Adsorption of Protein Fractions from Wheat onto Methylated Silica Surfaces

L. WANNERBERGER,[1] M. WAHLGREN,[1] and A.-C. ELIASSON[1]

ABSTRACT

The kinetics of the sequential adsorption of gliadins and water-soluble fraction (WSF) from the wheat variety Kosack onto hydrophobic silica (methylated silica) surfaces were studied by in situ ellipsometry. The measurements were made in water and in 0.01*M* acetate buffer, pH 4.0 and 5.0, with and without 0.1*M* NaCl. The concentration of gliadins and WSF was 1.7 μg/ml. The adsorption of gliadins in water was investigated at three different protein concentrations (0.17, 1.7, and 17 μg/ml), and the adsorption was found to be very high with regard to the low concentrations of protein in solution. The results of the sequential adsorption in water indicated that the WSF adsorbed to preadsorbed gliadins. When proteins of the WSF were adsorbed first, they blocked further adsorption of gliadins. Measurements in acetate buffer at different pH values showed that the effect of pH was larger in the experiments where the gliadins were adsorbed first, both for the initial adsorption and for the sequential addition of WSF. An increase in ionic strength also increased the amount of WSF adsorbed to preadsorbed gliadins. These results indicate that the sequential adsorption of WSF to gliadins is strongly affected by electrostatic interactions.

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A simplified model of a wheat flour dough consists of starch granules and air cells dispersed in a continuous gluten matrix. This leads to numerous possibilities for the proteins to adsorb at different interfaces. Adsorption of proteins to the air-water interface is an extensively studied phenomenon due to its important role in foam stabilization (de Feijter and Benjamins 1987, Dickinson et al 1988). The adsorption of protein reduces the surface tension; the reduction is dependent on the adsorbed amount, the hydrophobicity of the protein, and the capability of the protein to change conformation at the interface (Graham and Phillips 1979a–c). The behavior of cereal proteins at air-water interfaces has mainly been studied with the film balance (Lundh et al 1988, MacRitchie 1990, Wannerberger 1993). Interactions between proteins and the solid starch granules have been suggested to be important in baking (Lelievre et al 1987). The protein molecule is considerably smaller than the starch granule, and the situation can be described as protein adsorbing to a solid surface. The specific area of the total starch is around 0.4 m^2/g (Soulaka and Morrison 1985), which means that a substantial part of the gluten proteins in a wheat flour are required just to surround the starch granules. Predictions of how the protein interacts with the starch granule surface are complicated. One reason for this is the heterogeneity of the surfaces of both proteins and starch granules. Proteins in general display a very complex surface with differences in hydrophobicity and charge (Andrade 1985), while the surface of the starch granule is thought of as hydrophilic. However, it is possible to make starch hydrophobic by, for example, heat treatment (Seguchi 1984, Seguchi and Yamada 1988). Therefore, proteins are likely to be found at the important interfaces in a dough. The type of proteins adsorbed and their way of adsorbing possibly influence the quality of the dough, such as gas retention. Consequently, it is of interest to understand the interfacial behavior of cereal proteins.

Wheat proteins, like most protein systems, are mixtures of numerous proteins, varying, for example, in molecular weight, and they probably influence each other's adsorption behavior. While the general features of adsorption from single-protein solutions are understood, much less is known about adsorption from complex protein mixtures. When proteins are sequentially added, or proteins of a mixture reach a surface at different times (e.g., due to diffusion differences), there are three possibilities as to how this can affect the adsorption. In the first case, the protein first adsorbed is exchanged by the second one. This is exemplified by blood proteins (one of the most thoroughly studied complex protein systems), in which proteins with low molecular weights in high concentration are adsorbed initially. They are gradually replaced by proteins present in lower concentration but with higher surface affinity and higher molecular weight (Vroman and Adams 1986).

In the second case, adsorption of the first protein blocks further adsorption. One example is the sequential and competitive adsorption of β-lactoglobulin and κ-casein on metal surfaces, for which it was shown that κ-casein was preferentially adsorbed (Arnebrant and Nylander 1986). The conclusions were that the adsorption of κ-casein on the surface resulted in a hydrated surface structure, which made the surface less responsive to further adsorption.

The third case is that the second protein adsorbs onto the first one. This has been observed for adsorption of lactoferrin to preadsorbed β-lactoglobulin (Wahlgren et al 1993).

One established technique to study protein adsorption to solid interfaces is depletion (Norde 1976, Eirich 1977, Soderquist and Walton 1980), in which the residual amount of protein in solution is determined after adsorption. This was recently used by Eliasson and Tjerneld (1990) 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. They found that the adsorption was low for a wheat protein fraction composed of low molecular weight proteins and that for a fraction of high molecular weight proteins the amount adsorbed was much higher. The amount of protein adsorbed increased with pH, and the adsorbed amount increased with added NaCl, up to 0.0025*M*. Further increase in NaCl concentration gave a decrease in the amount of protein adsorbed.

