Lipid Binding by Protein Films Heated on Glass Beads and Prime Wheat Starch

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ABSTRACT

Heat treatment of glass powder or glass beads coated with bovine serum albumin (BSA) imparted strong oil-binding ability similar to that of heat-treated wheat starch. Heat-treated BSA alone showed strong oil-binding capacity (3.4–3.6 ml oil/g BSA) and almost no solubility in water. Other proteins (ovalbumin, casein, gluten, and soybean protein) also exhibited oil-binding ability after heat treatment, but polysaccharides (soluble starch, konjac glucomannan, cyclodextrin, Sephadex G-50, and pectin) did not.

Paper chromatography of 20 amino acids indicated that there was no change in amino acids after heat treatment. No differences were observed in the infrared spectroscopy and X-ray diffraction patterns of nonheated and heated BSA. Heated BSA showed far lower susceptibility to pepsin digestion than nonheated BSA, which would suggest the occurrence of conformational changes in BSA by heat treatment.

Cereal Chem. 63(4):311–315

Seguchi and Matsuki (1977) reported that chlorination of wheat flour imparted hydrophobicity (lipophilization) to the prime starch fraction. This hydrophobicity gave improved pancake textures such as increased springiness and reduced gumminess. Seguchi (1984a) suggested that the lipophilization of the prime starch was caused by chlorination of a protein film on the starch granules.

Seguchi (1984b) also indicated that heat treatment (100–160° C) of wheat prime starch imparted a hydrophobicity to starch similar to that caused by chlorination. Hydrophobicity induced by heating wheat prime starch might possibly be used in place of chlorinated flour (Russo and Doe 1970). The heat treatment lipophilization was assumed to be caused by heat denaturation of a protein film coated on the starch granule. In this work, the lipophilization was studied by preparing glass beads and glass powder coated with bovine serum albumin (BSA) as models of the starch granules. The oil-binding abilities of the BSA-coated glass samples were investigated after heat treatment. Some results of lipophilization of various proteins by heat treatment are also reported.

MATERIALS AND METHODS

Materials

Wheat prime starch was prepared from nonchlorinated wheat flour by an acetic acid fractionation technique previously described by Seguchi and Matsuki (1977). Protein (N × 6.25) of the prime starch was determined by the method of Smith (1964). Glass powder (10–50 μm) was prepared by grinding a detergent-washed Pasteur pipette in a mortar and pestle. Glass beads (1 mm in diameter), BSA, gelatin, casein, ovalbumin, gluten, soybean protein, rapeseed oil, and pepsin were purchased from commercial sources. Other materials were reagent grade products.

Preparation of BSA-Coated Glass Samples and Wheat Prime Starch

Samples of glass powder or beads (1 g) were suspended in 10 ml of 1% BSA and stirred for 2 hr at 25° C. The glass samples were then filtered and dried for one day at room temperature (31° C) at 22% relative humidity. The amounts of protein adhering to the glass powder or glass beads were determined by the method of Lowry et al (1951). BSA-coated prime starch was prepared by the same procedure used to coat the glass samples.

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Heat Treatment of BSA-Coated Glass Samples, BSA-Coated Wheat Prime Starch, Proteins, Polysaccharides, Amino Acids, and a Peptide

BSA-coated glass or wheat prime starch samples (500 mg) were placed in petri dishes (93 × 14 mm) and heated for 2 hr at 120° C as previously described by Seguchi (1984b). Powders (500 mg) of proteins, polysaccharides, 20 amino acids, and a peptide were placed in petri dishes and heated at 120° C for 2 or 5 hr.

Alkali Treatment of Wheat Prime Starch and Determination of Its Oil-Binding Ability

Alkali treatment of the wheat prime starch and determination of its oil-binding capacity were carried out as previously described by Seguchi (1984b). The standard deviation was 0.06 ml with this method.

