).

In starch science, the core of research lies in the nano-structural units composed of the polyglucopyranose chains (Buleon, Colonna, Planchot, & Ball, 1998; Bottomely, 1998;

* Corresponding author. Address: Food Science and Technology Department, Henan University of Technology, Songshan Road 140#, Zhengzhou Henan 450052, People's Republic of China. Tel.: +86 371 6778 8125; fax: +86 371 6778 9000.

E-mail addresses: liuzhongdong2345@163.com (L. Zhongdong), liuzhongdong2345@yahoo.com.cn (L. Zhongdong).

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Li, Hansma, Vesenka, Kelderman, & Hansma, 1992; Li, Xu, & Ilai, 1996; Hansma, 1996). Since the model of starch granules is deduced from the concept of these glucopyranose chains, principally obtained through the process of gelatinization and enzymolysis, there is no direct proof that substantive nano-structural units really exist in the starch granules. Furthermore, since these granular units are in nanometer size, they are a kind of effective nano-phase materials and have an extensive value in use. For example, they can be used as surface-active agents because of the large surface. The most effective practical utilization will be achieved by a clear knowledge of their structure and characteristics.

The traditional method to research the structure of starch granules is gelatinization and enzymolysis. But when gelatinizating, because the starch granules collapse instantly if the temperature reaches the point of gelatinization, only the fragments can be observed, not the nano-structural units (Zhongdong, 2003), see Fig. 1. And when enzymolysis is employed, it only can yield degradation or hydrolysis products down to glucopyranose again, not the nano-structural units (Manners, 1989).

1.4. The technology of molecular manipulation and modification assisted by microwaves

There are several kinds of effects in the microwave irradiation field, but in the research of nano-structural units of starch granules, it mainly uses that which effects the catalyzing of chemical reactions.

Intentionally, it should modify the parts of starch granules in order that the characteristics of the structure in this part are different from others. When one controls the condition of gelatinization, the different nano-structural units can dissociate from the granules, respectively. Using this method, one can observe in greater detail the process of starch gelatinization, and prove that the substantive nano-structural units belong to the starch granules.

Since microwaves have the effect of catalyzing the chemical reaction, they can be used to modify the starch granules exactly. Investigation involves mixing the starch and denaturant symmetrically and putting them into the microwave field. Under the catalysis of microwaves, starch and denaturant can react chemically in the place where the two come in contact with each other. Since the size of denaturant is different from that of the starch granules, even when they are mixed symmetrically, the reaction only happens in the parts of the starch granules. So the characteristics of this part are different from others and can dissociate from the granules firstly when gelatinisation occurs. Under this method, one can achieve the aim of modifying the starch granules in the fixed-point.

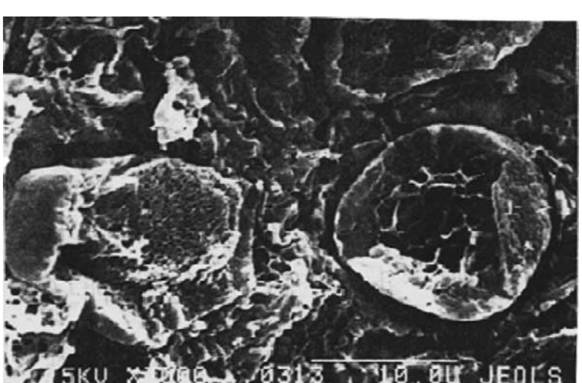

Fig. 1. Transmission Electron Microscope image showing the gelatinization of starch at 70 °C. This is the temperature suitable for gelatinization as tested by DSC.

2. Experimental

2.1. Reagents and apparatus

The following items were needed for this experiment: cornstarch (Henan Shangshui Starch Factory, ash > 0.4%, protein > 0.6%, cellulose > 0.1%); double distilled water (quartz evaporator, conductivity $<1.0\times10^{-7}\ \Omega\ cm^{-1}$); NaOH (AR, Beijing Chemical Plant, China); $ClCH_2COOH$ (AR, Jinshan Chemical Plant, China); CH_3CH_2OH (Anhydrous AR, Luoyang Chemical Plant, China); microwave oven (QIDAOHAIER ER-761MD, 2000 W input and 1000 W output, Qingdao, China); atomic force microscope (AFM) (Digital Instruments Co., St Barbara, CA, USA).