In the present study, ellipsometry was used to measure the adsorption of the water-soluble fraction (WSF) and gliadins onto methylated silica surfaces. The adsorption was investigated in water and acetate buffer and at different pH and ionic strength levels. We wanted to investigate the competitive adsorption of different wheat protein fractions, and for simplicity we chose those fractions that could be easily dissolved. The water-soluble proteins have a very heterogeneous composition. However, they have some properties that differentiate them from the storage proteins. The WSF proteins have a higher mobility than gliadins in electrophoresis at pH 3, and they also have the highest mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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(SDS-PAGE) (Wrigley and Bietz 1988). Most of the soluble proteins seem to have a molecular weight below 40,000 (Lásztity 1984), and their isoelectric point is in the range 5–8. Note that this fraction may contain soluble surface-active compounds other than proteins. The ethanol-soluble gliadin fraction contains single polypeptide chains that associate by hydrogen bonding and hydrophobic interactions (Shewry et al 1986). These were initially classified into the traditional four groups α-, β-, γ-, and ω-gliadins according to their mobilities at low pH in free boundary (Jones et al 1959) and starch gel electrophoresis (Woychick et al 1961). The molecular weight of gliadins varies from around 30,000 up to 200,000 (Bietz 1979). The gliadins are known to be very hydrophobic and to contain a low number of charged amino acids (Wrigley and Bietz 1988).

MATERIALS AND METHODS

Materials

The protein used in this study was purified from the Swedish winter wheat variety Kosack, supplied from Skånska Lantmännen (Malmö, Sweden). The protein content in the fractions was analyzed according to the Kjeldahl method (N × 5.7) by a Kjeltec Auto 1030 Analyser (Tecator, Hogänäs, Sweden). The protein content in the WSF was 95.3% on a dry basis and in the gliadin fraction, 97.8%. Water content was determined from weight loss after 16 hr at 105°C. The buffer used was an 0.01*M* acetate buffer, pH 4.0 and pH 5.0, with and without 0.1*M* NaCl. The water was distilled, passed through an ion exchanger and activated charcoal (Millipore Corporation, Bedford, MA), and finally double-distilled in a glass still. All glassware was cleaned in a 1:1 (v/v) mixture of concentrated sulfuric and nitric acids and then thoroughly rinsed in water. All chemicals were analytical grade.

Preparation of Protein Fractions

The fractions studied were obtained from freeze-dried defatted gluten. The gluten was prepared by extracting the flour lipids with chloroform, washing the gluten with water, and drying it.

The WSF was extracted with distilled water, and gliadins were prepared from defatted gluten by extraction with ethanol. Defatted gluten (4 g) was homogenized in water (60 ml) for 2 min using a homogenizer LX-1020 (Intern. Laborat. App. GmbH, Dottingen). The vessel was cooled with ice water during homogenization. The gluten sample was stirred for 30 min followed by centrifugation in a centrifuge (Sorvall, DuPont, Wilmington, DE) with a GSA rotor at 4,000 × *g* for 30 min. The supernatant, containing the WSF, was decanted and collected. The sediment was cut into pieces, and 60 ml of distilled water was added, followed by a second homogenization for 90 sec. The sample was stirred for 15 min before centrifugation at 4,000 × *g* for 30 min. Supernatants were collected, mixed with the previous supernatant, and then freeze-dried.

The sediment obtained above was cut into pieces, and 60 ml of 70% (v/v) ethanol was added, followed by homogenization for 2 min. The sample was stirred for 15 min before centrifugation at 4,000 × *g* for 30 min. Supernatants containing the gliadin fraction were decanted and collected. Homogenization in ethanol, followed by stirring and centrifugation, was repeated twice more. The supernatants were collected and stored. The ethanol was evaporated at room temperature, and the gliadins were freeze-dried.

The WSF and gliadins were dissolved in 70% (v/v) ethanol containing 0.3 m*M* HCl to a concentration of 0.5 mg/ml. To get the protein into solution, the gliadin solution was sonicated for 20 sec before use.

