NOTE

CARBOHYDRATES

Study of Wheat Starch Structures by Sonication Treatment

MASAHARU SEGUCHI,[1] TAKAHIKO HIGASA,[2] and TOMOHIKO MORI[2]

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Seguchi (1984a-c) reported that chlorination and heat treatment of wheat prime starch granules changed the surface characteristics from hydrophilic to hydrophobic, depending on changes of the starch granule surface proteins. Wheat prime starch granule surface proteins were extracted without swelling and gelatinization of the starch granules by using 1% sodium dodecyl sulfate (SDS) solution containing 1% 2-mercaptoethanol at room temperature (Seguchi and Yamada 1989). At the same time, soluble starch was also extracted with the same solution and subjected to Sepharose CL-2B gel-filtration chromatography. The chromatography profile of the soluble starch showed that the molecular weight was rather average and smaller (MW 20×10^4) (Seguchi 1991). However, this extraction step with the SDS solution took a long time, and there is a possibility that a side reaction, such as amylase degradation, would be present. We designed the sonication treatment on starch granules to obtain a soluble starch fraction in a short time. Sonication treatment of starch can cause the physical degradation of starch granules (Gallant et al 1972, Hagiwara et al 1984); a decrease in starch suspension viscosity (Basedow and Ebert 1977); and reduction in molecular sizes of starch polymer (Hagiwara et al 1984). Jackson et al (1988) showed that mild sonication increased the water solubility of starch, although extensive sonication appeared to depolymerize amylopectin. Basedow and Ebert (1977) suggested that ultrasonic depolymerization is a nonrandom process that produces fragments of specific molecular size. However, it was not clear how the starch polymer structures change by sonication. Yamaguchi et al (1979) dispersed waxy maize starch in hot dimethyl sulfoxide (DMSO) and autoclaved. They observed details of starch molecular structure by transmisson electron microscopy (TEM). The objectives of this study were to observe the changes of sonicated starch polymer from chromatography and TEM and to determine the structure details of starch polymer. Sonicated starch samples of a predetermined molecular size were used in the TEM experiments. The thickness and length of the visible starch molecules were measured and analyzed.

MATERIALS AND METHODS

Wheat starch was prepared from soft wheat flour (Alps, Nitto Flour Milling Co.) by the acetic acid (pH 3.5) fractionation technique (Sollars 1958). The crude protein (0.06%) and moisture contents (14.0%) of the starch were measured by the methods of Smith (1964) and Tsutsumi (1961), respectively. Sonication treatments were performed using a Yamato sonitech Powersonic model 50 (Yamato Co.) probe sonicator equipped with a 1/4-in. microtip. Wheat starch (250 mg) was suspended in 25 ml of 90% DMSO solution and boiled for 3 min. After cooling to room temperature, the sample solution was subjected to sonication treatment with stirring at 40°C. Sonication times were 300, 600, 900, 1,200, 1,500, 1,800, 2,100, 2,400 and 2,700 sec. Reducing power of the sonicated samples was measured by the method of Hizukuri et al (1981). Total sugar was measured by the phenol-sulfuric acid method (Dubois et al 1956). Gel-filtration chromatography of the starch sample was performed as follows: diameter and height of the column were 1.0 and 100 cm respectively; the column was equilibrated with 90% DMSO solution (Kobayashi et al 1985); flow speed was 24 hr per one column volume; sample (10 mg) was placed on the gel; sugar content in each eluted fraction (2.0 ml) was determined by the phenol-sulfuric acid method (Dubois et al 1956); starch molecular weight was determined by marker polysaccharides (pullulan [α-1,3 glucan], Shodex P-82, MW 10×10^4, 18.6×10^4, 38×10^4, 85.4×10^4). Within these standards, a straight-line graph was obtained. Light scattering was measured by a spectrophotometer (DLS-700 Dynamic, Otsuka Electronics Co.). The sample concentrations were 1, 2, and 3 mg/ml of 90% DMSO solution. The intensity of light scattering was measured at angles from 30° to 150° at 26.1°C, refractive index 1.477, $dn/dc = 0.1,165$, and cell length 21 mm. Molecular weight of the starch sample was determined from the Zimm plots. TEM observation was conducted using an Hitachi H-700H transmission electron microscope operated at 100 kV (Tani et al 1993). A 1% sample solution (90% DMSO solution) was placed on a carbon-coated electron microscope grid and negatively stained with 2% uracyl acetate.

