Model Experiments on Hydrophobicity of Chlorinated Starch and Hydrophobicity of Chlorinated Surface Protein

MASAHARU SEGUCHI[1]

Cereal Chem. 62(3):166–169

ABSTRACT

Chlorination of dried glass powder or glass beads, which were coated with gelatin, imparted strong oil-binding ability similar to that of chlorinated starch. Soluble proteins such as gelatin, bovine serum albumin (BSA), or ovalbumin formed hydrophobic, water-insoluble thin film after chlorination. These films could be dissolved in weak alkali or 1% sodium dodecyl sulfate (SDS) but became insoluble when dialyzed against water. SDS disc gel electrophoresis of chlorinated BSA showed no polymerization or decomposition. Ultraviolet spectra of BSA or gelatin showed an increased absorption between 290 and 400 nm by chlorination. Paper chromatograms of chlorinated tyrosine, lysine, or cystine showed new hydrophobic derivatives. It is assumed that these derivatives rendered the hydrophobic character to the protein.

Chlorinated prime starch in wheat flour shows strong oil-binding ability, which improves texture such as springiness or gumminess in pancakes, the so-called "hot cake" in Japan (Seguchi and Matsuki 1977). Corn, potato, rice, and arrowroot starches have also attained this ability by chlorination (Seguchi 1984a). Decrease in oil-binding ability by protease-digestion indicated that chlorination might have changed the protein film on the starch granule to a lipophilic (hydrophobic) form.

In order to ascertain the role of protein in the lipophilization, one model experiment of chlorination was designed and examined by using gelatin-coated glass beads or glass powder. The changes in protein and amino acids were also studied to clarify the mechanism of lipophilization of protein by chlorination.

MATERIALS AND METHODS

Materials

Glass beads (1 mm in diameter), gelatin, bovine serum albumin (BSA), ovalbumin, casein, polypeptone, rapeseed oil, soluble starch, and amino acids were purchased from commercial sources. Other materials were reagent-grade products.

Preparation of Gelatin-Coated Glass Samples and Chlorination

Glass powder (10–50 μm) similar to the size of starch granules was prepared by grinding a washed pasteur pipette in a mortar and pestle. Glass powder or beads (1 g) were suspended in 10 ml of 10–50 mg/ml gelatin solution and stirred for 2 hr at 25° C. Samples were then filtered and dried at room temperature. The ratio of gelatin to dried glass beads was 0.32 mg/g of glass beads as determined by the difference in weight. This gelatin-coated glass sample (500 mg) was chlorinated with 28.5 mg of chlorine gas in a test tube for 1 hr at room temperature as described by Seguchi and Matsuki (1977).

Oil-Binding Ability of Gelatin-Coated Chlorinated Glass Samples

Gelatin-coated chlorinated glass samples (500 mg) and rapeseed oil (0.5 ml) were mixed in a test tube and shaken vigorously for a few minutes at 3,200 rpm in a Yamato vibrio shaker with a horizontal vibration amplitude of 0.1 cm. Oil-binding ability of glass samples was observed microscopically (×50).

Preparation of Water-Insoluble Protein Film by Chlorination

Ten milliliters of protein solution (10 mg/ml) in a petri dish (93

[1]Seibo Women's Junior College, Tayamachi-1, Fukakusa, Fushimiku, Kyoto, Japan 612.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. American Association of Cereal Chemists, Inc., 1985.

×14 mm) was fan dried at room temperature. Water content of the protein film was 12% after drying, as determined by the method of Tsutsumi and Nagahara (1961). Chlorine gas (17.7 mg) was introduced into the petri dish, sealed with Parafilm, and then left for 1 hr at room temperature. After reaction, 10 ml of water was poured into the petri dish. When gelatin was used, the petri dish was warmed after chlorination in 40°C water for several minutes in order to release the film from the glass surface. Other protein films were easily released from the surface of the petri dish by water at room temperature. All recovered water-insoluble film was washed by centrifugation with a large volume of water.

Sodium Dodecyl Sulfate Disc Gel Electrophoresis of Protein

Sodium dodecyl sulfate (SDS) disc gel electrophoresis of protein was performed by the method of Weber and Osborn (1969).

Ultraviolet Spectra of BSA and Gelatin

The BSA (0.5 mg) or gelatin (0.27 mg) were dissolved in 1 ml of 0.1*N* NaOH and analyzed on a Shimazu UV-200 spectrophotometer.