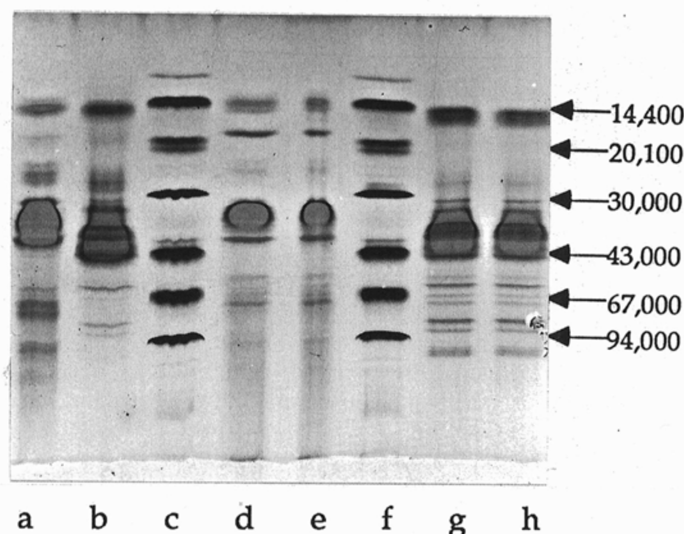

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis on the water-soluble fraction (WSF), gliadins, and sonicated gliadins. On each sample, two runs were made, one reduced and one unreduced. a, WSF, unreduced; b, WSF, reduced; c, reference; d, gliadins, unreduced; e, gliadins, unreduced, sonicated; f, reference; g, gliadins, reduced; h, gliadins, reduced, sonicated.

Electrophoresis

SDS-PAGE was performed on WSF, gliadin proteins, and sonicated gliadin proteins in PhastSystem electrophoresis equipment (Pharmacia, Uppsala, Sweden). The method was essentially as described elsewhere (Marchylo et al 1989) except for the staining procedure (see below). Gels with a concentration range of 8–25% (Pharmacia PhastGel gradient 8-25) and PhastGel SDS buffer strips (Pharmacia) were used. For each sample, two runs were made: one with 2-mercaptoethanol (5.0%, w/v) added and one without.

The following standard proteins were used as molecular weight markers: phosphorylase b (mol wt 94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soya bean trypsin inhibitor (20,100), and α-lactalbumin (14,400) (low molecular weight calibration kit, Pharmacia Sweden). The molecular weights of the WSF and gliadins were calculated from these calibration proteins.

The separation was made in two steps, first 400 V and 5.0 mA at 20°C and then 50 V and 0.1 mA at 15°C. The gels were stained under continuous stirring at room temperature with 0.1% (w/v) Coomassie Brilliant Blue R250 in 30% (v/v) ethanol and 10% (v/v) trichloroacetic acid for 2 hr. They were then destained in the PhastSystem development unit (Pharmacia) with 30% (v/v) methanol and 10% (v/v) acetic acid for 5 min at 50°C. After washing, the destaining was repeated for 8 min. The gels were preserved in 5% (v/v) glycerol and 10% (v/v) acetic acid for 35 min at room temperature.

Preparation of Surfaces

Silica surfaces (Okmetic OY, Espoo, Finland) with an oxide layer, 300–350 Å thick, were made hydrophobic by reaction with dichlorodimethylsilane (DDS) (Jönsson et al 1982, Wahlgren and Arnebrant 1990).

The silica surfaces were cleaned by washing witha mixture of NH_4OH and H_2O_2 and H_2O (1:1:5) (v/v/v) at 80°C for 5 min, and after that they were rinsed with double-distilled water. The surfaces were then immersed in a mixture of HCl and H_2O_2 and H_2O (1:1:5) (v/v/v) at 80°C for 5 min and rinsed in double-distilled water followed by ethanol.

Before treatment with DDS, the surfaces were rinsed twice with ethanol and then three times with trichloroethylene. The surfaces were immersed in a 0.1% solution of DDS in trichloroethylene for 1 hr. The methylated surfaces were then rinsed three times with trichloroethylene followed by ethanol, and then stored in ethanol.

Adsorption Measurements

The adsorption was measured using a null-ellipsometer (thin film ellipsometer, type 43603-200E, Rudolph, Fairfield, NJ), modified according to Arnebrant and Nylander (Arnebrant 1987, Nylander 1987), which records the change in ellipticity of polarized light upon reflection at an interface. Ellipsometry is an optical method that gives the ellipsometric angles Δ and ψ, from which it is possible to calculate the thickness and the refractive index of a film adsorbed on the surface. The model used to calculate refractive index and thickness is a three-component model containing the surface (silica, silicon oxide, and methylated layer), a protein film, and solution (Cuypers 1976, Azzam and Bashara 1977). The adsorbed amount was calculated from the refractive index and thickness of the adsorbed film according to Cuypers et al (1983). The values for partial specific volume (v) and ratio of the molar weight to molar refractivity (M/A) were 0.75 g/ml and 4.1 g/ml, respectively.

