

# Study of Wheat Starch Granule Surface Proteins from Chlorinated Wheat Flours

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ABSTRACT

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After wheat starch granules were collected from chlorinated wheat flour (chlorination level: 0.0, 0.5, 1.0, 1.5, and 2.0 g of Cl<sub>2</sub> gas per kilogram of wheat flour) by acetic acid fractionation, the starch granule surface proteins were successively extracted from them with a 1% sodium dodecyl sulfate (SDS) solution containing 1% 2-mercaptoethanol at room temperature. The treated prime starch granules could not be stained with amido black 10B, which shows that almost all of the starch granule surface proteins were excluded in this process. Ultraviolet spectra (200–420 nm) of this broad range of extracted surface proteins showed proportional increases of the absorbance of ultraviolet light with chlorination levels.

The amounts of these starch granule surface proteins per gram of wheat starch granules estimated by the Lowry and ninhydrin methods, respectively, showed a similar increase with chlorination levels. These surface proteins were further subjected to SDS slab gel electrophoresis and Sephadex G-150 column chromatography, which showed that chlorination produced a slight polymerization of some larger molecular weight proteins in the surface. The chlorine content in these starch granule surface proteins was then measured by fluorescent X-ray analysis, which showed that the content of molecular bound chlorine also proportionally increased with chlorination levels.

Previous studies at this laboratory (Seguchi and Matsuki 1977) indicated that chlorination of wheat flour improves characteristics of pancake texture, such as higher springiness and lower gumminess, and suggested (Seguchi 1984) that these improving effects were caused by a change in the wheat starch granule surface proteins from hydrophilic to hydrophobic by chlorination. Furthermore, our studies (Seguchi 1986, Seguchi and Yamada 1989) indicated the presence of wheat starch granule surface protein and its electrophoretic patterns, and using an experimental model (Seguchi 1985) showed the change of proteins from hydrophilic to hydrophobic by chlorination. Greenwell and Schofield (1986) reported that all soft wheats possess a prominent 15 K band in starch granule protein extracts, and this protein plays an important role in conferring endosperm softness on wheats.

For this study, surface proteins were extracted from wheat starch granules collected from chlorinated wheat flours using 1% sodium dodecyl sulfate (SDS) containing 1% 2-mercaptoethanol (2-ME) as reported by Seguchi and Yamada (1989). Differences in the amounts of starch granule surface proteins, electrophoretic patterns, Sephadex G-150 column chromatograms, and chlorine content of the proteins were examined.

## MATERIALS AND METHODS

### Materials and Reagents

Wheat flour (Alps brand, Nitto Flour Milling Co. Ltd.) from western white wheat was used in this study. The protein content was 7.2% and ash was 0.39% at 12.8% moisture. Wheat prime starch fractions were obtained from chlorinated flour (0.0, 0.5, 1.0, 1.5, and 2.0 g of Cl<sub>2</sub> gas per kilogram of flour) by the method of Sollars (1958). Other reagents were purchased from commercial sources.

### Extraction Method

Starch granule surface proteins were extracted with 1% SDS solution containing 1% 2-ME at room temperature as reported previously by Seguchi and Yamada (1989). The extracted starch granule surface proteins were determined by the Lowry et al (1951) and ninhydrin methods (McGrath 1972).

### Ultraviolet Spectra Analysis

Ultraviolet (UV) spectra (200–420 nm) of these extracted surface proteins, which were first dialyzed against water, were analyzed on a Shimadzu UV-200 spectrophotometer.

