

Adsorption Chromatography on Controlled-Pore Glass Beads of Acetic-Acid-Soluble Wheat Gluten Proteins

A. D. B. PERUFFO,^{1,2} A. CURIONI,¹ G. PRESSI,¹ N. E. POGNA,³ and A. ZAMORANI¹

ABSTRACT

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Adsorption chromatography on 2,000-Å controlled-pore glass beads, performed by frontal analysis in the absence of any detergent or chaotropic agent, was found to be capable of highly reproducible separation of the acetic-acid-soluble unreduced proteins from wheat gluten. Glutenin polymers with molecular mass $>10^7$ Da and free from both starch and monomeric proteins were recovered. Furthermore, a number of polymers with lower molecular mass differing in subunit composition were obtained.

The polymers retained their ability to make up the gluten. Gluten proteins were adsorbed on the glass beads with a sequential and competitive mechanism involving hydrophobic interactions. Proteins desorbed by increasing urea concentration in the eluent up to 6M were found to be γ -gliadins encoded by the group 1 chromosomes, along with glutenin polymers.

Wheat gluten is generally believed to be a mixture of monomeric proteins and polymers stabilized by interchain disulfide bonds.

Many studies have been made to gain information on both the subunit composition of the polymers and the precise nature of the links involved in making up the gluten mass. Most investigations have been performed after reduction of disulfide bonds, which causes breaking down of the gluten structure and the consequent loss of the possibility to elucidate it. Only the availability of gluten polymers in a pure, unreduced form will allow the study of the relationships between their structural features and their functional properties in dough-making.

Glutenin polymers are difficult to purify because of their insolubility in the buffers normally used for protein separation and analysis. Kobrehel and Bushuk (1977) suggested that the insolubility of glutenin polymers is due to their strong aggregation properties through hydrophobic interactions. This hypothesis is supported by the fact that several dissociating agents like cetyltrimethylammonium bromide (Meredith and Wren 1966), Na-stearate (Kobrehel and Bushuk 1977), and sodium dodecyl sulfate (SDS) (Danno 1981) have been successful in both extraction and fractionation of gluten proteins. However, these agents alter the native protein structure, preventing the possibility of structure-functionality studies.

Low-pressure gel filtration and size-exclusion high-performance liquid chromatography (HPLC) of unreduced gluten extracts, with a variety of dissociating and chaotropic solvents as eluents (Preston 1982, Bietz 1985, Kruger and Marchylo 1985, Dachkevitch and Autran 1989, Singh et al 1990), provide poorly resolved peaks in which proteins belonging to different solubility classes overlap. Consequently, these fractions are inadequate to determine, in reconstitution experiments, whether certain gluten proteins are more effective than others in contributing to breadmaking quality. Only hydrophobic interaction chromatography on Phenyl-Sepharose CL-4B allows recovery of a small fraction of the acetic-acid-soluble glutenin polymers in their native state, i.e., in the absence of any detergent and/or reducing agent (Chung and Pomeranz 1979, Popineau 1985).

The first step in explaining gluten structure is to understand the roles of the individual polypeptides in determining the biochemical properties of the resulting polymers.

Glutenin polymers contain two major size classes of polypeptides, the high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits (for a review, see Shewry and Tatham 1990). However, although the research on both the biochemical properties of HMW glutenin subunits and their relationships to breadmaking quality is extensive (for a review, see Wrigley and Bietz 1988), knowledge on the LMW glutenin subunits is minimal. In fact, the purification of these proteins is difficult because they are similar to gliadins in terms of molecular weight and biochemical characteristics.

Research interest is focused on gluten polypeptides able to aggregate through disulfide bonds; accordingly, some procedures to purify glutenin polymers free from monomeric proteins have recently been developed (Burnouf and Bietz 1989, Graybosch and Morris 1990, Gupta and MacRitchie 1991).

In this article, we describe a new chromatographic procedure, based on the use of controlled-pore glass (CPG) beads in the absence of any detergent or chaotropic agent, for the separation of a number of glutenin polymers differing in their physicochemical properties. Furthermore, since at the end of the chromatographic experiment about 35% of the protein loaded had tightly adsorbed onto glass, we report the results of their desorption using different solvents.

MATERIALS AND METHODS

Gluten Preparation

Gluten was separated from dough by hand-washing under a stream of tap water. The dough was prepared from flour of the Italian bread wheat cultivar Spada, which contains HMW glutenin subunits 2, 7, and 12 (nomenclature of Payne and Lawrence 1983).

