Adsorption of Wheat Proteins on Wheat Starch Granules[1]

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ABSTRACT

In order to study interactions between wheat starch and wheat protein, the adsorption of wheat proteins on wheat starch was measured. Starch was mixed with protein solutions differing in concentration, and protein content was determined before adding the starch and after centrifugation and removal of supernatant. Any decrease in protein content was calculated as the amount of protein adsorbed on the starch granules. The adsorption was found to be low for bovine serum albumin and for a wheat protein fraction composed of low molecular weight proteins. For a wheat protein fraction composed of high molecular weight proteins the amount of adsorbed protein was much higher (around 10 mg protein/m^2 starch). The amount of protein adsorbed increased with pH in the interval 3.1–7.6. It was also affected by the concentration of NaCl, and was found to be highest in 0.0025*M* NaCl. The amount of protein adsorbed increased when starch granules had been heated before adsorption but was not affected by heating the protein solutions. Wheat proteins were adsorbed to a much greater extent on potato starch than on wheat or maize starches.

In a wheat flour dough, starch granules are dispersed in a continuous gluten matrix. Due to the small size of the starch granule (in the range 1–30 μm), the surface area per gram of starch is considerable; values in the range 0.25–0.90 m^2/g of starch have been reported (Morrison and Scott 1986). These values are, of course, dependent on the proportion of the small B-granules. In dough there will thus be a great number of gluten/starch interfaces that might be of importance for baking performance.

The role of starch-protein interactions during baking has been discussed by Sandsted (1961) and Kulp and Lorenz (1981). It was suggested that the surface of the wheat starch granule, as well as the nature of gluten, are of importance for starch-protein interactions during dough formation and baking. Recently Lelievre et al (1987) discussed protein-starch interactions as an explanation for the results of baking tests where protein level and starch granule size were varied.

Starch-protein interactions have also been discussed in relation to wheat endosperm hardness (Barlow et al 1973, Simmonds et al 1973). In hard endosperms, proteins adhere so strongly to the starch granule surface that during milling breakage will occur through the starch granule. In the soft endosperm, the adhesion between starch and protein is weaker and breakage will occur along the granules. Typical starch proteins have been identified (Lowy et al 1981), and one protein of 15,000 daltons (Da) has been shown to correlate with endosperm softness (Greenwell and Schofield 1986).

The nature of the surface of the wheat starch granule has been the topic of several investigations. The zeta potential was determined for the small B-granules in wheat starch and was found to be negative above pH 3.7 (Marsh and Waight 1982). Extraction of the starch with different solvents showed that proteins as well as lipids were present on the starch granule surface. Also ESCA (electron spectroscopy for chemical applications) revealed that lipids (phospholipids) and proteins are present at the starch granule surface (Russell et al 1987). The presence of a protein film on the wheat starch granule has been demonstrated by protein-specific dye binding (Seguchi 1986).

Wheat starch-wheat protein interactions have been studied in solution by measuring absorbances of amylose-iodine complexes or amylopectin-iodine complexes in the presence of wheat proteins (Dahle 1971). Wheat starch-gluten interactions have also been studied by measuring the viscoelastic properties of starch-gluten gels (Lindahl and Eliasson 1986).

One way to study starch-protein interactions would be to treat the interaction as an adsorption of proteins on a solid phase (the starch granules). Adsorption of different proteins on hematite (Koutsoukos et al 1983) and of bovine serum albumin on silica (MacRitchie 1972) were measured by determining protein concentration in solution before and after adsorption. The difference in protein content is calculated as the amount of protein adsorbed on the solid surface. In the present study the same approach was used to study the adsorption of wheat proteins on wheat starch granules. The influence of time, pH, ionic strength, and temperature on the adsorption was investigated. A few experiments were also carried out to check the adsorption of proteins other than wheat proteins on wheat starch, and the adsorption of wheat proteins on other starches. During the course of this work, it was found that different protein preparations from the same flour gave rise to differences in the adsorption behavior. Reasons for this behavior, which clearly emphasizes the importance of the protein in the starch-protein interaction, will be discussed.

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MATERIALS AND METHODS

Material

The wheat flour used for protein preparation was a commercial flour (Kungsörnen, Sweden). Wheat starch used was A-starch from the wheat variety Amy prepared according to Meredith et al (1978). Commercial samples of potato starch (Lyckeby National, Kristianstad, Sweden) and maize starch (CPC, England) were also used. Bovine serum albumin (BSA) was from Sigma (lot A4503). Distilled-deionized water was used. All other chemicals were analytical grade.

Specific areas (determined according to the Brunauer-Emmett-Teller equation) of the starches were 0.52 m^2/g for maize starch, 0.47 m^2/g for wheat starch, and 0.26 m^2/g for potato starch.

Preparation of Wheat Protein Fractions

Wheat protein fractions were prepared according to the procedure described by Clements (1973). The protein fractions extracted in 1*M* NaCl were discarded, whereas the three first fractions obtained in distilled water were collected. The first of these three fractions (WP1) was treated separately, whereas the second and third fractions were combined. The combined fraction (WP2) was used for the main part of this work.