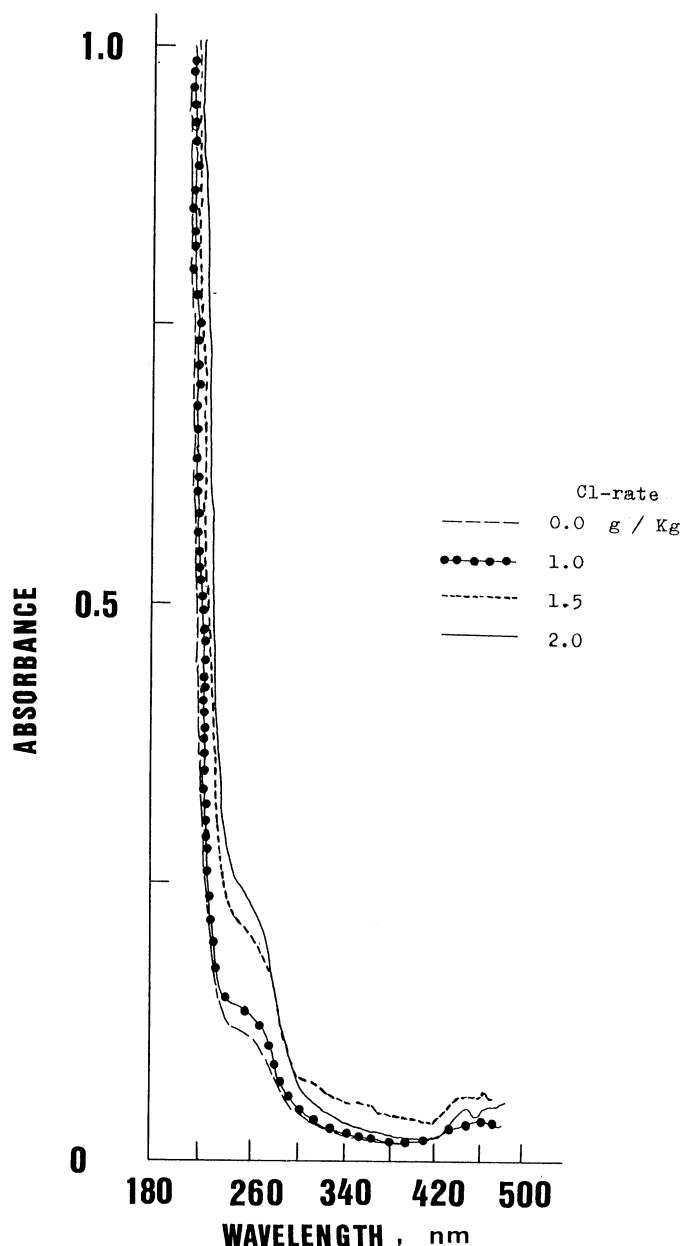


Fig. 1. Spectra of surface proteins of starch granules that are collected from chlorinated wheat flours. Chlorination levels are 0.0, 1.0, 1.5, and 2.0 g of Cl<sub>2</sub> gas per kilogram of wheat flour.

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### SDS Slab Gel Electrophoresis

SDS slab gel electrophoresis (10%) of these extracted surface proteins was performed by the method of Laemmli (1970) and stained with silver (Sammons et al 1981) as reported by Seguchi and Yamada (1989). Surface protein (20  $\mu$ g) of each sample was charged on each slab gel lane.

### Gel Filtration by Sephadex G-150

The starch granule surface proteins were dialyzed against water and freeze-dried. The entire amount (milligrams) of the surface proteins per 6.5 g of prime starch granules was subjected to Sephadex G-150 column (0.5  $\times$  95 cm) chromatography, which was equilibrated with 1% SDS containing 1% 2-ME. The protein content of each fraction was determined by the Lowry method (1951).

### X-ray Fluorescent Analysis

X-ray fluorescent analysis of the chlorine content in the starch granule surface proteins was performed on a Rigaku X-ray spectrometer type 3080 E (Rigaku Industrial Corp.). In order to exclude the starch fraction in the sample, the Sepharose CL-2B column chromatography step was inserted before X-ray fluorescent analysis. Carbohydrate content of the fractions was determined by the phenol-sulfuric acid method (Dubois et al 1956).

## RESULTS AND DISCUSSION

From previous work (Seguchi and Yamada 1989), it was known that the wheat starch granule surface proteins could be extracted with 1% SDS containing 1% 2-ME at room temperature without gelatinization. Using the same extraction conditions, the wheat starch granule surface proteins were extracted from the chlor-