The pseudorefractive index for the bare surface was determined before the addition of protein. In the sequential studies, 20 μl of a 0.5 mg/ml solution of protein was added to the cuvette, giving a final concentration of 1.7 μg/ml. The adsorption was measured for 60 min, and then the cuvette was rinsed for 5 min at a flow rate of 20 ml/min, followed by measurement of the desorption for an additional 10 min. In the sequential measurements, this procedure was repeated once more for adsorption of the second protein. Experiments were performed in water or acetate buffer at pH 4 and 5 at 23°C. The stirring rate was 325 rpm. All experiments were made in duplicate.

RESULTS AND DISCUSSION

Electrophoresis of the unreduced and reduced protein fractions is shown in Figure 1. The results of the electrophoresis showed that there is almost no difference between the sonicated gliadins and the ones not sonicated. The problem of getting the gliadin fraction into solution is probably due to aggregation of the proteins. By using sonication, it is possible to break these aggregates, but there is also a risk that intermolecular bonds will be cleaved (Singh and MacRitchie 1989, Singh et al 1990). The electrophoresis shows that the main purpose of the sonication was achieved without cleavage of covalent bonds. However, aggregates kept together with hydrophobic and other noncovalent bonds can be broken.

The bands visible in the low molecular range between 14,400 and 20,100, obviously show that these proteins are difficult to wash out with water or ethanol. The WSF seems to consist of more high molecular weight proteins in the unreduced sample, while the gliadin fraction seems to consist of more high molecular weight proteins in the reduced one. It was also observed that the WSF has some visible bands in the low molecular weight range that are not present in the gliadin. The results of the gel emphasize that both the WSF and the gliadin fraction are complex mixtures of proteins. The differences observed between the bands for the reduced and unreduced gliadin obviously show that the gliadin fraction consists of some polymeric proteins.

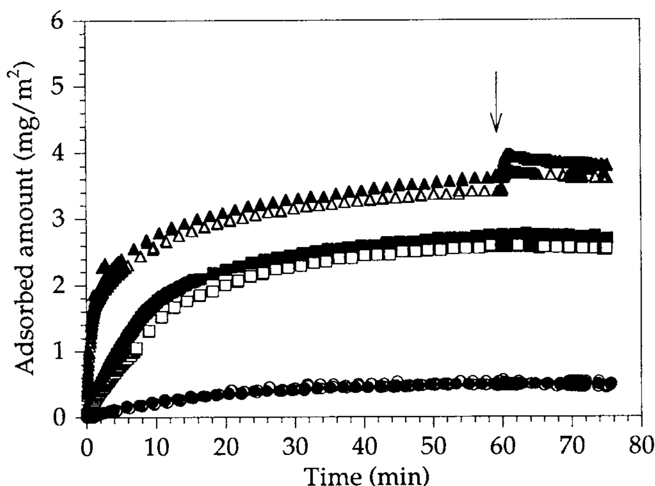

Fig. 2. Amount of different concentrations of gliadins adsorbed onto hydrophobic surfaces versus time. Concentrations of gliadins were 17.2 (▲ and △), 1.72 (□ and ■) and 0.172 μg/ml (● and ○). Measurements were performed as duplicates and carried out at 23°C in water solution. Rinsing was performed after 60 min at a rate of 20 ml/min for 5 min, followed by a 10-min equilibration period. Arrow indicates start of rinse.

Figure 2 shows the adsorption curves for three different concentrations (0.172, 1.72 and 17.2 μg/ml) of gliadins in water. The figure shows two replicates of each experiment. It is evident that the reproduction is good for the whole experimental series. The maximum variation in adsorbed amounts between the replicates is 0.25 mg/m^2, which is less than 8%. The mean plateau values for the adsorbed amounts, together with the time taken to reach half these plateau values are shown in Table I. The amount of adsorbed protein is very high considering the low concentration of protein in solution. This might be an effect of the low solubility of the gliadins in water, reflecting their high hydrophobicity (Pomeranz et al 1970). The adsorbed amount increases with protein concentration, and thus the value for maximum adsorbed amount could not be determined in the study. For total irreversible adsorption, the adsorbed amount should level off, either because the solution is depleted of proteins or because the maximum surface coverage is reached. Considering the small surface area available, depletion is unlikely in this case, and the reasons for difference in adsorbed amount must be found elsewhere. These could be differences in structure and orientation of proteins adsorbed at different concentrations or, due to heterogeneity of the sample, the composition of adsorbed protein might vary. However, the increase in adsorbed amount between 17.2 and 1.72 μg/ml is smaller than that between 1.72 and 0.172 μg/ml, indicating that the adsorption is approaching the plateau value for the isotherm. There is a slight increase in adsorbed amount upon dilution in the case of the highest protein concentration. It has earlier been observed in mixtures of surface-active components that dilution leads to increased adsorbed amount (Wahlgren and Arnebrant 1992, Wahlgren et al 1993). The decrease in total concentration during rinse might influence interaction between compounds in solution, e.g., dimerization, subsequently influencing the adsorption. The dilution might also influence the competition between surface-active compounds at the interface, as the concentration dependence of adsorption might differ strongly among different molecules in a mixture. Although the protein concentration is low, the possibility cannot be totally excluded that it might influence the refractive index of the solution, which might influence the ellipsometric calculations.