Protein Extraction

Wet gluten (from 10 g of flour) was mixed with 40 ml of 0.1N acetic acid. The suspension was stirred overnight at 4°C and centrifuged at $31,000 \times g$ for 20 min. The opalescent supernatant was used for the chromatographic separations. Protein content estimations indicated that about 83% of the protein in the original gluten was extracted.

Protein Determination

Total proteins of the gluten extracts were determined by the Kjeldahl procedure ($N \times 5.7$). The protein content of the chromatographic peaks was estimated by a urea-ammonia kit (Boehringer). The sample, after the mineralization step, was neutralized with 33% (w/v) NaOH instead of 10M KOH.

Fractionation on CPG Beads

The acetic-acid-soluble gluten proteins were fractionated at 25°C onto a column (1 × 60 cm) of CPG beads (2,000 Å pore

¹Dipartimento Biotecnologie Agrarie, Università degli Studi di Padova, Via Gradenigo 6, 35131 Padova, Italy.

²Author to whom correspondence should be addressed.

³Istituto Sperimentale Cerealicoltura, Roma, Italy.

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size, 100–200 mesh, surface area 12.1 m²/g, Sigma Chemical Co.) equilibrated with 0.1N acetic acid. Chromatography was monitored at 280 nm in a flow-through cell (40 μl) at a constant flow rate (0.5 ml/min). Fractions (0.6 ml) showing an absorbance higher than 0.02 were pooled for each peak.

Desorption of Proteins from Saturated Column

After saturation of the glass beads, the adsorbed proteins were eluted by sequential washings with 2, 4, and 6M urea and 6M urea plus 0.5% (w/v) SDS in 0.1N acetic acid.

Used CPG beads were reclaimed by mineralization with H₂SO₄-H₂O₂ (Hach et al 1985), followed by repeated washings with water to neutrality.

Gel Filtration on Sephadex G-150

Gel filtration of peak II proteins was performed on a Sephadex G-150 column (1.6 × 65 cm) equilibrated with 0.1N acetic acid. The chromatography conditions were those used in the fractionation on CPG beads.

Gel Filtration on Sepharose CL-4B

Acetic acid gluten extract and the peaks obtained by CPG beads chromatography were freeze-dried, solubilized with 0.1N acetic acid-3M urea-0.01M cetyltrimethylammonium bromide (AUC), and chromatographed on a Sepharose CL-4B column (1 × 60 cm) equilibrated with AUC. The effluent was collected in 0.3-ml fractions.

Analytical Gel Electrophoresis in the Presence of SDS

SDS-polyacrylamide gel electrophoresis (PAGE) on 11% acrylamide gels was done according to Laemmli (1970). A 90-

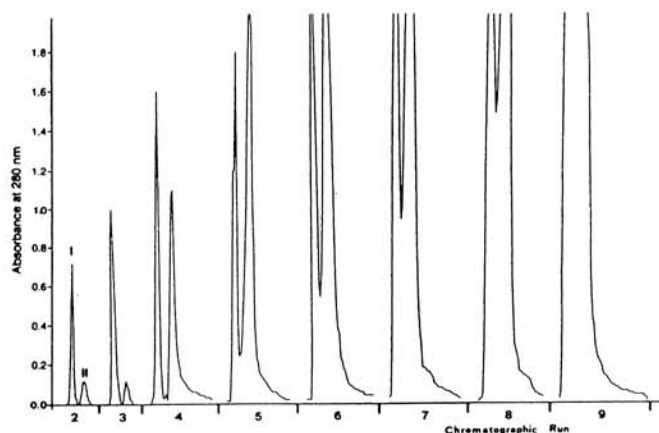


Fig. 1. Controlled pore glass chromatography profiles of acetic-acid-soluble proteins from gluten (cv. Spada). Column: 1 × 70 cm; bed height 60 cm; flow rate 30 ml/hr.

μl aliquot of each CPG chromatographic peak was placed in a plastic test tube, to which was added 40 μl of a solution containing 1.33M Tris-Cl (pH 8.6), 8% (w/v) SDS, and 40% (v/v) glycerol. For SDS-PAGE in reducing conditions, the solution was made 9% (v/v) in 2-mercaptoethanol and the samples were boiled for 5 min. When the protein concentration of the peak was too low, an appropriate sample volume containing 70 μg of protein was freeze-dried and then dissolved in 70 μl of the solution described above but diluted 1:4 with water.