Electrophoresis

Composition of protein fractions was studied by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in the Pharmacia Phast System. Precast gradient gels 10–15%, 45 × 43 × 0.45 mm, were used. SDS-PAGE was performed both with and without the addition of 2-mercaptoethanol (2-ME). The following molecular standards were used (Pharmacia LMW calibration kit): phosphorylase b 94,000 Da, albumin 67,000 Da, ovalbumin 43,000 Da, carbonic anhydrase 30,000 Da, trypsin inhibitor 20,000 Da, and α-lactalbumin 14,000 Da. The gels were stained in 0.04% Coomassie Brilliant Blue R250 in 12% (w/w) trichloracetic acid, 1 hr at 50° C and destained in 12% trichloracetic acid.

Protein Determination

Protein content of protein solutions was determined with the Biuret method (Thorne 1978) using bovine serum albumin as a standard. For wheat protein fractions, the factor 5.7 was used to convert nitrogen to protein.

Adsorption Experiments

Wheat starch granules swell when water is added. To avoid this as a source of error during the adsorption experiments starch was first hydrated by mixing 0.5 g of starch (dry matter) with 5 ml of water for 30 min at room temperature. The suspension was then centrifuged (15 min, 1,100 × *g*) and the supernatant was discarded. Prior to adsorption experiments the hydrated starch was suspended in water (2 ml).

Protein (300 mg of WP1 or WP2) was dissolved in water or HCl ($1 \times 10^{-5}M$, 29.7 g), and stirred for 2.5 hr. The solution was centrifuged (15 min, 1,100 × *g*) before further use in order to remove aggregates that otherwise might settle together with starch granules without being adsorbed. The supernatant was diluted with the same solvent as was used to dissolve the protein to give six concentrations in the range 0.2–5 mg/ml. Solutions of the highest concentrations were turbid. Protein concentration was determined with the biuret method. BSA solutions were prepared without the prolonged stirring and centrifugation.

Protein solution (5 ml) was added to a test tube containing starch (0.5 g dry matter) suspended in water (2 ml). Unless otherwise specified, adsorption was allowed to continue for 30 min at room temperature. The test tubes were shaken gently during the adsorption. The test tubes were then centrifuged as described above, and the protein content of the supernatant was determined. The amount of protein adsorbed was calculated from the decrease in protein content and expressed as milligrams of protein per gram of starch. In a few cases the adsorbed amount was expressed as milligrams per square meter of starch. Control experiments were carried out with protein solutions without starch, and with starch in water, respectively. When protein solutions without starch were used, a decrease in protein content was observed. However, this decrease, which could be attributed to adsorption on test tubes and so on, never exceeded 3% of the amount of protein present. This corresponded to a change in the adsorbed amount of at most 0.1 mg/g starch and was not compensated for in the calculations. When starch was treated in water or in $1 \times 10^{-3}M$ HCl, there was no detectable protein in the supernatant.

Desorption

After the protein containing supernatant had been removed, the same volume of water was added. The starch granules were suspended and desorption was allowed to occur during the same conditions as the adsorption. After centrifugation the protein content of the supernatant was determined. The amount of protein desorbed, as well as the amount of protein remaining on the starch granules after desorption were calculated.

Time, pH, and Salt Concentration

Usually the adsorption was allowed to proceed during 30 min. The influence of adsorption time was checked by using shorter or longer times (up to 24 hr). Values in the pH range 3.1–7.6 were obtained by using HCl or NaOH as solvents. The ionic strength was changed by using NaCl at different concentrations (0.001–0.035*M*).

Temperature

The influence of temperature was investigated by using heated starch granules or heated protein solutions. Heated starch granules were prepared by keeping starch suspensions (1 g of starch in 100 ml of water) in a waterbath for 30 min. Temperatures investigated were 40, 50, 60, 70, 80, and 90° C. After cooling to room temperature, the samples were centrifuged (15 min, 1,100 × *g*), the supernatant was discarded, and the remaining starch was resuspended in the same volume of water and centrifuged again. This procedure was repeated once and then the starch (suspended in water) was freeze-dried. The freeze-dried starch was hydrated as described above. When the starch was heated to 90° C, a gel formed when water was added, and thus it was not possible to study protein adsorption for this starch.

Protein solutions, after dilution, were heated for 15 min at 60 and 90° C, respectively. Before further use, the heated protein solutions were centrifuged (15 min, 1,100 × *g*). These solutions were then added to hydrated starch and the adsorption performed as described.

Statistical Evaluation

An average value of adsorbed amount was calculated for protein concentrations ≥1.5 mg/ml. Student's *t* test was used to calculate if observed differences in adsorbed amount were statistically significant. Significance levels of 5, 1, and 0.1% were tested.

RESULTS AND DISCUSSION

The first problem to solve in this kind of experiment is the formation of gluten when water is added to a wheat protein fraction. The fractions obtained with the salt treatment described by Clements (1973) gave turbid solutions but no detectable gluten formation. At low protein concentrations and low pH values, clear solutions were obtained.