2.2. Method

Using microwaves to denaturalize the natural starch and convert it to partially carboxymethyl starch, the specific processes are as follows:

1. The technology of molecular manipulation and modification assisted by microwave (Zhongdong, 1998; Hongxiu & zhaotan, 1996) involves: mixing the starch (16.8 g), NaOH (8.8 g) and $ClCH_2COOH$ (8.5 g) symmetrically, then using a spray catcher to put ethanol (50 ml) into the mixture. Then, the mixture is subjected to microwaving (hold stability, without moving), for 5–10 min.
2. The technology of direct gelatinisation (Hongxiu & Zhaotan, 1996) involves: putting 0.1–0.3 mg of starch sample into a 2.5 ml covered vial and adding 2 ml of double distilled water. Then, the vial is placed in a water bath 90 °C for 3–5 min.
3. The technology of molecule combing (Zhongdong, 2001) is achieved by a pipetting the starch solution at a concentration of 0.02% w/v and at a temperature of 90 °C after gelatinisation (2 μl), depositing it instantly onto the surface of newly cleaved natural mica. Then, the mica surface is promptly dried with a blast of air from a blastball (a normal tool in lab with high elasticity that is made of rubber, so the air in it can be squeezed out

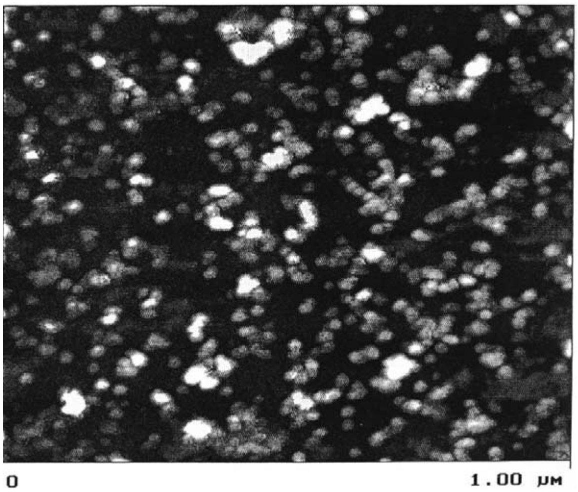

Fig. 2. Deliberate over gelatinisation of starch. The time for heating in a water bath was more than 5 min and the granule had broken down thoroughly, into fragments.

as a strong airflow) in a certain single direction. It should be noted that this step must be finished within 5 s.

4. Testing (Zhongdong, 2004). All samples ready to be tested are observed using the AFM, working in tapping mode in air.

3. Results and discussions

The process should modify the parts of starch granules in order that the characteristics of the structure in this part are different from others. It uses chloroacetic acid as the denaturant to modify the starch granules partly. It can also use other denaturants to achieve this purpose. Also when one controls the condition of gelatinization, the different nano-structural units can dissociate from the granules, respectively. The purpose of the gelatinization is to break down the starch granules, to make the nano-structural units dissociate. As is well known, the starch granules will swell in water and collapse if the temperature reaches the point of gelatinization. Then one can only observe the fragments, not the nano-structural units. Controlling the time of gelatinization in order to reach the moment that the gelatinization is just taking place and the granules are not broken down thoroughly is important. A time of heating of 3–5 min has been deduced from many of our own of experiments, not from other articles, because this kind of experiment has not been carried out by others and the nano-structural units are newly discovered. If the time is more than 5 min, what we can see is the fragments, just as can be seen in the following picture (Fig. 2). If the time is less than 3 min, the granules do not collapse and the chains cannot be discovered.

In an AFM photograph of starch granules under the technology of molecule manipulation and modification assisted by microwave (Fig. 3), it can be observed that several starch granules are collapsing, that some nano-structural chains are just flowing out of the granules, and that a few chains have already dissociated from the collapsed granules. It displays a special and specific moment in the gelatinisation progress. Both granules and chains in the picture are under the effect of molecule combing, so that they are stretched to lines. In some granules, only one chain is flowing out, showing that the chemical reaction is happening in only one point of the starch granules, and that molecular modification assisted by microwave is a very precise process.

Production of another AFM photograph (Fig. 4) of starch granules under the same conditions as in Fig. 3 but with