Determination of Oil-Binding Ability of BSA-Coated Glass Samples

BSA-coated glass samples (500 mg) and 1 ml of rapeseed oil were mixed with 10 ml of water and shaken vigorously for 2–3 min at 3,200 rpm in a Yamato vibrio shaker with a horizontal vibrational amplitude of 0.1 cm. The oil-binding ability of the BSA-coated glass samples was observed microscopically (×50) and was identified by precipitated oil droplets adhering to the BSA-coated glass samples.

Determination of Lipophilization of Heated BSA

The oil-binding capacity of heated BSA (120° C for 5 hr) was determined by the following procedure. Heated BSA (500 mg), 5 ml of water, and 2 ml of rapeseed oil were mixed in measured conical centrifuge tubes and shaken vigorously for 2–3 min. After centrifugation at 2,000 rpm for 20 min, the volume of the free oil layer above the water and insoluble protein was measured and the oil-binding capacity of heated BSA (ml of oil/g of protein) was calculated as: (2 ml − ml of free oil) × 2.

Paper Chromatography of Amino Acids and Peptide

Paper chromatography of 20 amino acids and the peptide was performed as previously described by Seguchi (1985).

Infrared Spectrum and X-Ray Diffraction of BSA

Infrared spectra of nonheated and heated BSA (120° C for 5 hr) in KBr pellets were recorded from 600 to 4,000 cm^{-1}. X-ray diffraction patterns of nonheated and heated BSA (120° C for 5 hr) were recorded with a Rigaku Denki Flux diffractometer. The packed disks were exposed to Ni-filtered Cu alpha-radiation. The diffractometer was operated at 35 kV and 30 mA.

Susceptibility of Nonheated and Heated BSA (120° C for 2 hr) to Pepsin Digestion

Nonheated and heated BSA (10 mg) were suspended in 10 ml of

0.07*M* Na-acetate buffer (pH 4.0) containing 50 m*M* NaCl. The samples were digested with 0.1 mg pepsin at 37° C for 60 min and then boiled for 5 min. The digested products were dialyzed against 100 ml of 0.9% NaCl overnight, and the dialyzable products were determined by the method of Lowry et al (1951).

RESULTS

Experiment I: Oil-Binding Capacity of Wheat Prime Starch Following Various Treatments

In this experiment, the heating conditions (120° C for 2 or 5 hr) were selected for low damage to starch. As previously reported by Seguchi (1984b), heated wheat prime starch with a moisture content of almost zero percent showed strong oil-binding capacity (Table I). The lipophilization was lowered by treatment with 0.2% alkali. This effect was assumed to result from solubilization of the surface protein by the alkali. However, heat treatment of the alkali-treated and dried prime starch again brought some lipophilization (Table I). This may indicate that a small amount of protein remained on the starch granule.

Alkali-treated and dried prime starch that was coated with BSA lost oil-binding ability (Table I) but regained strong oil-binding ability upon reheating. From the results of the following experiments with BSA-coated glass samples, this change in oil-binding ability was assumed to depend on heat-induced lipophilization of the BSA on the surface of the starch granule. Figure 1 shows these lipophilic starch granules adhering to oil droplets.

Experiment II: The Lipophilic Properties of Heated BSA-Coated Glass Samples

Because the amount of protein film on wheat starch granules is small, it is difficult to determine the mechanism of the change in hydrophobicity (lipophilization) of the protein on the starch granules. Protein-coated glass samples were prepared to use as an

TABLE I
Effects of Various Treatments on Oil-Binding Capacity of Wheat Prime Starch[a]

Treatment of Wheat Prime Starch	Oil-Binding Capacity (ml of oil/g of starch)
Nonheated	0.2
Heated 120° C for 2 hr[b]	0.8
0.2% NaOH[c]	0.4
0.2% NaOH, reheated	0.6
0.2% NaOH, BSA-coated	0.3
0.2% NaOH, BSA-coated, reheated	0.9

[a] The standard deviation was 0.06 ml with this method.
[b] Seguchi 1984a.
[c] Alkali treatment with 0.2% NaOH (Seguchi 1984a).