The adsorption of the two wheat protein fractions in water and of BSA at different pH values on wheat starch granules are shown in Figure 1A. Little BSA seemed to be adsorbed, and the small differences in protein concentration before and after adsorption resulted in scattering of the data. It was not possible to differentiate between the different pH values. Little WP1 was adsorbed, whereas the second wheat protein fraction (WP2) was adsorbed to a much greater extent on wheat starch granules. Although there is some scattering in the data at the higher protein concentrations, Figure 1A seems to indicate that the adsorbed amount leveled off at around 5.5 mg/g of starch. The amount

of protein remaining on the wheat starch granules after desorption is shown in Figure 1B. WP1 and BSA seemed to be more or less completely desorbed from the starch, whereas WP2 was desorbed to only a small extent.

Two independent adsorption experiments are shown for WP2 in Figure 1A. It is evident that reproducibility is good at low protein concentrations, whereas there is considerable scattering in the results obtained at higher protein concentrations. The average values for adsorbed amount in the plateau region were not significantly different.

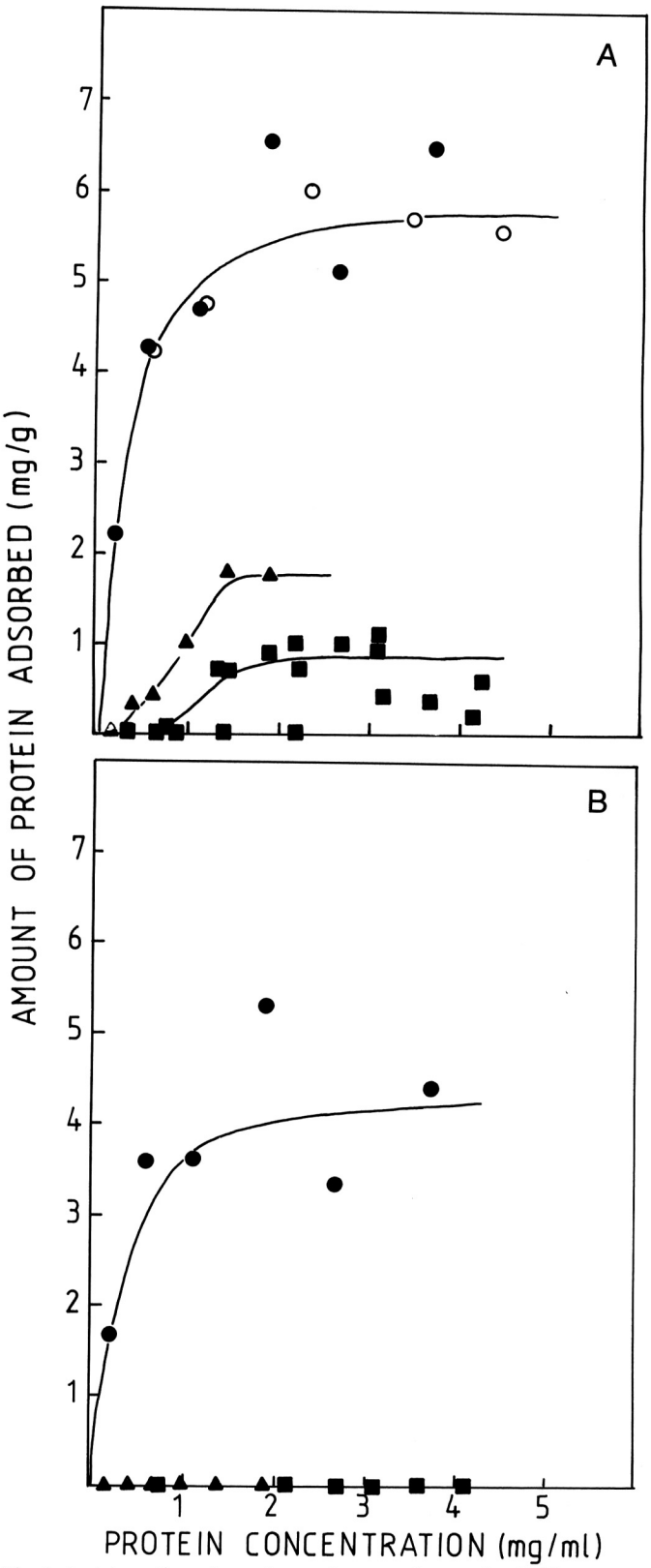

Fig. 1. A, Adsorption of protein on wheat starch granules. **B,** The amount of protein remaining on starch granules after desorption in water. Wheat protein fraction 1 dissolved in water (▲); wheat protein fraction 2 dissolved in water, two independent experiments (○, ●); bovine serum albumin at different pH values (■).

The results presented in Figure 1 show that the interaction between wheat starch and wheat proteins might be discussed in terms of protein adsorption on solids. The amount of protein adsorbed seemed to level off at higher protein concentrations. An isotherm with a plateau region could then be constructed in accordance with results obtained in other protein-solid systems (Koutsoukos et al 1983, MacRitchie 1972). However, the possibility of coincidental precipitation of protein could not be ruled out. In fact, precipitation might explain the scattering in results at high protein concentration. Although there was scattering, the same type of isotherm could always be repeated. It should be pointed out that the lines drawn in Figure 1 and the following figures are not meant to represent a "true" isotherm, but are there in order to make the figures more easily comprehensible.