The kinetics during the initial part of the adsorption were much faster for the highest concentration than for the lower concentrations (Table I). The adsorption rates can be controlled either by the adsorption step or by the transport of molecules to the surface. If the initial adsorption is compared to diffusion-controlled adsorp-

TABLE I
Adsorption Mean Values for Gliadins at Different Concentrations in Water

	Concentration, μg/ml		
	0.172	**1.72**	**17.2**
Adsorbed amount, mg/m^2			
Before rinse (60 min)	0.49	2.69	3.59
After rinse (75 min)	0.47	2.63	3.74
Time to reach half plateau value, sec	803	538	85

tion, it appears that the two lowest concentrations could be diffusion-controlled. This is only an indication of diffusion-controlled adsorption, as the gliadin fraction contains a large number of molecules for which the concentration or the diffusion coefficient is unknown. The calculations of the diffusion-controlled adsorption were based on diffusion through a stagnant layer. The thickness of the stagnant layer has been estimated as 20 μm by Cuypers (1976) for a similar experimental setup. The diffusion constant employed was 5×10^{-7} cm²/sec, which is in the range of globular proteins with a molecular weight corresponding to that of gliadins. The results also show that the adsorption is irreversible with respect to dilution with water at all three concentrations (Fig. 2). Gliadins are rather large, compared to many soluble proteins, and studies have shown that large proteins are hard to remove (Andrade and Hlady 1986, Norde 1986, Wahlgren and Arnebrant 1990).

The results of sequential adsorption of gliadins and WSF in water to the hydrophobic surfaces are shown in Figure 3. The mean values for the adsorbed amount of gliadins and WSF from 1.72 μg/ml solutions before and after rinsing are shown in Table II. The WSF was adsorbed in considerably higher amount than the gliadins. It should be noted that the WSF might contain other compounds than proteins, such as water-soluble pentosans. If this is the case, these are surface-active and might be a part of the adsorbate. The adsorption of WSF had not leveled off after 1 hr of adsorption, in contrast to that observed for the gliadins. Rinsing after 60 min led to partial desorption of the adsorbed WSF, and

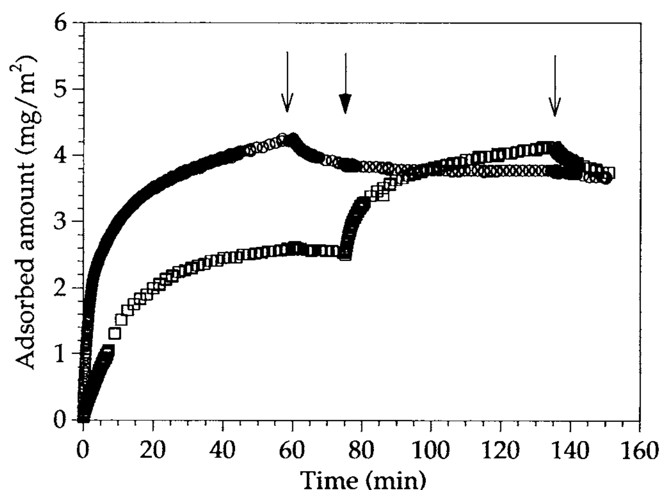

Fig. 3. Adsorbed amount versus time for sequential adsorption of gliadin (1.72 μg/ml) and water-soluble fraction (WSF) (1.72 μg/ml) onto a hydrophobic surface. Adsorption of gliadins followed by adsorption of WSF is indicated by □, and the adsorption in reversed order is indicated by ○. Measurements were carried out at 23°C in water solution. Rinsing was performed after 60 min at a rate of 20 ml/min for 5 min, and after another 60 min, rinsing was performed again. After each rinse, there was a 10-min equilibration period. Open arrows indicate start of rinse, and closed arrow indicates sequential addition.

the sequential adsorption of gliadins did not result in higher adsorbed amounts. This indicates that the preadsorbed WSF proteins block the adsorption of gliadins. Even if exchange reactions cannot be totally excluded, they are still unlikely as the adsorbed amount is unaffected by the addition of gliadins. Rinsing after 135 min hardly affected the adsorbed amount.