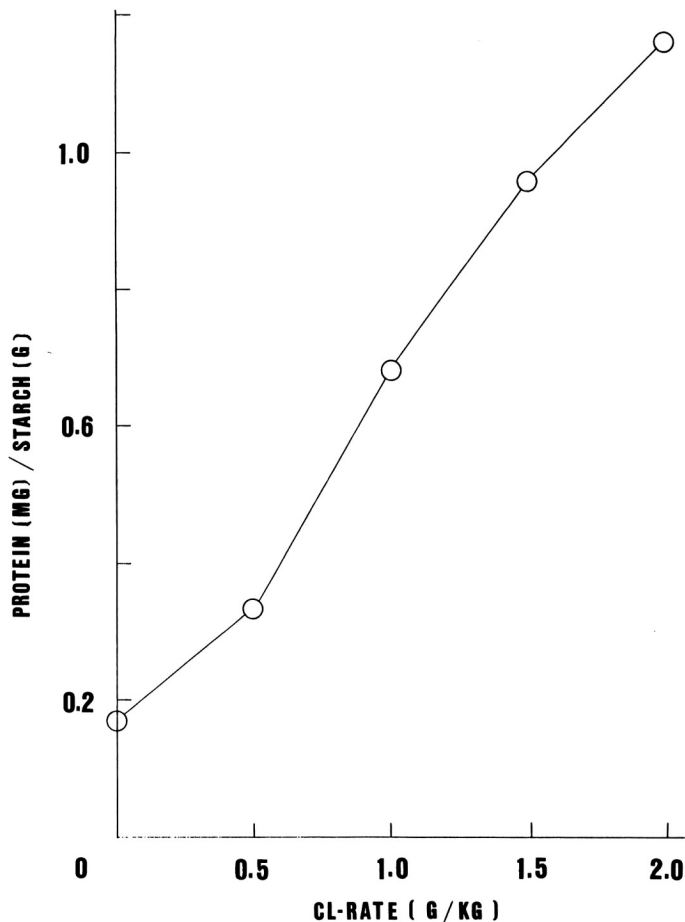


Fig. 2. Relationship between the amount of starch granule surface proteins and chlorination level of their original wheat flour (three replicates, standard deviation  $\pm$  0.06).

inated starch granules. It was ascertained that the results of the amido black 10B staining test (Seguchi 1986) on these extracted starch granules showed almost no surface proteins present.

### UV Absorption of the Starch Granule Surface Proteins

The extracted surface protein solutions were subjected to measurement by UV absorption ranging from 200 to 410 nm. It was clearly observed that the UV absorption of these solutions proportionally increased with chlorination levels, in which the range of increase was from 220 to 300 nm and its midpoint was about 260 nm (Fig. 1). Tsen and Kulp (1971) and Seguchi (1985) reported that the increase in UV absorption of chlorinated proteins would be caused by binding of the chlorine molecule to the proteins. In this case, it is possible that the chlorine-bound surface granule proteins increased this absorption, and the amount of proteins

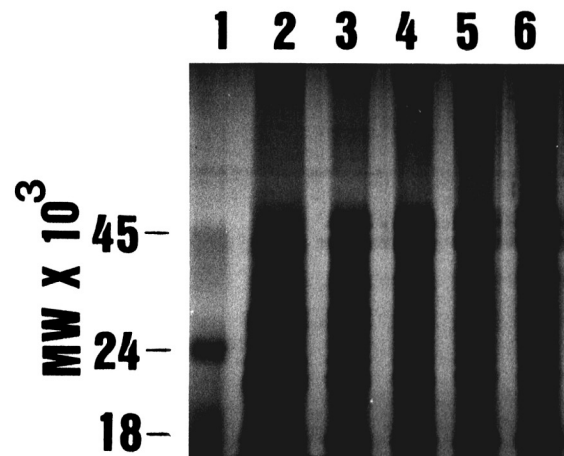


Fig. 3. Sodium dodecyl sulfate slab gel electrophoresis patterns showing expression of marker proteins (lane 1) and surface proteins of starch granules collected from chlorinated wheat flours. Chlorination levels are 0.0 (lane 2), 0.5 (lane 3), 1.0 (lane 4), 1.5 (lane 5), and 2.0 (lane 6) g of  $\text{Cl}_2$  gas per kilogram of wheat flour.