SDS-PAGE showed that the composition of WP1 and WP2 differed (Fig. 2). SDS-PAGE performed in the absence of 2-ME showed that WP1 contained several proteins in the molecular weight (MW) range 45,000–30,000. There was also one protein of higher molecular weight (~60,000), and one of lower molecular weight (~14,000). The sample application spots were not stained, and it might thus be concluded that the largest protein in this fraction had a molecular weight of around 60,000. The SDS-PAGE pattern of WP1 was essentially the same when SDS-PAGE was performed in the presence of 2-ME, except that mobility was somewhat higher in the absence of 2-ME. The SDS-PAGE pattern of WP2 showed a greater number of bands, especially in the high molecular weight (HMW) region. In the absence of 2-ME, the sample application spots were heavily stained and there was diffuse streaking of the gel. In the presence of 2-ME the number of bands increased. Furthermore, in the presence of 2-ME the sample application spots were not stained. It might thus be concluded that WP1 contained LMW proteins that do not form intermolecular disulfide bridges to a great extent, whereas WP2 contained HMW proteins that were formed from LMW subunits through intermolecular disulfide bridges.

When the experiments described above were repeated with an equivalent protein fraction (WP2) from the same flour, the appearance of the isotherm was different (Fig. 3). A plateau was not observed, instead the amount of protein adsorbed continued to increase. A third protein preparation gave the same appearance, i.e., the amount of protein adsorbed continued to increase with increasing protein concentration. When the experiments were

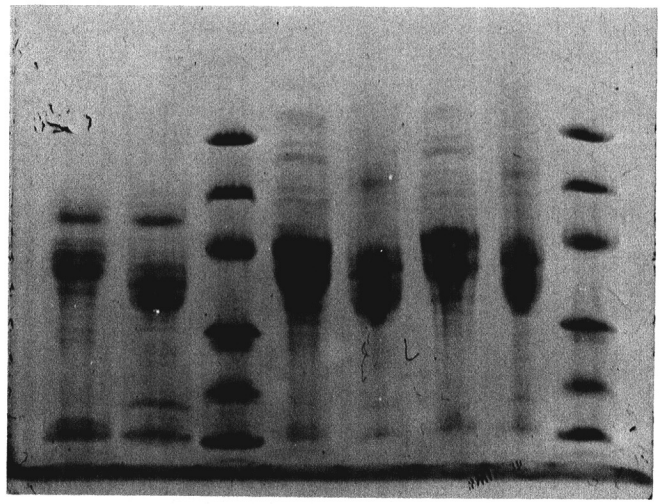

Fig. 2. Sodium dodecyl sulfate-electrophoresis of wheat protein fractions. From left to right: wheat protein fraction 1 (WP1) with 2-mercaptoethanol (2-ME), WP1 without 2-ME, calibration kit, WP2 (first batch) with 2-ME, WP2 (first batch) without 2-ME, WP2 (2nd batch) with 2-ME, WP2 (2nd batch) without 2-ME, calibration kit. The molecular weights indicated are, from the top down: 94,000; 67,000; 43,000; 30,000; 20,000; and 14,400.

repeated, the same type of isotherm was always obtained for the same protein fraction. The average value for the amount of protein adsorbed at protein concentrations above 1.5 mg/ml was not significantly different between duplicates of the same type of isotherm. The SDS-PAGE patterns were identical for the different WP2 fractions (Fig. 2). As the variation evidently was due to the protein fraction and not to the experimental conditions during adsorption, both types of protein fractions were used in other experiments.

Different Starches

In Figure 4 is shown the adsorption of WP2 on wheat, maize, and potato starch, respectively. It is evident that the adsorption depends not only on the type of protein, but also on the starch. Maize and wheat starches adsorbed wheat proteins to about the same extent, whereas potato starch adsorbed wheat proteins to a much greater extent. However, the amount of protein adsorbed is expressed as milligrams of protein per gram of starch in Figure 4 and the adsorption is a surface phenomenon. Differences in specific area thus affect the results. The plateau values from Figure 4 were recalculated and expressed as milligrams of protein per square meter of starch in Table I. In this case, the amount of protein adsorbed on potato starch was about 10 times the amount adsorbed on the cereal starches.

Time

The adsorption of wheat proteins on wheat starch granules as a function of time is shown in Figure 5. The experiments were carried out at two protein concentrations, 0.7 and 1.4 mg/ml, respectively. It is evident from Figure 5 that the amount of protein adsorbed continued to increase during considerable time. In other systems, e.g., BSA on silica (MacRitchie 1972), 30 min to 1 hr was sufficient time for a constant value to be reached. To avoid keeping starch-protein solutions at room temperature for long periods of time, 30 min was chosen as a fixed adsorption time in all other experiments.

pH

The adsorption of wheat protein (WP2) on wheat starch as

TABLE I
The Amount of Wheat Protein Adsorbed on Wheat, Maize, and Potato Starch Granules

Starch	BET[a]-Area (m^2/g)	Amount of Protein Adsorbed	
		mg/g Starch[b]	mg/m^2 Starch
Maize	0.52	4.2*[c]	8.1
Wheat	0.47	4.6**	9.8
Potato	0.26	25.0**	96.2

[a] Determined according to the Brunauer-Emmett-Teller equation.
[b] Estimated from Fig. 4.
[c] Significant difference at * = 5% level and ** = 0.1% level.