A convenient volume (20–40 μl containing about 20 μg of protein) was loaded into each slot of a Dual-Mini slab electrophoresis apparatus (Bio-Rad) and electrophoresed at 35 mA per gel until the tracking dye (bromophenol blue) reached the bottom of the gel.

A 16% acrylamide gel was also used to fractionate proteins with molecular mass smaller than 30 kDa.

The gels were stained with Coomassie R-250 as reported by Koenig et al (1970).

Two-Step One-Dimensional SDS-PAGE

The two-step one-dimensional SDS-PAGE procedure was performed as described by Singh and Shepherd (1988). After the electrophoresis in unreduced conditions of peak I, two gel pieces (2 mm wide) were cut along the entire width of each lane, one at the base of the sample well and the other at the top of the separating gel. The gel pieces were placed in 0.5 ml of 50 mM Tris-Cl buffer (pH 7.4) containing 2.5% (w/v) SDS and 2.5% (v/v) 2-mercaptoethanol, boiled for 5 min, and submitted to the second SDS-PAGE fractionation.

Acid Electrophoresis

Acid electrophoresis (A-PAGE) at pH 3.1 was performed in a Pharmacia GE 2/4 LS vertical cell as previously described (Peruffo et al 1981).

Free-Flow Isoelectric Focusing

Free-flow isoelectric focusing of the CPG chromatographic peaks was performed in a Bio-Rad Rotoform cell as previously described (Curioni et al 1990). The freeze-dried peaks were solubilized by stirring for 2 hr at 37°C with 50 ml of 10 mM Tris-Cl (pH 7.4) buffer containing 3M urea and 25 mM dithiothreitol.

Determination of Starch Content

The starch content of each chromatographic peak was determined by an enzymatic method using the native flour amylases. One gram of flour was stirred for 1 hr at 4°C with 10 ml of water. The suspension was centrifuged (10,000 × g, 10 min), and the supernatant was then dialyzed against water for 48 hr with several changes. The amylolytic activity at pH 5.5 of the resulting solution was checked by the dinitro-salicylic acid method (Snell and Snell 1971), using a 1% soluble starch solution.

TABLE I
Protein Recoveries from Peaks I and II Obtained by Controlled-Pore Glass Beads Chromatography of Acetic-Acid-Soluble Gluten Proteins

| Chromatographic run | Protein Loaded (mg) | Sample Volume (ml) | Peak I | | Peak II | | Protein Adsorbed ^a |
|---------------------|---------------------|--------------------|--------------|-----------|--------------|-----------|-------------------------------|
| | | | Protein (mg) | Yield (%) | Protein (mg) | Yield (%) | |
| 1 | 16 | 1 | ... | ... | ... | ... | 100.0 |
| 2 | 16 | 1 | 1.64 | 10.2 | 0.13 | 0.8 | 88.9 |
| 3 | 16 | 1 | 2.06 | 12.8 | 0.13 | 0.8 | 86.3 |
| 4 | 24 | 1.5 | 2.67 | 11.1 | 3.01 | 12.5 | 76.3 |
| 5 | 32 | 2 | 3.16 | 9.9 | 14.23 | 44.5 | 45.6 |
| 6 | 48 | 3 | 8.27 | 17.2 | 26.26 | 54.7 | 28.1 |
| 7 | 64 | 4 | 13.09 | 20.4 | 43.90 | 68.6 | 10.9 |
| 8 | 80 | 5 | 15.01 | 18.8 | 59.35 | 74.2 | 7.0 |
| Total | 296 | 18.5 | 45.90 | 15.5 | 147.01 | 49.7 | 34.8 |
| 9 | 96 | 6 | 94 | 97.9 | | | 2.1 |

^aPercent protein adsorbed = $\frac{\text{protein loaded} - (\text{protein peak I} + \text{protein peak II})}{\text{protein loaded}} \times 100$.

Native starch obtained by centrifugation of the gluten wash water was used as a positive control.

RESULTS

Preliminary chromatographic experiments demonstrated that 1 g of CPG beads completely adsorbed 2.3 mg of the unreduced gluten proteins soluble in 0.1N acetic acid. When the amount of the loaded protein exceeded this value, the proteins were only partially adsorbed and the electrophoretic pattern of the eluted protein solution was different from that of the original solution.

To elucidate these facts, we performed some frontal analysis chromatography experiments in which the sample mixture was loaded onto the column stepwise rather than continuously. In particular, nine sequential loadings containing 16, 16, 16, 24, 32, 48, 64, 80, and 96 mg of protein respectively, were made. After each loading, the column was washed with 0.1N acetic acid until the absorbance at 280 nm of the effluent was <0.02 . The elution profiles of a typical experiment are reported in Figure 1.