The adsorption of gliadins to the clean silica surface reached a plateau value after 60 min, and no desorption was recorded on rinsing with water, in accordance with the results in Fig. 2. When WSFs were added to the gliadins, the adsorbed amount was increased, and rinsing after 135 min resulted in a small desorption. The gliadins probably remain at the surface, and the WSF adsorbs to the preadsorbed gliadin layer. This cannot be verified by these measurements, as only the total amount adsorbed is obtained. However, the strong binding of the gliadins to the surface, indicated by the fact that they are hard to remove by rinsing, and their high adsorption at low concentrations, make it unlikely that they are removed. Thus the WSF most likely adsorbs on top of the gliadin-covered surface. On the contrary, the WSF adsorbs faster and in higher amounts, but as this takes place in the range of diffusion-controlled adsorption, the presumed lower molecular weight of the WSF could explain the higher adsorption rate. A higher adsorbed amount does not necessarily mean a stronger interaction but rather reflects the dimensions and packing of the molecules. Since the gliadin proteins are very hydrophobic, the adsorbed layer provides a hydrophobic surface for further adsorption. The pH values for the gliadins and the WSF water solutions varied between 5.6 and 6.0, which means that it was close to the pH value measured for the water used and the isoelectric point reported for gliadins (Lindahl 1990). To avoid pH variations in the following experiments, they were carried out in acetate buffer at fixed pH.

To investigate the effect of electrostatic interactions, experiments were made at different pH values and ionic strengths. In Figure 4, the sequential adsorption of gliadins and WSF in 0.01*M* acetate buffer at pH 4.0 and 5.0 respectively, are shown. The curves are representative experiments chosen from at least two replicates. The variation in adsorbed amount between the replicates was at a maximum 0.18 mg/m², which is lower than in water (Figs. 2 and 3). The values of adsorbed amounts are shown in Table II. The adsorbed amounts were higher at pH 5 regardless of whether gliadins or WSF were adsorbed first. At pH 5, the mean values of adsorbed amounts of gliadins and WSF after 60 min were almost the same. After rinsing for 5 min, partial desorption was found for the WSF, whereas the adsorbed amount of gliadins was not affected. The sequential adsorption of the WSF to the preadsorbed gliadins resulted in an increase in total adsorbed amount of 0.88 mg/m². Rinsing did not affect the system. The increase of adsorbed amount when gliadins were adsorbed to preadsorbed WSF were much lower, around 0.37 mg/m², and also in this case rinsing did not substantially affect the adsorbed amount. At pH 4, the mean values of adsorbed amount of gliadins and WSF after 60 min were 2.27 and 2.79 mg/m², respectively, and almost no decrease in adsorbed amount was found upon rins-

TABLE II
Adsorption Values (mg/m²) for Sequential Adsorption of Gliadins and Water-Soluble Fractions in Different Solutions and at Different pH Levels

			0.01*M* Acetate Buffer					
	Water		pH = 4			pH = 5		
Time, min	G[a] + W[b]	W + G	G + W	W + G	G + W (NaCl)	G + W	W + G	G + W (NaCl)
60	2.69	4.28	2.27	2.79	3.57	3.47	3.35	3.42
75	2.63	3.89	2.26	2.71	3.48	3.44	3.24	3.30
135	4.24	3.76	2.47	2.97	5.58	4.32	3.61	5.44
150	3.92	3.64	2.47	2.92	5.27	4.19	3.54	5.11

[a] G = gliadin fraction.
[b] W = water-soluble fraction.

ing. For both WSF and gliadins, the addition of the other protein resulted in a minor increase in adsorbed amount (Table II), which was not affected by the following rinse.

The effect of pH is much larger in the experiments where the gliadins were adsorbed first, both for the initial adsorption and the sequential addition of the WSF. The smallest effect of pH was observed when gliadins were added to the preadsorbed WSF. The higher amounts of protein adsorbed to clean surface at pH 5 compared to pH 4 might be due to the proteins approaching their isoelectric point. It is well known that protein adsorption tends to be at a maximum at the isoelectric point because the protein has zero net charge (Andrade and Hlady 1986).