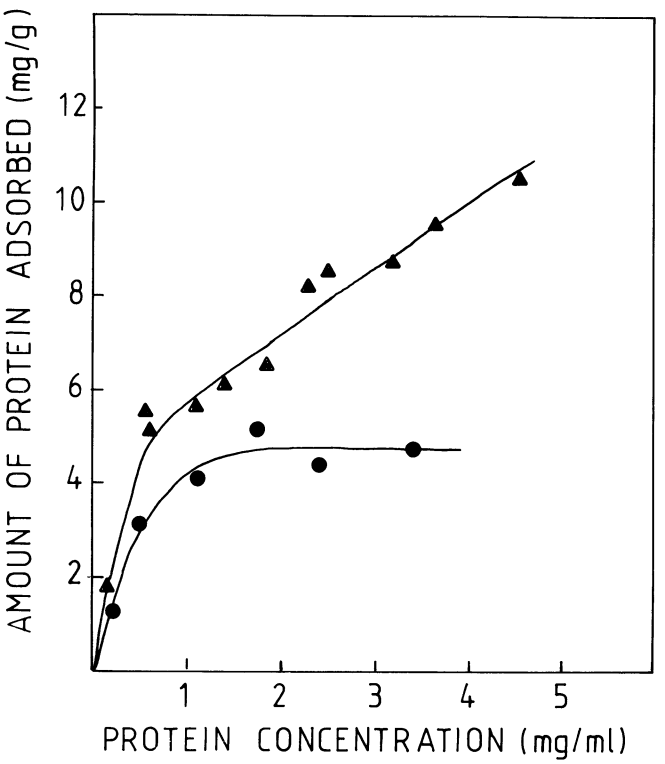

Fig. 3. Adsorption of wheat protein fraction 2 (in $1 \times 10^{-5} M$ HCl) on wheat starch. The curves represent two different batches of the same protein fraction.

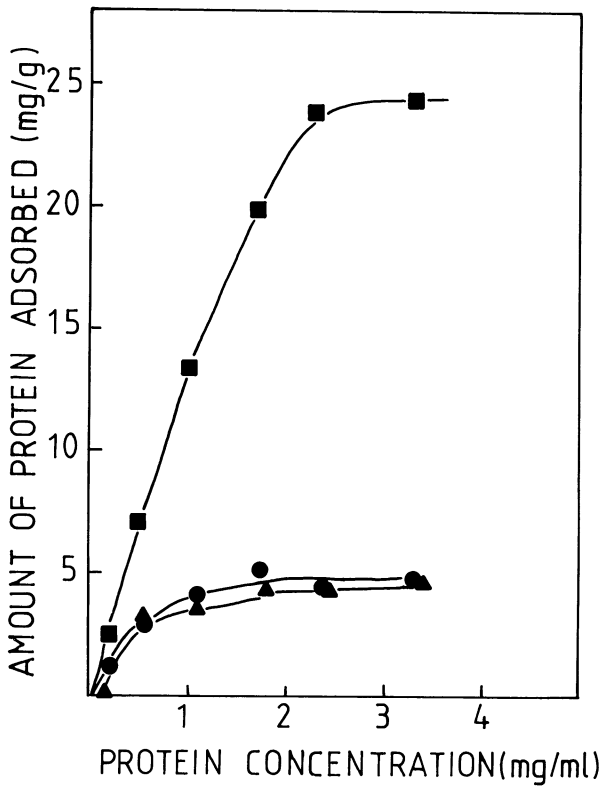

Fig. 4. Adsorption of wheat protein fraction 2 ($1 \times 10^{-5} M$ HCl) on different starches. Wheat starch (●), maize starch (▲), and potato starch (■).

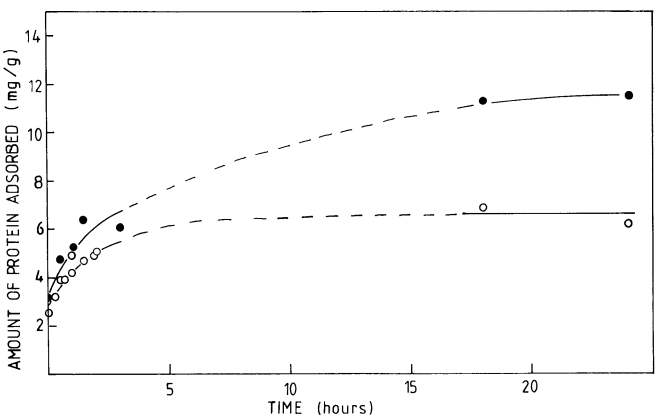

Fig. 5. The adsorption of wheat protein fraction 2 (water) on wheat starch granules as a function of time: 0.7 mg protein/ml (○), 1.4 mg protein/ml (●).