After the first loading (16 mg of protein), no peak at 280 nm is observed, indicating that all the proteins were adsorbed onto the glass beads. On the contrary, two distinct peaks (I and II) are obtained from each of the subsequent seven loads. The areas of the peaks increase with the increasing number of the chromatographic run. In particular, the increase in height of peak I is evident from the third run, whereas that of peak II begins in the fourth run. Furthermore the broadening of the peaks begins at the fifth chromatographic run and increases so strongly that peaks I and II blend in a single peak in the last run. Thus, the observed increase in both height and broadening of the peaks indicates a progressive drop in the percentage of protein adsorbed in each run (Table I).

This behavior is independent of the loading volume, as was proved by performing a chromatographic experiment in which the same volume (containing 16 mg of protein) was loaded onto the column in each step. A slow increase in the broadening of the peaks occurred, but more than 23 chromatographic steps were necessary to bring the two peaks together (data not shown).

In the second and third runs (Table I), only peak I contains a significant amount of protein, whereas peak II does not contain any polypeptide, as demonstrated by SDS-PAGE (see below). In the fourth run, peak II contains a significant amount of protein, slightly exceeding that of peak I, whereas in the subsequent chromatographic steps its protein content becomes three to five times larger than that of peak I. In addition, the yield of peak I, related to unitary sample volume, is nearly constant from the second to the fifth chromatographic run, after which it doubles and keeps rather constant through the following chromatographic steps.

Table I also indicates that, during the eighth chromatographic run, the glass beads are apparently saturated, since recovery of the protein loaded in the following step can be considered complete. Moreover, the total recovery of the protein loaded, calculated as the quantity of protein eluted in the first eight runs, is about 65%, indicating that 14.7 mg of protein is bound to 1 g of CPG beads.

Furthermore the enzymatic assay for starch determination showed that peaks I and II did not contain any starch. The peak I solutions were milky and opaque. Addition of NaCl permitted the recovery of a coagulated elastic protein mass. In the presence of SDS, the solutions turned clear. Also peak II solutions, which appeared clear, provided a coagulated elastic mass after addition of salt.

The reproducibility of the chromatograms was very high.

Electrophoretic Analysis of Peak I

The unreduced and reduced SDS-electrophoretic patterns of peak I are reported in Figure 2A and B, respectively. The unreduced patterns from the second to the eighth run (Fig. 2A, lanes 2-8) clearly show that the proteins are present in two different molecular mass classes; in fact, there is evidence of a considerable quantity of protein at the point of sample application as well as some streaking in the upper part of the running gel. The two-

step one-dimensional electrophoretic analysis of these two groups of polymers demonstrated that they are made up of the same glutenin subunits (not shown). Furthermore, the unreduced patterns do not show any monomeric protein, except for peaks I from the seventh and eighth run, where, when the protein loading onto the gel is heavy, some faint bands with molecular mass similar to that of α -gliadins appear. A-PAGE fractionation strengthens these results (data not shown).

The SDS-PAGE protein pattern of the sole peak from the ninth chromatographic run (Fig. 2, lane 9) coincides with that of the unfractionated gluten (lane 1). Moreover the acidic pattern of the same peak (Fig. 3, lane 9) lacks the 1D chromosome ω -gliadins as well as the γ -gliadins 43.5 and 45.5 (Peruffo et al 1981). Thus, in contrast to the quantitative data, the chromatographic data show that the glass beads cannot be considered fully saturated at the end of the ninth run.

SDS-PAGE fractionation in reducing conditions (Fig. 2B) shows HMW and LMW glutenin subunits (A, B, and C subunits) along with some medium molecular weight (D zone) bands with isoelectric points that, as determined by free-flow isoelectric focusing, are between pH 5 and 6.