RESULTS AND DISCUSSION

Since the temperature of the sample solutions could be raised by continuous sonication, the treatments were performed with the sonicator on for 0.2 sec and off for 0.8 sec. Sonication times listed do not include the resting period. Sample temperatures were thus maintained at 40°C throughout the sonication time. With increased sonication time, the solution gradually turned more transparent, its viscosity decreased, and the linear increase

[1]Faculty of Domestic Science, Kobe Women's University, Kobe, Hyogo 614, Japan.
[2]Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan.

of starch reducing power was observed. The change of molecular weight of the sonicated starch samples was calculated from the total sugar and reducing power. The average MW of the starting starch sample was ~25 × 10^4, which reduced to 1–2 × 10^4 after 2,700 sec of sonication. The reducing sugar in the starting starch samples was 1 μg/mg of total sugar (reducing sugar was less than 0.1%) and increased to ~1.5% after 2,700 sec of sonication treatment. The quantity of the reducing sugars increased up to 15× after 2,700 sec. Figure 1 shows the changes of starch gel-filtration chromatography profiles at six durations of sonication. The large amylopectin peak (with a MW >10^8), changed to a broad peak with MW 27–150 × 10^4 after 600 sec. Upon additional sonication, those peaks gradually formed one sharp peak. At 1,800 sec, MW was 28–30 × 10^4. At 6,300 sec, the peak had a slightly smaller MW of 12 × 10^4. The reduced power of the sonicated (2,700 sec) starch sample was only 15× higher than that of the original starch, but the chromatogram data showed a decrease of MW from 10^8 to 10^4 after 1,800 sec of sonication. This suggests that some starch molecular linkages could be cleaved by sonication. In addition, randomly aggregated amylopectin molecules would be dispersed and separated from one another by the sonication treatment. A cluster model of starch molecules has been proposed by many starch scientists (Robin et al 1974, Hood and Mercier 1978, Manners and Matheson 1981, Hizukuri 1986). When a cluster model of starch molecules is cleaved by the shear force of sonication, the weakest areas and likely cleaved area may be the linkage portions between the clusters. The resulting cleaved cluster fragments showed one chromatograph profile peak. Further sonication would cleave the additional weak areas in the cluster and make smaller fragments. In the chromatographic profile of starch sonicated at 1,800 sec, one sharp peak (MW 28–30 × 10^4) could be observed. This was compared with molecular weight values determined by light scattering. This sample had a MW 28.9 × 10^4 from the Zimm plot that was almost identical to the molecular weight value obtained by chromatography. The aggregation of starch molecules in a 90% DMSO solution was observed by TEM (Fig. 2). Aggregated starch in 90% DMSO solution looks like a cotton flower. Many starch molecules were aggregated with large random agglomerates. However, sonication treatment gradually separated the agglomerates. This supports the conclusion that DMSO does not fully solubilize starch (Jackson 1991). After 300 sec of sonication (Fig 2B), whitish agglomerates were gradually untied, separated, and reduced in size. This is reflected by the chromatograph profiles. Figure 2C is a photograph after 600 sec of sonication. The starch samples were more separated from each other, and minute starch structures could be observed. Fig. 2D is a photograph after 900 sec of sonication. The aggregated starch molecules were almost completely separated into a single strand that shrank. Almost all starch strands are single and are further composed of some starch double helix chains (Zobel 1988a,b). Various forms and sizes of the strands were observed. Some strands are long, some are short. Some strands look like they have branches. When some starch strands were further magnified, we see that the strand is composed of clusters, one cluster is attached to the next one. Connected clusters make one strand, like a string of beads. This is similar to the observation of waxy maize starch by Yamaguchi et al (1979). They observed the starch strand after homogenation with a glass homogenizer. We examined the relationship between the molecular weight and the size of the starch strand. The chromatogram profile after 300 sec of sonication (Fig. 1) shows the starch sample in fraction 19 has MW 360 × 10^4. The sugar concentration was 0.04%, which suggests that overlapping of starch strands is unlikely because the concentration was low. TEM observation of the sample showed that almost all of the starch strands were the same size, and they were not shrunken but straight. We could then measure the length and thickness of the starch strands. We could not find any strands that branched clearly. The average strand had a thickness of 110 ± 10Å, which is consistent with the observation of waxy maize starch (Yamaguchi et al 1979). Its length was 910 ± 220Å. The starch strand is composed of several clusters like a string of beads. The thickness and length