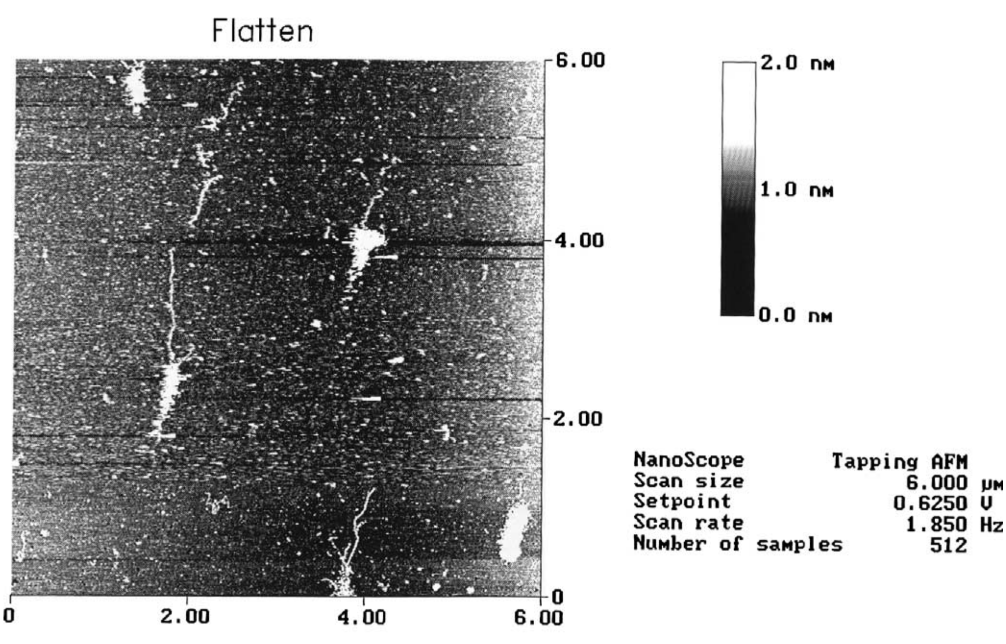

Fig. 3. AFM image shows several starch (CMC) chains flowing out of the starch granules. Size by 6.0×6.0 μm.

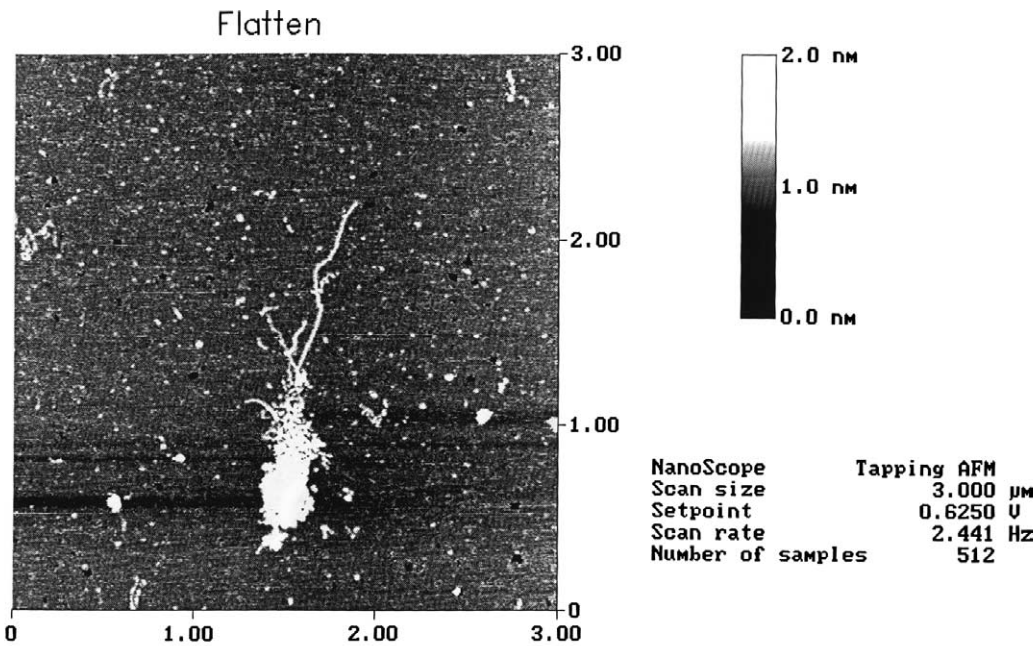

Fig. 4. AFM image shows several chains flowing out of one starch granule. Size 3.0×3.0 μm.

a larger scan size allows visualization of different aspects of phenomenon of the emergent chains. From Fig. 3, one can see the moment of gelatinization and from Fig. 4 one can see the origin of structures more clearly. From this picture (Fig. 4), it can be seen very clearly that a long chain and some short chains are all dissociating from the same granule. Besides this, it also shows that these chains are of uniform width and long, so it can be considered that such complicated structures may result form the regular entanglement of the glucose monochains and that they may be secondary units or structures of the starch granule.

In more detail, from the vertical sizes bars (Figs. 3 and 4), it can be seen clearly that the height of the chains is approximately 2 nm. Moreover, since the AFM is being used in the tapping mode, the sample has to become thinner by virtue of the pressure of the tip. So the actual height of the chains is more than 2 nm, which is bigger than the helix diameter of V_H-amylose. That means the chains in Figs. 3 and 4 are neither amylose nor amylopectin. They are not glucose monochains. This, therefore, also proves that there exist in the starch granules complicated structures and that is what it was intended to show in this experiment, besides showing that microwaves can be used to do molecular manipulation and modification exactly. This is the first time that humans can directly observe the structure of chains from the starch granules and it proves that complicated structures exist in at least cornstarch granules.

4. Conclusions

In summary, this work proves that microwaves can be used to modify the parts of starch granules exactly. This new method can improve the technology of molecule manipulation and modification. Besides, from the AFM photographs of starch granules, it proves that there are substantive nano-structural units in the granules and the granules are composed by these units. This is a powerful proof for the model of starch granules. Then it raises some new subjects, such as the molecular weight, the sequence of glucose chains, the configuration and constitution of the nano-units. Meanwhile, the characteristics of the nano-units should be researched in order to find some utilization of this new nano-phase material.

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