Chlorination and Paper Chromatography of Amino Acids

Ten milliliters of amino acid solution or suspension (10 mg/ml) was chlorinated in the same manner as described for protein in the previous section. Paper chromatography of amino acids was performed by the ascending method, prepared with double developments on Whatman No. 51 filter paper with a solvent system of *n*-butanol, acetic acid, and water (4:1:2). Amino acids were detected by the ninhydrin reaction test.

RESULTS AND DISCUSSION

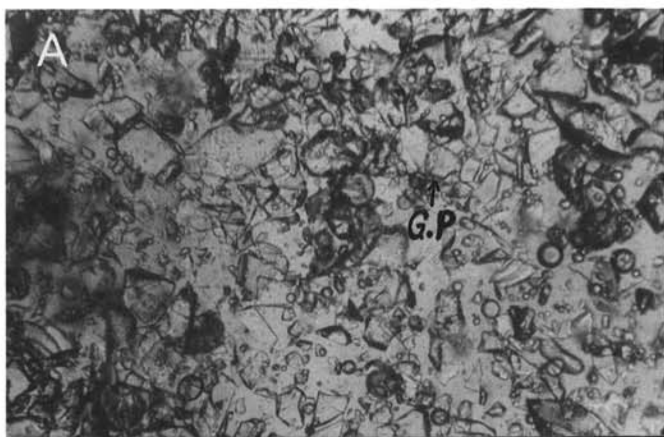
Lipophilic Properties of Chlorinated Gelatin-Coated Glass Samples

Seguchi (1984a) reported that chlorinated starches showed high oil-binding ability, and it was thought that the protein film on the starch granule was involved in acquiring hydrophobicity. As all starches show this ability, protein specificity to chlorination is probably small. Because the amount of protein film on a starch granule is small, it is difficult to study the mechanism of lipophilization (hydrophobicity) of starch-protein directly.

To study the mechanism of lipophilization, an experiment was designed using glass powder or glass beads and gelatin as a model of the starch granule. When dried, gelatin-coated glass beads were tested with Coomassie Brilliant Blue, they stained blue, indicating the presence of protein (data not shown). Figure 1A and B showed no oil-binding ability for nonchlorinated glass samples after vigorous mixing with oil in water; however, chlorinated, gelatin-coated glass samples showed strong oil-binding ability (Fig. 2A and B). This phenomenon is similar to that reported previously for chlorinated starch (Seguchi 1984b). Therefore, it seemed that the chlorinated gelatin-coated glass samples were an appropriate model of chlorinated starch for this study.

Preparation of Hydrophobic Protein by Chlorination

A water-insoluble gelatin film was prepared as described in the previous section. Gelatin formed no film when chlorination was omitted. Other water-soluble proteins, such as BSA (Fig. 3) or ovalbumin, also formed water-insoluble film by chlorination. Recoveries of insoluble protein from chlorinated gelatin and BSA were 2.9 and 2.7%, respectively. This low recovery could result from chlorination occurring only on the surface of the protein in the petri dish. Casein or gluten were also chlorinated and formed films. Low molecular weight protein such as polypeptone did not form a film by chlorination (data not shown). The possibility that chlorinating carbohydrate gives a more hydrophobic surface was checked by using soluble starch in the same manner. No film formation or increase in hydrophobicity was observed with



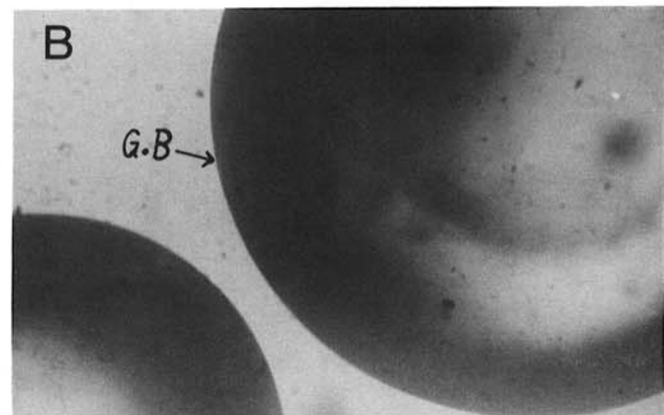
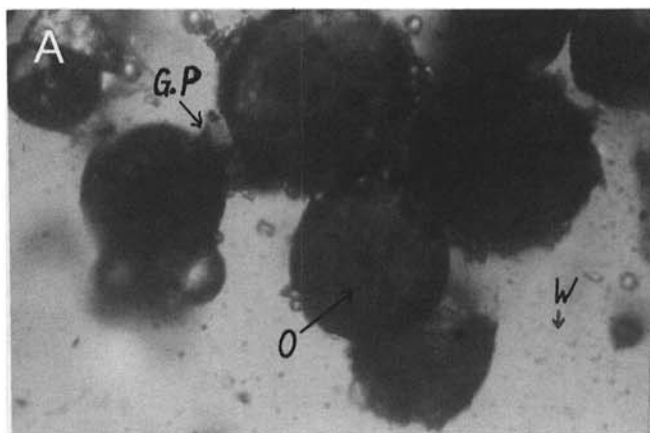


Fig. 1. Photomicrographs of **A,** nonchlorinated, gelatin-coated glass powder (**G.P**) and **B,** nonchlorinated, gelatin-coated glass bead (**G.B**) after mixing with oil and water.