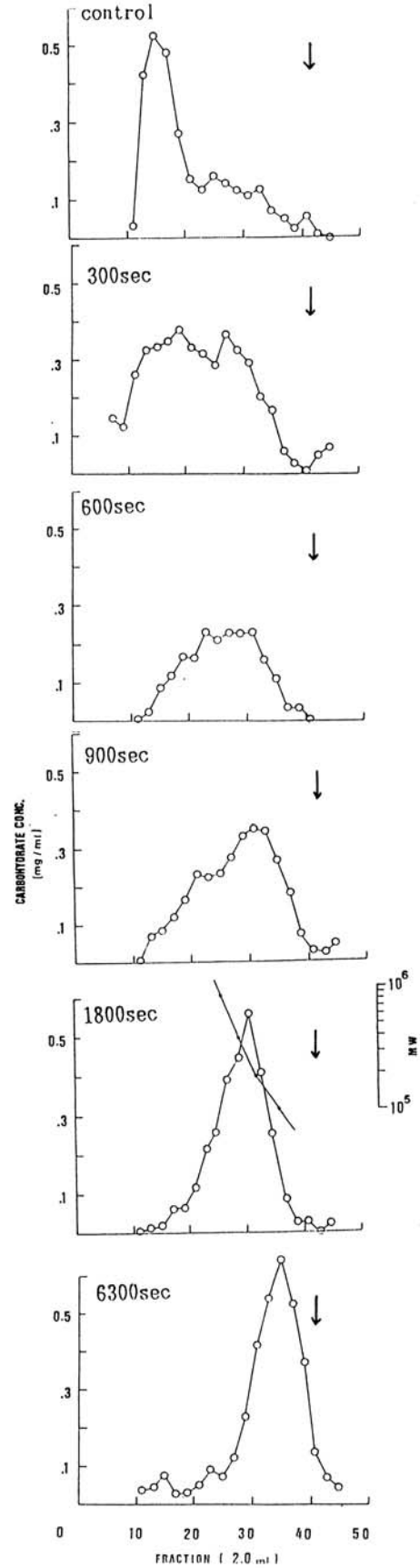

Fig. 1. Changes of starch chromatography profiles after sonication. From top to bottom: control, 300 sec, 600 sec, 900 sec, 1,800 sec, and 6,300 sec. Arrow = V_t.