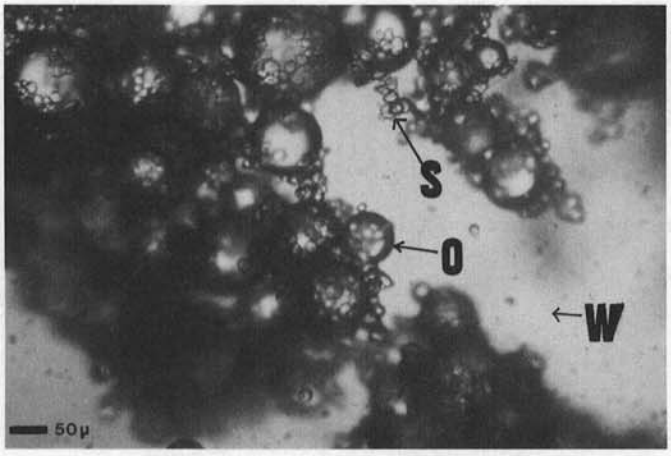

Fig. 1. Photomicrograph of heated wheat prime starch granules coated with BSA after mixing the granules with oil droplets (**O**) and water (**W**).

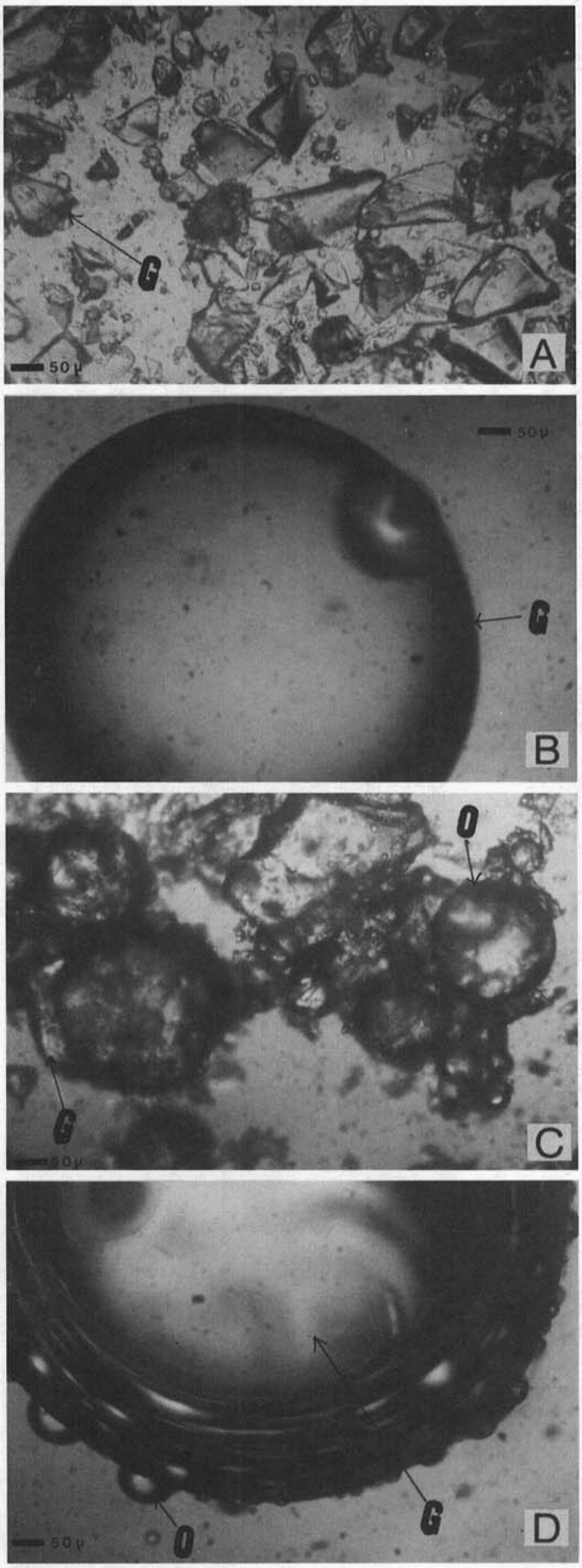

Fig. 2. Photomicrographs of **A,** nonheated BSA-coated glass powder (**G**); **B,** nonheated BSA-coated glass bead (**G**); **C,** heated BSA-coated glass powder; and **D,** heated BSA-coated glass bead mixed with oil (**O**) in water.