a function of pH is given in Figure 6. The protein solutions of different concentrations were prepared by dissolving protein in HCl or NaOH and diluting this solution. The pH measurements were made on these diluted solutions, and due to the buffering capacity of the protein each adsorption isotherm will, thus, represent a pH range. An average value of the adsorbed amount of protein was calculated for each isotherm. These values were significantly different at the 5% level in distilled water and $1 \times 10^{-5}M$ HCl, at the 1% level in $1 \times 10^{-4}M$ HCl and $1 \times 10^{-3}M$ HCl, and in $1 \times 10^{-4}M$ HCl and $1 \times 10^{-5}M$ HCl. The value in $1 \times 10^{-5}M$ HCl was not significantly different from the values obtained in $1 \times 10^{-4}M$ NaOH or in $1 \times 10^{-6}M$ NaOH.

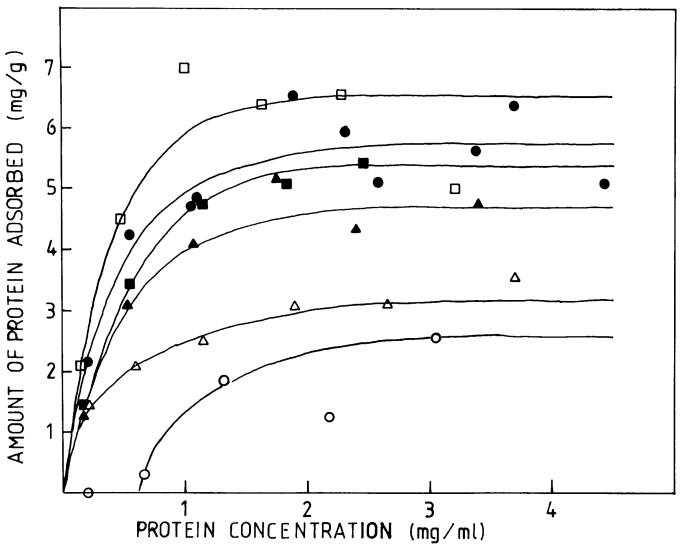

Fig. 6. The adsorption of wheat protein fraction 2 on wheat starch granules as a function of pH: (○) pH 3.1–4.3 ($1 \times 10^{-3}M$ HCl), (△) pH 4.3–5.5 ($1 \times 10^{-4}M$ HCl), (▲) pH 5.5–5.6 ($1 \times 10^{-5}M$ HCl), (■) pH 5.5–5.7 ($1 \times 10^{-6}M$ NaOH), (●) pH 5.6 (water), (□) pH 5.7–7.6 ($1 \times 10^{-4}M$ NaOH).

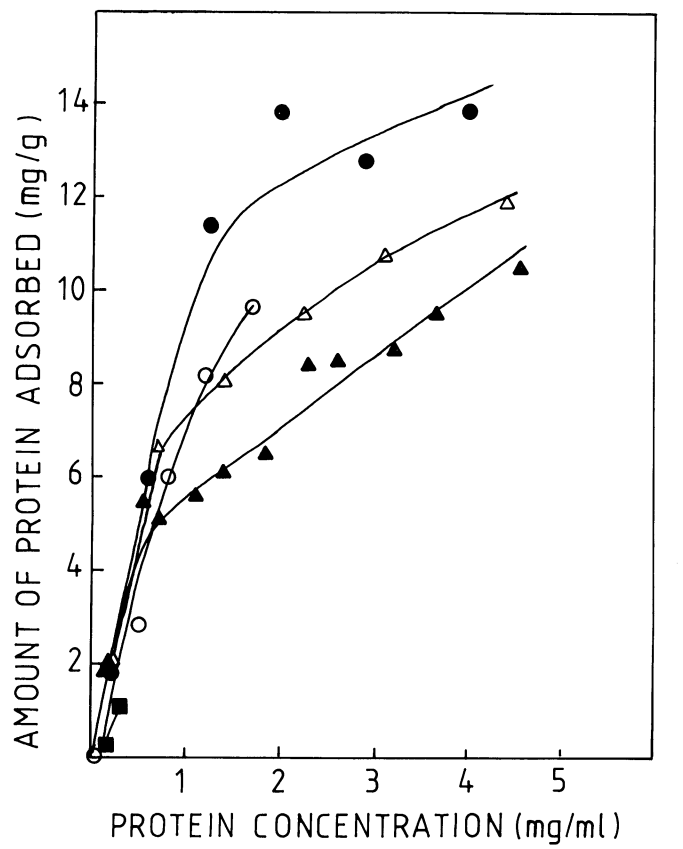

Fig. 7. The adsorption of wheat protein fraction 2 on wheat starch granules in NaCl solutions of varying concentration: (▲) 1×10^{-5} HCl, (△) $+0.001M$ NaCl, (●) $+0.0025M$ NaCl, (○) $+0.005M$ NaCl, (■) $+0.015M$ NaCl.

The results in Figure 6 indicate that the adsorption is dependent on pH, and evidently the adsorption is low at low pH values. It is known that the amount of protein adsorbed is highest around the isoelectric point of the protein (MacRitchie 1978), and the results presented in Figure 6 thus indicate that the isoelectric point of this wheat protein fraction was close to or above neutral. Higher pH values were not investigated because they are not reasonable in baking.

