NOTE

Dye Binding to the Surface of Wheat Starch Granules

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ABSTRACT

Protein-specific dyes (Coomassie brilliant blue, eosin Y, and amido black 10B) were bound to wheat prime starch granules. Microscopic observation of dye-treated starch granules showed color on their surface. Fluorescamine-treated starch granules showed fluorescence under a fluorescence microscope. Glass beads coated with bovine serum albumin could be stained with amido black 10B, but glass beads without this coating could not be stained. Phase contrast microscopic observation of amido black 10B-stained and dried starch granules showed peeling of their colored surface protein film from starch granules, indicating the presence of a protein film on the surface of the starch granule. After various treatments (7.0% HCl, 0.2% NaOH, and 1.0% sodium dodecyl sulfate) of wheat prime starch, dye-binding capacity (with amido black 10B) decreased to about 70% of the nontreated sample. Complete exclusion of the surface protein was not observed. After extensive treatment, the starch granules swelled and completely lost dye-binding capacity.

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The degree of wheat starch gelatinization in bakery products varies from almost complete in wafers to incomplete in puff pastry, for example. Texture in bakery products is greatly influenced by gelatinization resulting from the nature of the starch granule surface. As reported previously (Seguchi 1984a,b), chlorination or heat treatment changes the surface characteristics of wheat prime starch granules from hydrophilic to lipophilic. Seguchi and Matsuki (1977) reported that in the pan-cake baking test of chlorinated wheat flour such lipophilic starch granules are important to the improvement of texture factors such as springiness or gumminess. Therefore, it is necessary to study the surface of wheat starch in order to understand the texture of a bakery product.

In other work (Seguchi 1984a,b; 1985; 1986) I suggested that the protein film on wheat starch granules is changed from hydrophilic to lipophilic by chlorination or heat treatment. Lowy and coworkers (1981) reported on salt-extractable protein from starch granules and tentatively concluded that it was associated with the surface of the starch granules.

In this paper I demonstrate a protein film on the starch granule seen by protein-specific dye binding.

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MATERIALS AND METHODS

Wheat prime starch was prepared from nonchlorinated wheat flour by an acetic acid fractionation technique previously described by Sollars (1958). Protein (N × 6.25) of the prime starch was determined by the method of Smith (1964). Glass beads (1-mm diameter), bovine serum albumin (BSA), soluble starch that did not contain surface proteins, dyes (Coomassie brilliant blue, eosin Y, amido black 10B, and fluorescamine), and other reagents were purchased from commercial sources.

BSA-coated glass beads were prepared as described earlier (Seguchi 1986). Soluble-starch-coated glass beads were prepared by suspending the beads (1 g) in 10 ml of 1% soluble-starch aqueous solution and stirring for 2 hr at 25° C. Then the beads were filtered and dried at room temperature for 24 hr.

Coomassie brilliant blue (0.25% in a mixture of 45% methanol and 9% acetic acid), eosin Y (0.07% in water), and amido black 10B (1.0% in 3% acetic acid) solutions were prepared by the methods of Weber and Osborn (1969), Vanderzant and Tennison (1960), and Racusen (1973), respectively. Wheat prime starch (500 mg) was suspended in 3 ml of each dye solution and left overnight at room temperature. Stained prime starch was washed with 10 ml of water by centrifugation (600 × *g*, for 5 min) 12 times until the supernatant became colorless, followed by dialyses against 10 L of water for three days, and finally dried in a small petri dish (3-cm diameter) at room temperature.

Fluorescamine solution (0.03% solution in acetone) was prepared according to the method of Weigele et al (1972). Prime starch (10 mg) suspended in 1.5 ml of 50 m*M* sodium phosphate

buffer (pH 8.0) was mixed with 0.5 ml of the fluorescamine solution by vigorous shaking. Treated prime starch was washed, dialyzed, and dried as described above.

BSA-coated, soluble-starch-coated, and noncoated glass beads were stained with amido black 10B as wheat prime starch and washed with water until water became colorless. The reflux index (%) of the dye-treated prime starch in petri dishes was measured by a Photovolt type reflectometer (Toyoseiki Co.).