experimental model. The protein content of the BSA-coated glass beads was 1.98 mg/g of glass beads, which is comparable to the protein (N × 6.25) of wheat prime starch (2.5 mg/g).

After vigorous mixing with oil in water, BSA-coated glass samples showed no oil-binding ability (Fig.2A and B), but heated BSA-coated glass samples showed strong oil-binding ability (Fig. 2C and D). This response was similar to that of heated starch (Seguchi 1984b). Therefore, the heated BSA-coated glass samples were studied as a model of the heat-treated starch.

Experiment III: Lipophilization of Heated BSA

Heated BSA showed reduced solubility in water, sodium dodecyl sulfate solution, weak alkali, or acid solutions. No definite changes of appearance in the crystalline BSA were observed after heat treatment; however, the white powders had become slightly yellow.

The oil-binding capacity of heated BSA (120° C for 5 hr) was 3.4–3.6 ml of oil/g of BSA. Figure 3B shows heated BSA cohering to oil droplets in water. The original, nonheated BSA had no oil-binding ability and easily dissolved in water (Fig. 3A).

Experiment IV: Heat Treatment of Other Proteins and Polysaccharides

The same heat treatment (120° C for 5 hr) was applied to other proteins (ovalbumin, casein, gluten, and soybean protein), and all showed oil-binding ability after heating (Fig. 4B, D, F, and H). The original, nonheated proteins showed no lipophilization (Fig. 4A, C, E, and G). When the protein, gelatin, was heated, no clear evidence of lipophilization was observed, but the gelatin became water insoluble.

To test if polysaccharide was involved in the lipophilization of heated starch granules, various polysaccharides (soluble starch, konjac glucomannan, Sephadex G-50, cyclodextrin, and pectin) were heated under the same conditions. No lipophilization of the heated polysaccharides was observed. The results suggested that lipophilization by heat treatment was a specific property of the protein.

Experiment V: Effects of Heating on Amino Acids, a Peptide, and BSA

Seguchi (1985) suggested that the water-insolubility of chlorinated BSA was caused by chlorinated tyrosine, lysine, and cystine in the protein. Because of these results, the possibility that the water insolubility of heated BSA also depended on changes of amino acids in the protein was investigated. Twenty amino acids and a peptide (glutathione) were heated (120° C for 2 hr). Paper chromatograms of these heated amino acids showed no new derivatives indicating that the mechanism causing water-insolubility of protein by heat treatment was different from that of chlorination. No differences were shown between heated and nonheated glutathione by paper chromatography. These results probably indicate that the polypeptide when heat treated as a solid phase did not change or interreact.

Infrared spectroscopy and X-ray diffraction patterns of nonheated and heated BSA were recorded, but no differences were observed by these analytical methods.

To ascertain if a conformational change had occurred in heated BSA, the susceptibility of nonheated and heated BSA to pepsin digestion was examined. After pepsin digestion of nonheated BSA, the amount of dialyzable products was 23.5%. No dialyzable products of heated BSA were found. Although heated BSA showed no change in appearance, these results indicate that pepsin could not digest the heated BSA because of a change in conformation.

Recently many proteins have been processed by extruders under conditions of heat, low moisture, and high pressure and have been used as texturized proteins in various foods. Because heated BSA showed low susceptibility to pepsin in this experiment, the digestibility and nutritional value of these heated proteins should be examined.