The increase in adsorbed amount upon addition of WSF to preadsorbed gliadins indicates that the adsorption occurs either to the preadsorbed gliadin layer or directly to bare spots on the silica surface. Exchange reactions are less likely considering the levels of the adsorbed amount. As both proteins give similar amounts adsorbed at pH 5, a complete exchange reaction would not affect the values for the total adsorbed amount. Another interesting observation is that the change in adsorbed amounts when pH is shifted from 4 to 5 is larger for WSF adsorbed to preadsorbed gliadins than for WSF adsorbed to a clean silica surface. This effect can probably be related to a decrease in the charge of the protein upon increasing pH, which decreases the electrostatic repulsion between preadsorbed gliadins and the adsorbing WSF. The initial pH in a normal dough is around 5.5 (Eliasson and Larsson 1993). Fermentation processes do, however, lower the pH, and the lowest pH, around 3.5, might be obtained in sour doughs (Eliasson and Larsson 1993). The results here show that it is likely that adsorption of wheat proteins differs in doughs with different pH levels.

The results presented in Figure 5 show the effect of increased ionic strength at pH 4 and 5 on the sequential adsorption of gliadins and WSF in 0.01*M* acetate buffer. At pH 4, both the initial adsorption of gliadins and the subsequent addition of WSF were affected by the increase in ionic strength. The mean plateau value for the amount of adsorbed gliadins was 2.27 mg/m^2, and, when the ionic strength was increased by addition of 0.1*M* NaCl, the adsorbed amount was drastically increased (Table II). When the ionic strength was low, the sequential adsorption of WSF hardly affected the adsorbed amount, but when the ionic strength was high, the sequential addition of WSF resulted in a large increase. At pH 5, increased ionic strength affected only the sequential addition of WSF to the preadsorbed gliadin layer, not the initial adsorption of gliadin. These results show clearly that an increase in ionic strength, which shields charges in the protein and reduces the electrostatic repulsion between the molecules, controls the adsorption behavior. The lower repulsion between the protein molecules allows the protein to be more closely packed at the surface or facilitates the formation of multilayers. The dough always contains salt, both added salt and salt from the water used. The addition of salt is known to affect the gas retention of the dough (Finney 1984). It is possible that the higher amounts adsorbed, observed for increased salt concentration, lead to a film that is less penetrable to air. The latter could be due to influences on protein adsorption at the air-water interface.

When the two proteins were added simultaneously in 0.01*M*

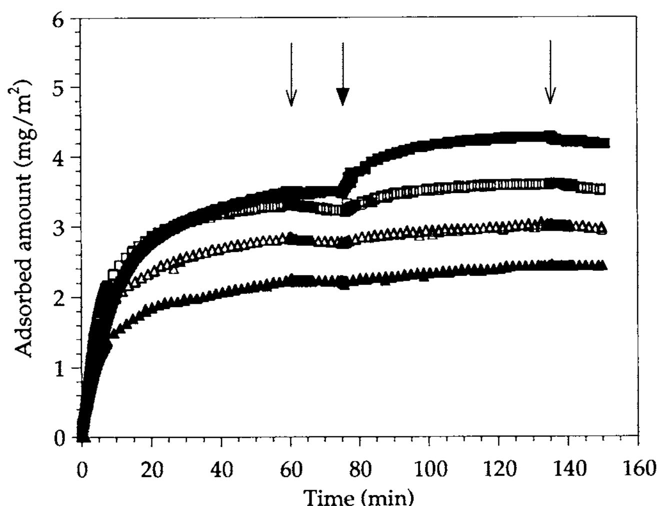

Fig. 4. Adsorbed amount versus time for sequential adsorption of gliadins and water-soluble fraction (WSF) onto hydrophobic surfaces at pH 4 and 5. Measurements were carried out in 0.01*M* acetate buffer at 23°C. Adsorption of gliadins followed by WSF at pH 4 is indicated by ▲ and at pH 5 by ■. Adsorption of WSF followed by gliadin at pH 4 is indicated by Δ and at pH 5 by □. Experimental conditions were the same as in Figure 3. Open arrows indicate start of rinse, and closed arrow indicates sequential addition.

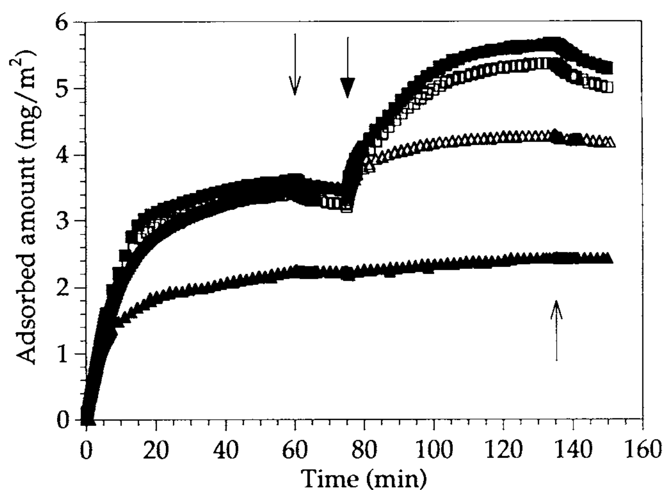

Fig. 5. Adsorbed amount versus time for sequential adsorption of gliadins and water-soluble fraction onto hydrophobic surfaces at pH 4 and 5 and with different ionic strengths. Measurements were carried out in 0.01*M* acetate buffer with (■, pH 4 and □, pH 5) and without (▲, pH 4 and Δ, pH 5) 0.1*M* NaCl. Experimental conditions were the same as in Figure 3. Open arrows indicate start of rinse, and closed arrow indicates sequential addition.