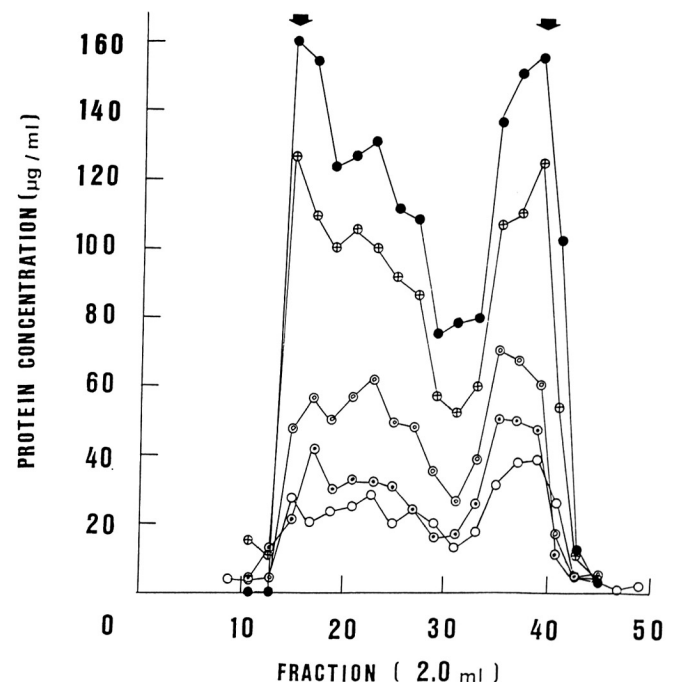


Fig. 4. Sephadex G-150 column chromatography of surface proteins of starch granules collected from chlorinated wheat flours chlorination levels are 0.0 ( $\circ$ ), 0.5 ( $\odot$ ), 1.0 ( $\oplus$ ), 1.5 ( $\oplus$ ), and 2.0 ( $\bullet$ ) g of  $\text{Cl}_2$  gas per kilogram of wheat flour.

**TABLE I**  
**Chlorine Content in Surface Proteins**  
**of Starch Granules**  
**Collected from Chlorinated Wheat Flours**

Chlorination Level (g/kg)	Chlorine Content <sup>a</sup> (%)
0.0	0.47
0.5	0.42
1.0	0.64
1.5	0.95
2.0	1.49

<sup>a</sup>Numbers represent averages of two replicates.

bound to the starch granules would also be expected to increase with chlorination. In order to ascertain the possibility of increase in surface proteins, protein content in these extracted solutions was examined by the Lowry method (1951). Figure 2 shows the amounts of these starch granule surface proteins were proportionally increased with chlorination levels. It is known that a number of materials can interfere with the Lowry technique and that the color is not strictly proportional to concentration. But the same results could be obtained by the ninhydrin method, which suggests that the chlorine molecule does not interfere with the Lowry protein assay method. From these results, the increased absorbance observed between 220 and 300 nm is apparently due to both an increased chlorine molecule binding and an increase in the amount of starch granule surface proteins.

#### SDS Slab Gel Electrophoresis and Sephadex G-150 Column Chromatography of the Surface Proteins

The extracted surface proteins were subjected to SDS slab gel electrophoresis as reported previously by Seguchi and Yamada (1989). Figure 3 shows the pattern of these chlorinated surface proteins. The higher molecular weight protein bands (near  $M_r$  45,000) were gradually increased with chlorination; protein bands near the midpoint from  $M_r$  24,000 to 45,000 also increased with chlorination, and the position of the bands moved slightly toward the higher molecular weight side. However, on the lower molecular weight side, no clear difference in protein bands could be observed by chlorination. The 15 K band reported by Greenwell and Schofield (1986) is present below  $M_r$  18,000 in every gel pattern. Figure 4 shows the patterns of Sephadex G-150 column chromatography of the starch granule surface proteins. Every pattern shows two distinct groups of larger (fraction nos. 13-31) and smaller molecular weights (fraction nos. 31-45). The patterns of the larger molecular weight group gradually changed with chlorination, and fraction no. 15 (chlorination level 1.5 and 2.0 g/kg near void volume) remarkably showed a sharp protein peak, which demonstrates the occurrence of polymerization of proteins by chlorination. However, I could not observe clear changes in the surface proteins of the smaller molecular weight protein group, which is similar to the result of SDS slab gel electrophoresis.

#### Chlorine Content of the Surface Proteins

Chlorine content in starch granule surface proteins was determined by X-ray fluorescence analysis. Table I results show that the amount of chlorine molecularly bound to surface proteins progressively increased with chlorination level. Seguchi (1985)

suggested that the chlorine molecule could bind to amino acids such as tyrosine, lysine, or cystine in the protein molecule, increasing their hydrophobicity. These chlorinated surface proteins may cause the hydrophobic (lipophilic) character of the chlorinated starch granules (Seguchi 1984), and this hydrophobic character would interact with other flour components such as proteins and lipids, or effect swelling of the starch granules in flour.

#### General Observation

From these results, it was observed that the amount of starch granule surface proteins clearly increased with chlorination, and the amount of chlorine molecules per gram of protein also increased. The increase of the amount of the surface proteins by chlorination would come from chlorination of other wheat flour proteins. Both patterns of SDS slab gel electrophoresis and gel filtration by Sephadex G-150 showed that chlorination would result in the polymerization of higher molecular weight proteins. Matoba et al (1985) indicated the cross-linking of  $\alpha_{s-1}$  casein by sodium hypochlorite, but in this case the mechanism of polymerization is not known.

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