DISCUSSION

I would like to hypothesize that the starch granule is covered by a thin protein film and that this film has a large role in transforming the starch granule from hydrophilic surface behavior to lipophilic behavior. This hypothesis is based on the following results: 1) In experiment I, the loss of the oil-binding ability of the heated starch granule (Table I) could be explained if 0.2% NaOH extracted a protein film from the starch granule. The regeneration of oil-binding ability after reheating appears contradictory, but it may be due to residual protein film that was not dissolved completely by 0.2% NaOH. 2) BSA-coated prime starch (experiment I, Table I) and BSA-coated glass samples (experiment II) became highly lipophilic after heat treatment. 3) BSA and other proteins (ovalbumin, casein, gluten, and soybean protein) became lipophilic after heat treatment (experiments III and IV). 4) Heating did not bring about lipophilization of polysaccharides (soluble starch, konjac glucomannan, cyclodextrin, Sephadex G-50, or pectin; experiment IV).

This hypothesis should be tested by an experiment that would confirm the presence of a protein film over the starch granule. Gallant et al (1972) reported the existence of surfaces in starch grains that showed resistance to α-amylase attack. Sasaki et al (1980) observed that starch granules solubilized by 10*M* urea pullulanase treatment left empty granular bags of the outside layers. However, the chemical composition of these granular bags is unknown. Some evidence indicates that there may be a protein membrane on starch granules. Lowy et al (1981) reported a salt-extractable protein from wheat starch granules. MacDonald and Preiss (1985) partially purified and characterized granule-bound starch synthases from normal and waxy maize.

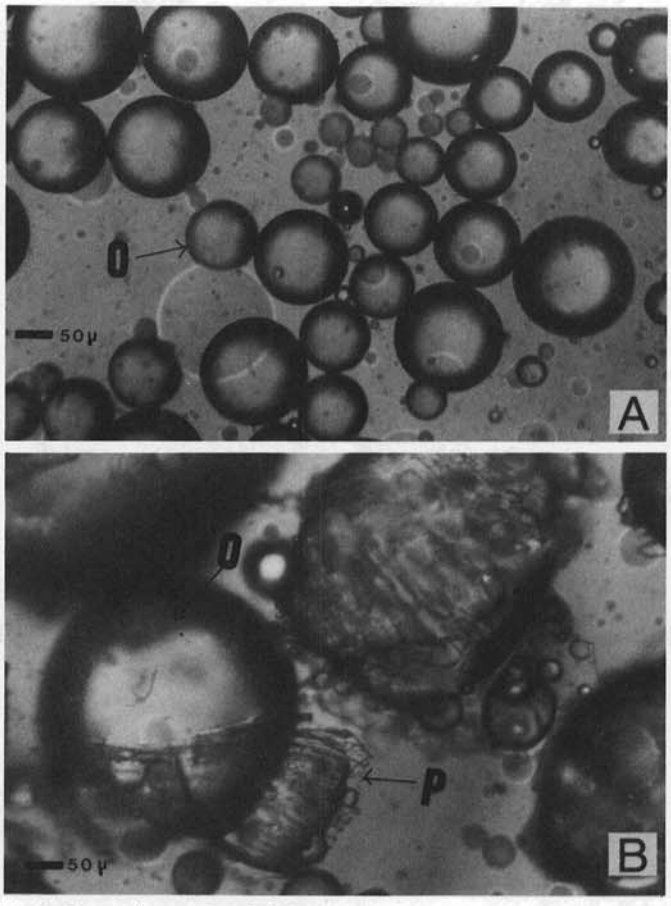

Fig. 3. Photomicrographs of oil droplets (**O**) and water after mixing with **A,** nonheated BSA and **B,** heated BSA **(P).**