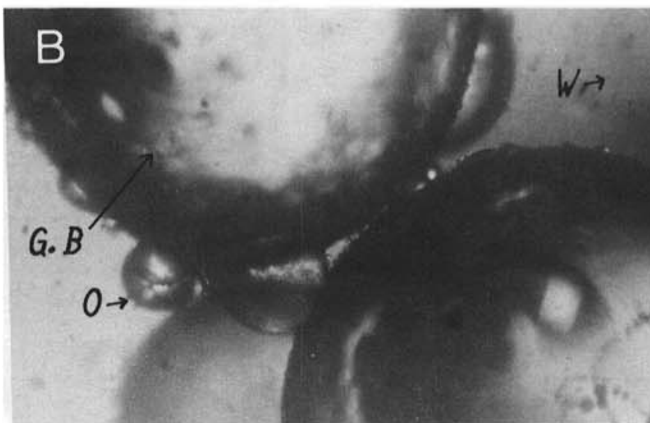


Fig. 2. Photomicrographs of **A,** chlorinated, gelatin-coated glass powder (**G.P**) and **B,** chlorinated, gelatin-coated glass bead (**G.B**) after mixing with oil (**O**) and water (**W**).

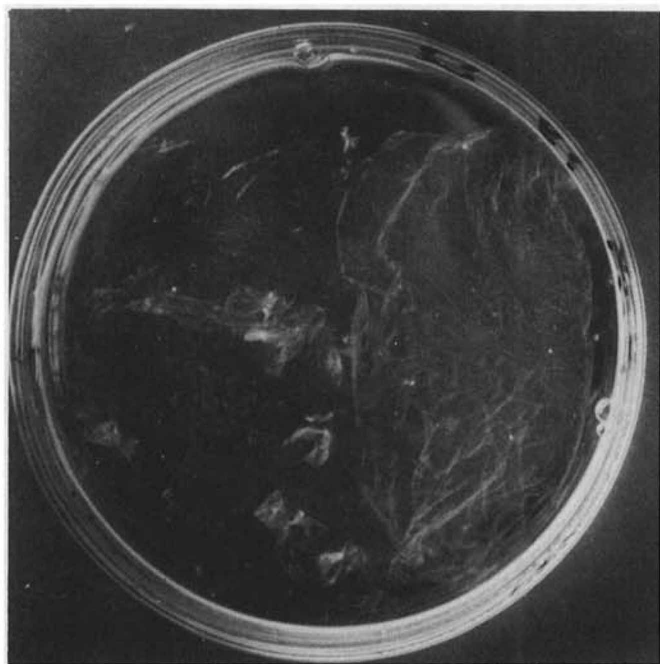


Fig. 3. Water-insoluble film of chlorinated bovine serum albumin in water.