Ionic Strength

For these experiments, the second wheat protein preparation described in Figure 3 was used. The increased salt concentration affected the protein adsorption in two ways. First, the protein solubility decreased considerably when the salt concentration increased (Table II). It was thus not possible to obtain an adsorption isotherm over a broad protein concentration range. Secondly, the isotherm was affected directly (Fig. 7). The adsorbed amount increased with added NaCl, up to $0.0025M$. Further increase in NaCl concentration gave a decrease in the amount of protein adsorbed. The sensitivity of protein adsorption to salt concen-

TABLE II
Solubility of a Wheat Protein Fraction in Salt Solutions of Varied NaCl Concentrations

Solvent	Solubility[a] (mg protein/ml solution)
Water	7.0
HCl, 1×10^{-5} M	6.0
NaCl	
$0.001M$	6.2
$0.0025M$	5.6
$0.005M$	2.4
$0.01M$	0.6
$0.015M$	0.3
$0.035M$	0.2

[a] Determined by dissolving 10 mg of protein in 10 ml of solvent. The protein concentration in supernatant after centrifugation was determined with the biuret method.

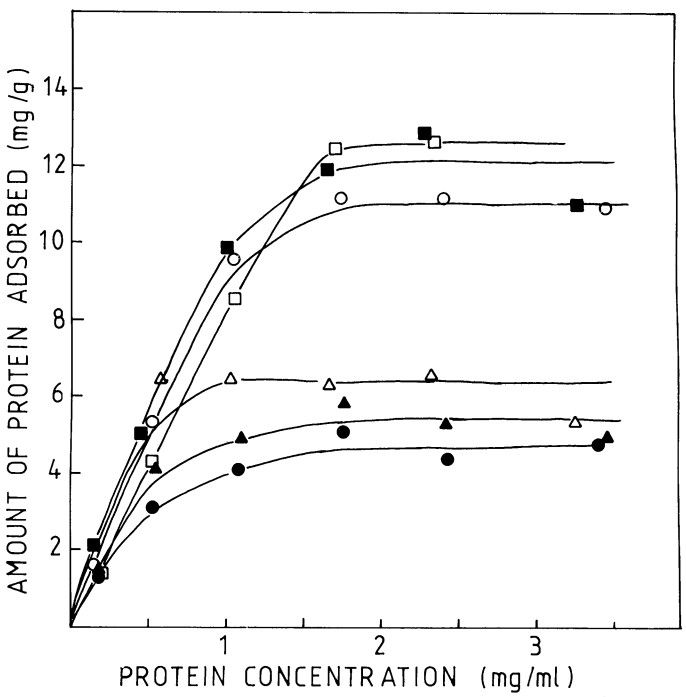

Fig. 8. The adsorption of wheat protein fraction 2 (in $1 \times 10^{-5}M$ HCl) on wheat starch granules that have been heated to different temperatures before adsorption. Control without heating (●), 40°C (▲), 50°C (△), 60°C (■), 70°C (○), 80°C (□).

tration might explain the discrepancies in adsorption isotherms between different batches of WP2 (Fig. 3).

Temperature

To study the adsorption of wheat proteins on wheat starch in relation to baking, it is of course necessary to consider the effects of heat. However, it is not possible just to heat the protein-starch mixture and then measure the protein concentration in the supernatant. A decrease in protein concentration might certainly be due to adsorption of protein on starch granules but might also be due to entrapment of protein in the starch gel formed as a result of gelatinization, or to heat precipitation of protein. The effect of temperature was studied indirectly; starch granules, or protein solutions, were heated separately before the adsorption isotherm was measured.

The adsorption of wheat protein on wheat starch granules heated to different temperatures is shown in Figure 8. The highest temperature investigated was 80° C, because starch granules heated to 90° C before freeze-drying gave a gel when hydrated. It is evident from Figure 8 that the amount of protein adsorbed increased when the starch granules had been heated before adsorption. The increase in adsorbed amount was highest between 50 and 60° C. It is not possible from the present measurements to prove if this increased adsorption resulted from changes in the nature of the starch granule surface as a result of heating or if the increase in adsorbed amount resulted simply because swelling of starch granules during gelatinization made a larger surface available for adsorption.

When heated protein solutions were used for adsorption experiments, the isotherms shown in Figure 9 were obtained. There were no significant differences between the isotherms obtained for unheated protein solutions and the solutions heated to 60 and 90° C.