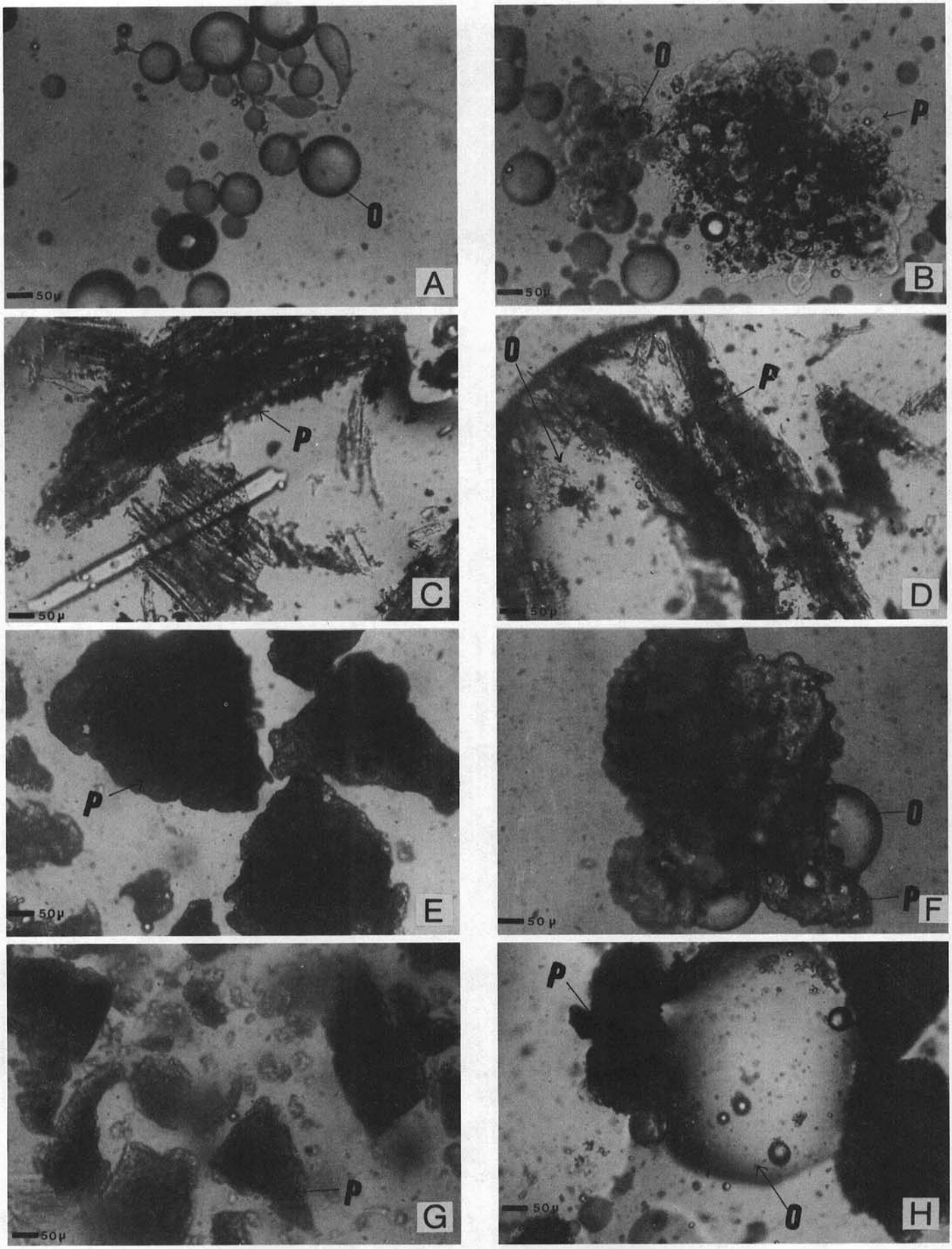

Fig. 4. Photomicrographs of **A,** nonheated ovalbumin; **B,** heated ovalbumin (**P**); **C,** nonheated casein (**P**); **D,** heated casein (**P**); **E,** nonheated gluten (**P**); **F,** heated gluten (**P**) **G**, nonheated soybean protein (**P**); and **H**, heated soybean protein (**P**) mixed with oil (**O**) in water.

ACKNOWLEDGMENT

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

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[Received September 10, 1985. Revision received January 8, 1986. Accepted January 9, 1986.]