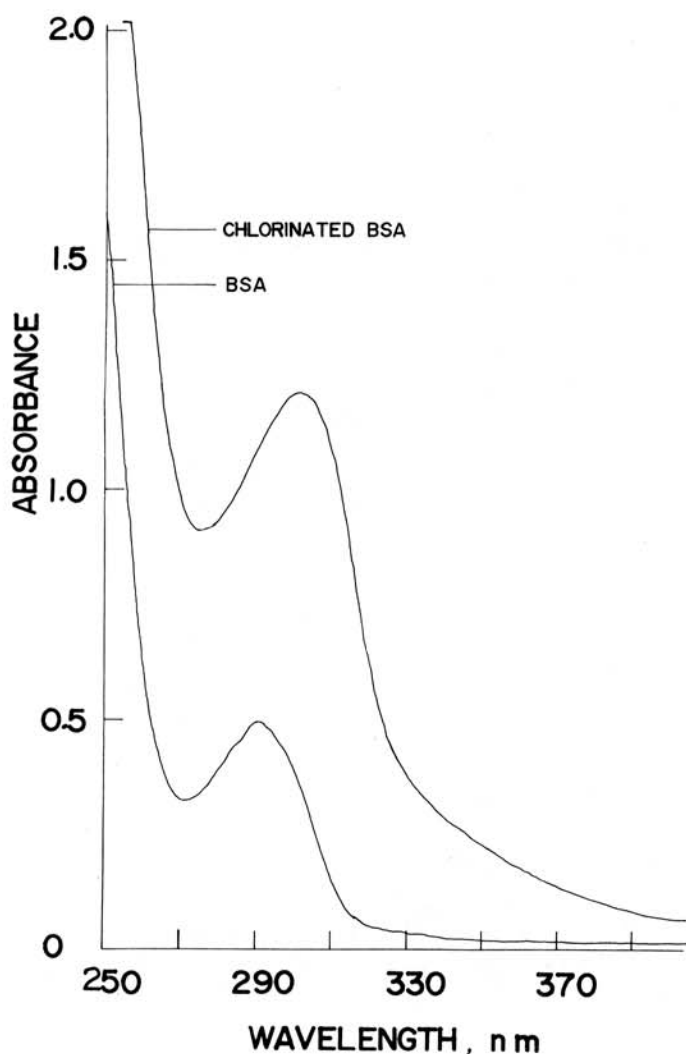


Fig. 4. Spectra of nonchlorinated and chlorinated bovine serum albumin (BSA).

carbohydrate (data not shown). The change in hydrophobicity by chlorination seemed to be specific to protein.

Nature of the Water-Insoluble Protein Film

The water-insoluble washed protein films could be dissolved in weak alkali (0.4% NaOH) or 1% SDS solution, but when dialyzed against water they formed a flocculant protein precipitate.

The BSA sample used in SDS disc gel electrophoresis showed one band in electrophoresis gel when stained with Coomassie Brilliant Blue. Water-insoluble washed protein film of chlorinated BSA was dissolved in SDS and used as an electrophoresis sample. SDS disc gel electrophoresis of these samples showed no difference in molecular weight between nonchlorinated and chlorinated BSA, indicating that the water insolubility after chlorination was not caused by polymerization or decomposition. There was no material that would not migrate into the electrophoresis gel.

The change in the ultraviolet absorption spectrum was determined upon chlorination of BSA. As presented in Figure 4, upon chlorination an increase was observed in the absorption between 260 and 400 nm. Gelatin also indicated the same increase. Tsen and Kulp (1971) found the same result with water extracts from treated flour. They also reported that the increase in absorbance at 280 nm by chlorination was caused by tyrosine only. It is known that tyrosine shows absorbance at near 290 nm in alkali; therefore an increase or shift of 290–300 nm by chlorination would depend on tyrosine residue only in protein. The chemical significance of these spectroscopical changes might be responsible for the hydrophobicity of protein by chlorination.

Paper Chromatography of Chlorinated Amino Acids

Amino acids were chlorinated and their hydrophobicity examined by paper chromatography (Table I). Almost all amino acids—except tyrosine, lysine, and cystine—did not change with chlorination. The R_f values of the chlorinated derivatives of these three amino acids were higher than the nonchlorinated ones. This indicates that tyrosine, lysine, and cystine were changed by chlorination to more hydrophobic molecules.

Chlorination of tyrosine made two new derivatives with R_f values of 0.65 and 0.77 (Fig. 5A). Aleksiev (1968) reported transformation of tyrosine to 3,5-dichlorotyrosine by chlorination. Since authentic chlorinated derivatives of tyrosine are not commercially available, the R_f values were compared with derivatives of mono- and diiodotyrosine. These results suggested the possibility of R_f 0.77 for 3,5-dichlorotyrosine and R_f 0.65 for

TABLE I
R_f Values of Chlorinated Amino Acid

Amino Acid	R_f value: Nonchlorinated	R_f value: Chlorinated
Alanine	0.48	0.48
Arginine	0.36	0.36
Aspartic acid	0.35	0.35
Cysteine	0.34	0.34
Cystine	0.20	0.20, 0.27
Glutamic acid	0.42	0.42
Glycine	0.24	0.24
Histidine	0.33	0.32
Isoleucine	0.79	0.79
Leucine	0.80	0.80
Lysine	0.28	0.27, 0.74
Ornithine	0.28	0.28
Methionine	0.68	0.68
Phenylalanine	0.75	0.75
Proline	0.44	0.44
Serine	0.34	0.34
Threonine	0.42	0.42
Tryptophan	0.70	0.70
Tyrosine	0.52	0.52, 0.65, 0.77
3,5-Diiodotyrosine	0.76	0.76
3-Iodotyrosine	0.65	0.64, 0.74
Valine	0.66	0.66