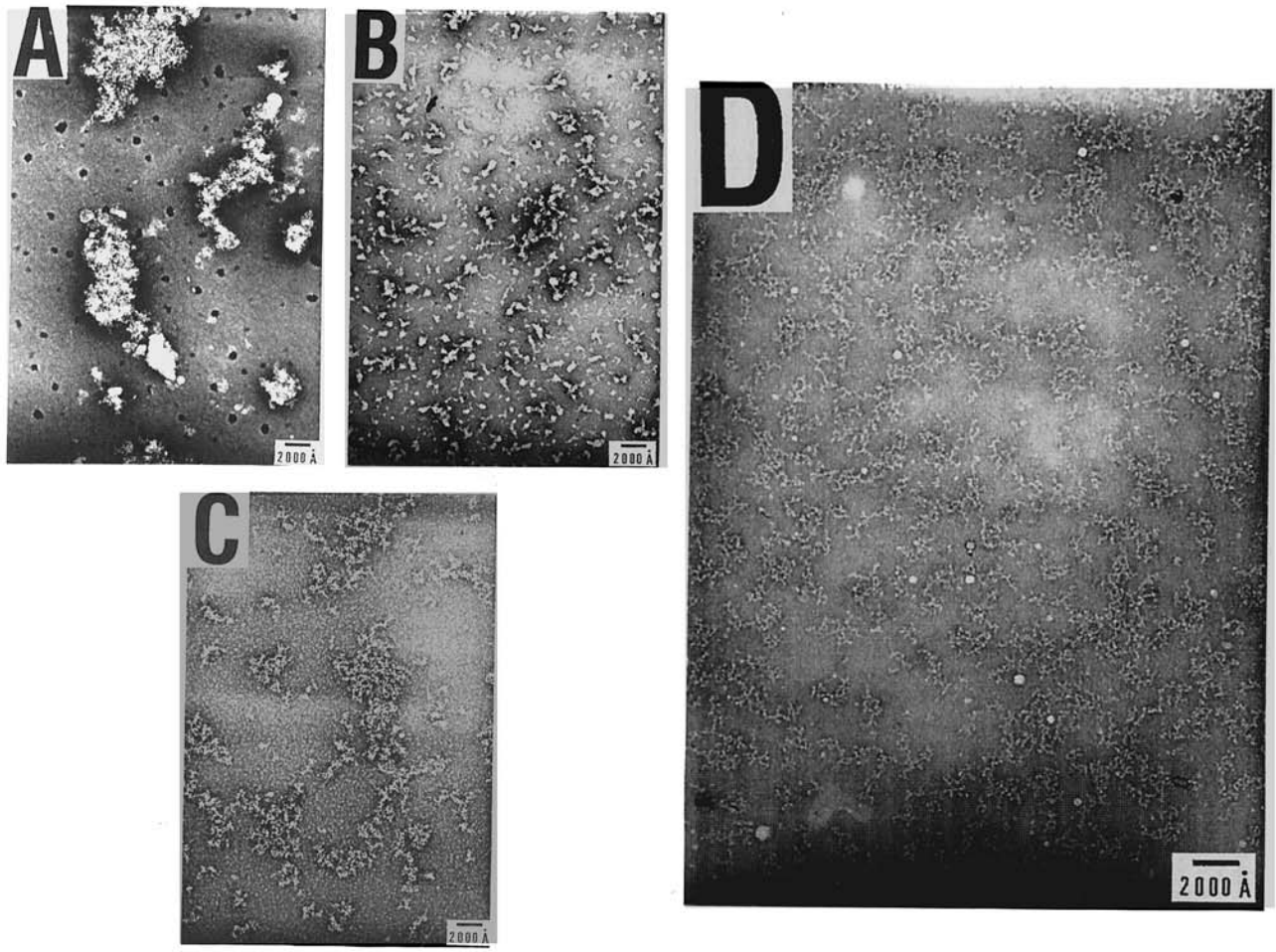

Fig. 2. 2 Transmission electron micrographs of sonicated starch granules with negative staining. Control (**A**), 300 sec (**B**), 600 sec (**C**), 900 sec of sonication (**D**). The scale indicates 2,000Å.

of the cluster were about 100 ± 10 Å and 133 ± 3Å, respectively. Calculated MW of one cluster was 50–80 × 10^4.

CONCLUSIONS

Wheat starch agglomerates were gradually reduced in size by the shear force of sonication, resulting in a single starch strand. Sepharose CL-28 gel-filtration chromatography of these strands showed a single sharp chromatographic peak. TEM observation of the sonicated starch strand (MW 360 × 10^4) showed that the thickness of the starch strand was 110 ± 10Å and the length was 910 ± 220Å, respectively. It was composed of five to six clusters.

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