Fluorescamine-treated prime starch was observed with a fluorescence microscope (Olympus BHF type), and micrographs were taken.

Wheat prime starch (2 g) was suspended in 40 ml each of 7.0% HCl, 0.2% NaOH, 1.0% sodium dodecyl sulfate (SDS), or water-saturated *n*-butanol (WSB). Aliquots (10 ml) from each mixture were stirred at room temperature for the indicated time. Finally, the prime starch was repeatedly washed with water by centrifugation and stained with amido black 10B.

The dye-binding capacity (%) was determined as follows: (100 − reflux index of treated and stained prime starch) × 100 ÷ (100 − reflux index of nontreated and stained prime starch). The standard deviation was 2.3% with this method.

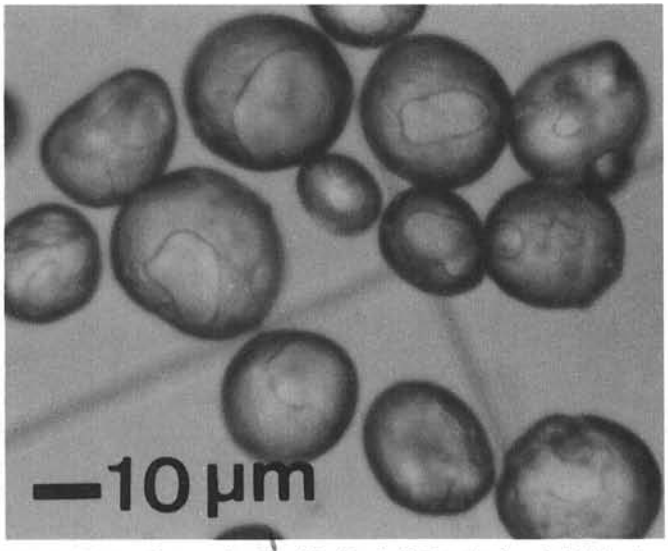

Fig. 1. Photomicrograph of amido black 10B-stained and dried prime starch granules.

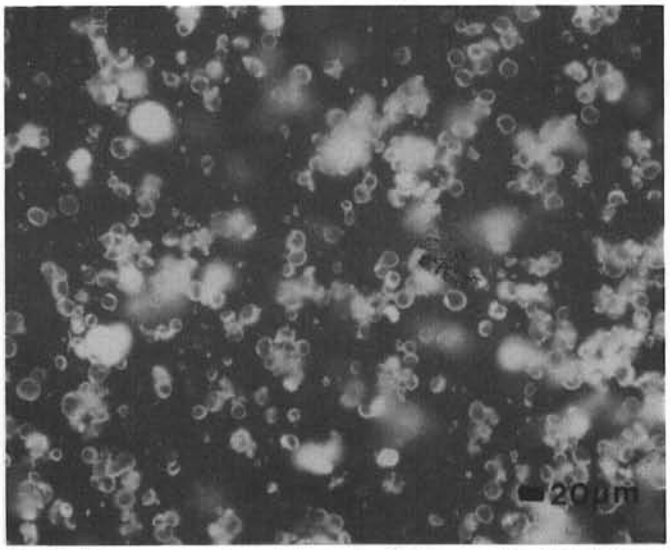

Fig. 2. Fluorescence microphotograph of fluorescamine-treated wheat prime starch.

RESULTS AND DISCUSSION

Wheat prime starch prepared by an acetic acid fractionation technique (Sollars 1958) contained 0.21% protein (N × 6.25). The prime starch stained with Coomassie brilliant blue, eosin Y, and amido black 10B showed violet blue, yellowish red, and greenish blue color, respectively (data not shown).

Amido black 10B-stained prime starches dried on slide glass and observed by phase contrast microscopy (Fig. 1) showed peeling of colored surface protein film from starch granules, indicating that the protein stains were on the starch surface only. These data indicated the presence of surface protein on the wheat starch granule.