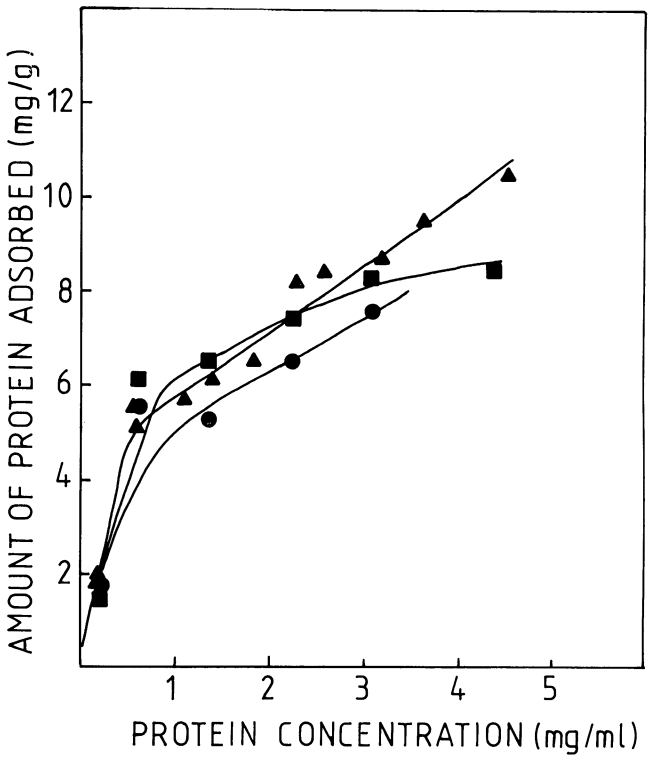

Fig. 9. The adsorption of heated wheat protein solutions (wheat protein fraction 2 in $1 \times 10^{-5} M$ HCl) on wheat starch granules. Control without heating (▲); 60° C (●); 90° C (■).

DISCUSSION

The results presented in Figures 1A, 4, 6, and 8 support the hypothesis of protein-starch interaction as a protein adsorption on a solid surface. This interpretation seems very plausible at low protein concentrations, whereas precipitation of protein cannot be ruled out at higher concentrations. A microscopic examination of some of the centrifuged starch-protein mixtures, after staining with protein-binding dyes (Coomassie Brilliant Blue, Seguchi 1986), did not reveal any blue particles. Dark lumps were occasionally observed that, when the focus was changed, turned out to be composed of starch granules. When starch granules were mixed with protein that precipitated during centrifugation, intense blue structures were observed in the light microscope after staining. The mixtures were, thus, homogeneous at the microscopic level.

When the results obtained for the other protein fractions also are taken into account (Figs 1, 3, 7, and 9), the picture becomes more complex. The discrepancies between the WP2 fractions could result from differences in ionic strength. The results in Table II and Figure 7 show that the solubility as well as the appearance of the isotherm depends on the salt concentration. The difference in solubility of the WP2 samples due to ionic strength could mean that the protein composition of the solution used in the adsorption experiments (i.e., after centrifugation) differed. The difference between WP1 and WP2 (Fig. 1A) might be explained by the differences in molecular weight, as there are indications that the plateau concentration for different proteins increases with increase of molecular weight (MacRitchie 1978).

In conclusion, this type of adsorption experiment can be used in the study of protein-starch interactions. The results in Figure 3 even suggest that the method is very sensitive.

When the amount of wheat protein adsorbed on starch granules is compared with results obtained in other systems, the values for the wheat protein are unusually high. MacRitchie (1978) reported plateau values in the range 0.4–11.8 mg/m^2 for several proteins on glass, silica, or polystyrene. The high values reported here for wheat proteins might be related to the MW, as discussed above, but also to the disulfide bridges present. SDS-PAGE revealed that WP1 contained mostly intramolecular disulfide bonds, whereas WP2 contained also intermolecular disulfide bonds. At the high concentration and after reorientation at the liquid-solid interface, sulfhydryl-disulfide exchange might occur. Cross-linking of wheat proteins has been shown to occur at the air-water interface (Lundh et al 1988). In that study, an HMW protein fraction was compressed at the air-water interface to about 0.05 m^2/mg without any indications of collapse of the film. For the wheat protein adsorbed on wheat starch, the area per milligram of protein was calculated to be 0.10 m^2 from Table I. For potato starch, the corresponding value was 0.01 m^2/mg protein.

The area available per protein molecule in a monolayer present at the starch granules was calculated for wheat and potato starches. As a model for the wheat protein, an HMW glutenin subunit described by Field et al (1987) was used. The molecular weight was 84,000; the length of the molecule was taken as 500 Å and the diameter as 17.5 Å. If such a molecule is adsorbed on the starch granule surface, two extremes exist: the molecule is adsorbed either with its long side or its end to the starch granule surface. The cross-section area would in the first case be 8,750 Å^2 and 240 Å^2 in the second case. The area available per molecule was calculated from the data in Table I to be 1,400 Å^2 for wheat starch, and 145 Å^2 for potato starch. In wheat starch it might thus be possible to pack protein molecules in such a way that a monolayer of protein was formed on the starch granule surface. However, this is not possible for potato starch, and the high amount of protein adsorbed might then be due to the formation of multilayers. A strong adsorption of wheat protein to potato starch during the present conditions might be expected due to electrostatic interactions. Potato starch contains negatively charged phosphate groups, whereas the proteins probably carry a positive net charge at the actual pH. Why such an interaction should result in the formation of multilayers is not clear. On the other hand, incompatibility between potato starch and gluten, observed after heating of a potato starch-wheat gluten suspension (Lindahl and Eliasson 1986), might result in precipitation of gluten proteins.

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