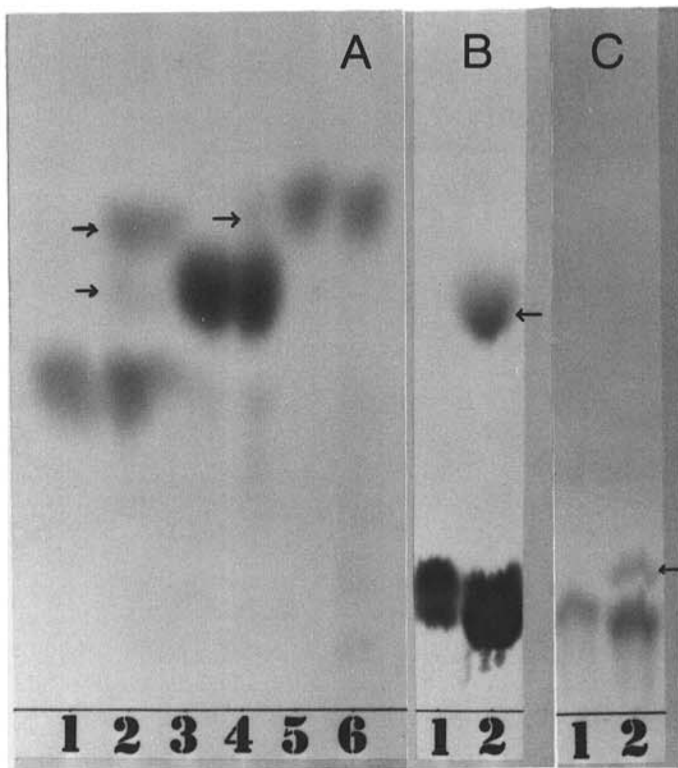


Fig. 5. Paper chromatograms of **A,** tyrosine **(1)** and chlorinated tyrosine **(2)**, monoiodotyrosine **(3)** and chlorinated monoiodotyrosine **(4)**, diiodotyrosine **(5)** and chlorinated diiodotyrosine **(6)**; **B,** lysine **(1)** and chlorinated lysine **(2)**; **C,** cystine **(1)** and chlorinated cystine **(2)**.

monochlorotyrosine. Figure 5B indicates a new derivative of lysine by chlorination with an R_f value of 0.74.

Aleksiev (1968) reported that chlorination changed cystine to cysteic acid. Since the R_f value of cysteic acid is lower than cystine in this solvent system, the chlorinated derivative of cystine that gave a higher R_f value in this study (Fig. 5C) is different from cysteic acid.

The hydrophobicity of tyrosine, lysine, and cystine were increased by chlorination. The chlorinated derivatives of these three amino acids could cause hydrophobicity in protein and show oil-binding ability. Because gelatin-coated glass samples became hydrophobic and showed oil-binding ability when they were chlorinated, the same mechanism should also work for starch.

CONCLUSIONS

Experimental models of chlorinated, protein-coated glass powder support the hypothesis that lipophilic properties acquired when wheat starch is chlorinated result from the lipophilic protein film on the starch granule. Also, the lipophilization (hydrophobicity) of chlorinated protein film was assumed to be induced by hydrophobic molecules of chlorinated amino acids such as tyrosine, lysine, or cystine.

ACKNOWLEDGMENT

I thank H. Matsumoto, Kobe Women's University, for his valuable advice and encouragement.

LITERATURE CITED

ALEKSIEV, B. V. 1968. Reaction of sulfur-containing aminocarboxylic acid, peptides, and proteins with chlorine. I. Stoev, S. (Hochsch, Chem. Technol:Sofia, Bulgaria). Pharmazia 23(11):641.

SEGUCHI, M., and MATSUKI, J. 1977. Studies on pan-cake baking. I. Effect of chlorination of flour on pan-cake qualities. Cereal Chem. 54:287.

SEGUCHI, M. 1984a. Comparison of oil-binding ability of different chlorinated starches. Cereal Chem. 61:244.

SEGUCHI, M. 1984b. Oil-binding capacity of prime starch from chlorinated wheat flour. Cereal Chem. 61:241.

TSEN, C. C., and KULP, K. 1971. Effects of chlorine in flour proteins, dough properties, and cake quality. Cereal Chem. 48:247.

TSUTSUMI, C., and NAGAHARA, T. 1961. Effect of drying temperatures on the moisture determination of starches by oven drying method. Shokuryo Kagaku Kenkyusho Hokoku 15:118.

WEBER, K., and OSBORN, M. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406.

[Received June 14, 1984. Revision received December 7, 1984. Accepted December 7, 1984.]