Because fluorescamine has high specificity to protein, it was used for the detection of surface protein on starch granules. The treated starches showed fluorescence on the starch granule surface under a fluorescence microscope (Fig. 2). These data, together with the data presented above, confirmed the presence of protein film on starch granules.

It is necessary to make clear that these dyes bind protein specifically. Glass beads that were preliminarily coated with BSA or soluble starch and noncoated glass beads were stained with amido black 10B as prime starch granules. After washing with water, only the BSA-coated glass beads showed staining (Fig. 3), indicating that amido black 10B was specifically bound to protein.

To study the protein film binding to starch, I excluded the protein film from prime starch by treating the prime starch for

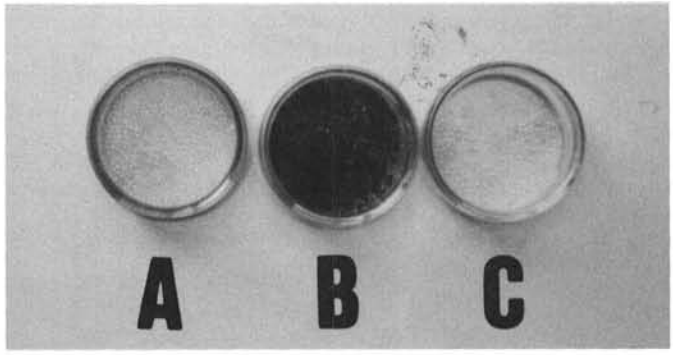

Fig. 3. Photograph of noncoated (**A**), bovine serum albumin-coated (**B**), and soluble-starch-coated (**C**) glass beads after being stained with amido black 10B, washed with water, and dried in a petri dish.

TABLE I
Dye-Binding Capacity After Various Treatments of Wheat Prime Starch

Treatment[a]	Dye-Binding Capacity (%)
None	100
1.0% SDS	
12 hr	80.3
24	74.6
36	71.9
60	Swelling
7.0% HCl	
12 hr	94.0
24	88.4
36	76.3
60	Swelling
0.2% NaOH	
12 hr	88.2
24	74.6
36	Swelling
WSB	
12 hr	98.7
24	96.2
48	88.7
72	88.4

[a] SDS, Sodium dodecyl sulfate; WSB, water-saturated *n*-butanol. Experiments were replicated three times.

various times with 7.0% HCl, 0.2% NaOH, 1.0% SDS, and WSB. The starches were stained with amido black 10B and the dye-binding capacity (%) measured (Table I).

WSB, one of the most efficient polar lipid extractants, was not effective in extracting the protein film from starch granules, and its dye-binding capacity decreased slightly (Table I).

Weak acid (7.0% HCl) or alkali (0.2% NaOH), used to wash the adhering protein from the starch granule, extracted some portion of the protein film from the prime starch, and dye-binding capacity gradually decreased to above 75% after a certain time. Beyond that time, the starch granules swelled and completely lost dye-binding capacity, indicating that protein film is indispensable for the tertiary structure of the starch granule. If it is removed, gelatinization occurs.

The anionic detergent, SDS, extracted the protein film as the weak acid or alkali did. However, microscopic observation showed that SDS-treated prime starch swelled slightly but not as heavily as weak acid or alkali when the dye-binding capacity reached 70%. This indicated that the capacity of SDS to remove protein film is less than that of acid or alkali.

Surface proteins in weak acid-, alkali-, or SDS-extracted solutions were ascertained by spectrophotometric absorption at 280 nm or a colorimetric method, which utilized the biuret reagent or the Folin-Ciocalteu phenol reagent, indicating that portions of the proteins were actually removed from the starch granules by those chemicals. Viscosity of extracted solutions rose with reaction time, and positive color reaction by phenol-sulfate reagent showed that starch granules were also gradually hydrolyzed in the presence of weak acid or alkali with release of surface proteins.

These data show that the extraction of the protein film from starch granules beyond a certain extent triggered swelling. This protein film seemed to control the permeation of water into the starch granule, thus protecting it from swelling.

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