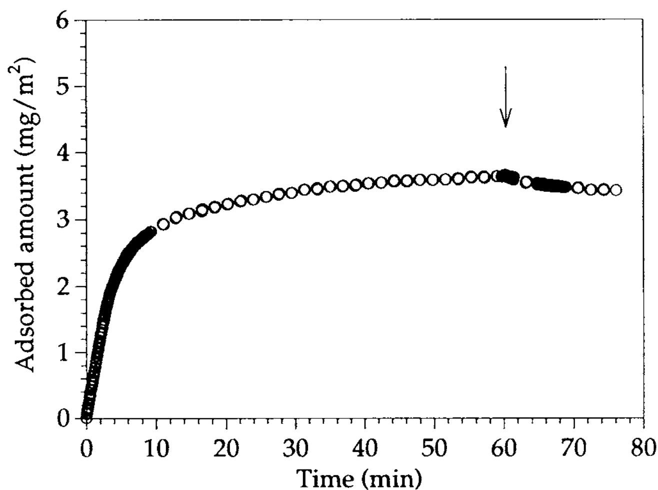

Fig. 6. Simultaneous addition of gliadins and water-soluble fraction. Measurements, performed as duplicates, were carried out in 0.01*M* acetate buffer, pH 5, at 23°C. Rinsing was performed after 60 min at a rate of 20 ml/min for 5 min, followed by a 10-min equilibration period. Arrow indicates start of rinse.

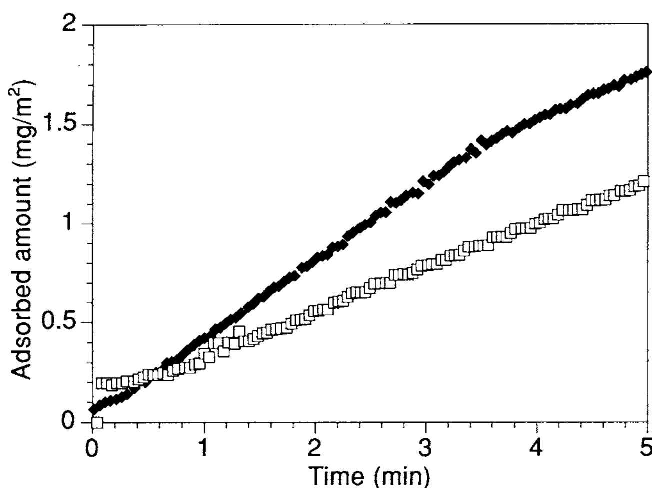

Fig. 7. Initial adsorption kinetics for gliadins (□) and WSF (◆) to methylated silica surfaces. Measurements, performed as duplicates, were carried out in 0.01*M* acetate buffer, pH 5, at 23°C.

acetate buffer, pH 5, the curve in Figure 6 was recorded. The mean plateau value of adsorbed amount after 60 min was 3.61 mg/m^2, and the following rinse decreased the adsorbed amount slightly. The variation in adsorbed amount between the replicates was a maximum of 0.06 mg/m^2, which is less than 2%. The level of adsorbed amount for the mixture of gliadin and WSF was very close to that found when WSF proteins were adsorbed followed by the adsorption of gliadins in 0.01*M* acetate buffer. This indicates that the WSF adsorbs faster to the surface than gliadins, blocking adsorption of the latter. This is also shown in Figure 7, where initial adsorption levels of WSF and gliadins are compared.

CONCLUSIONS

Wheat proteins adsorb readily and in high amounts to hydrophobic interfaces. Preadsorbed WSF seems to block further adsorption of gliadins. Preadsorbed gliadins, on the other hand, can adsorb WSF under certain conditions. The adsorption is influenced by pH and salt concentration. Increased pH increases the adsorbed amount, especially for the addition of WSF to preadsorbed gliadins. The adsorbed amount is also increased by the addition of salt in the case of addition of WSF to preadsorbed gliadin. This is most likely due to reduced electrostatic interactions between adsorbed gliadins and WSF in solution. In mixtures of WSF and gliadins, the WSF is preferentially adsorbed. This is probably due to a higher initial adsorption rate.

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