Electrophoretic Analysis of Peak II

The unreduced and reduced SDS-PAGE patterns of peak II are reported in Figure 4A and B, respectively. Under unreducing conditions peak II from the second to the third run does not show any protein (Fig. 4A, lanes 2 and 3), whereas the electrophoretic profiles of the peak II from the fourth to the

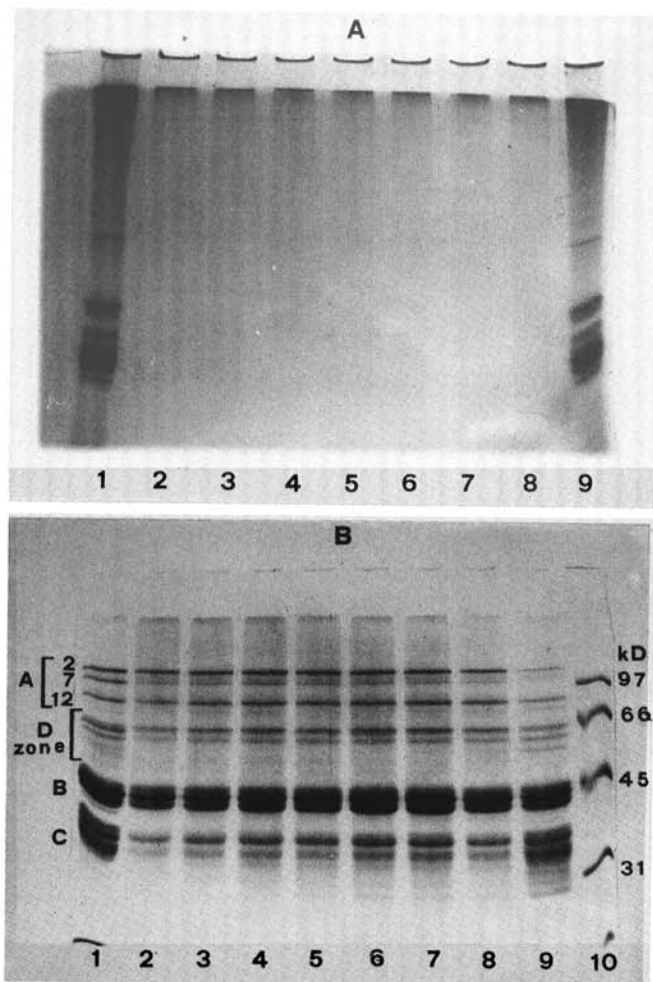


Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of unreduced (A) and reduced (B) proteins of peak I from the second (lane 2) to the ninth (lane 9) chromatographic run. The high molecular weight glutenin subunits (A subunits) are numbered according to Payne and Lawrence (1983). Lane 1, unfractionated gluten extract; lane 10, molecular mass standards.

eighth run (Fig. 4A, lanes 4–8) are quite distinct from each other both qualitatively and quantitatively. In particular, protein patterns show new bands as well as a more prominent anodal streaking. A-PAGE analysis of peak II (Fig. 3) demonstrates that some of the proteins that gradually appear with the increasing number of the chromatographic step are gliadins. Among these proteins, the first emerging are α -gliadins, followed by β - and finally some γ -gliadins. Chromosome 1D ω -gliadins and γ -gliadins 43.5 and 45.5 (arrowed in Fig. 3) are again absent. Note that the bands that appear in each lane are always accompanied by those obtained in the previous chromatographic steps, suggesting that the glass beads have been saturated with respect to the latter.

The reduced electrophoretic patterns of peak II (Fig. 4B) prove that the anodal streakings observed in unreduced conditions contain disulfide-linked polymers constituted of HMW and LMW glutenin subunits. The pattern of the fifth run peak (lane 5) lacks HMW subunits 2 and 7 and some LMW subunits of glutenin. In contrast, the patterns of peak II from the sixth through eighth runs (lanes 6–8) show all the HMW glutenin subunits, although the proportion of subunit 2 in the sixth run peak is low compared with that found in the seventh and eighth run peaks.

The SDS-PAGE patterns of peak II from the sixth to the eighth run contain a major component indicated by k in Figure 4B. This band has a mobility equal to that of the K3 protein described by Kazemie and Bushuk (1992). The K protein was also present in trace amount in the SDS-PAGE patterns of the peak I fractions under reducing conditions (Fig. 2B). Peak II also contains a low amount of subunit 7 compared with the total protein extract (Fig. 4B, lane 1) and peak I (Fig. 2B, lanes 2–9).

Like peak I, the reduced peak II contains some medium molecular weight proteins (D zone in Fig. 4B) the identification of which is uncertain. Their molecular masses are very similar to those of HMW albumins (Gupta et al 1991) and D group glutenin subunits (Jackson et al 1983). However, HMW albumins were found to be absent in the native glutenin extracted in 0.1N acetic acid (Tao et al 1989). Therefore, taking into account their solubility in 0.1N acetic acid and their acidic isoelectric point, proteins in the D zone may correspond to glutenin subunits of group D. Furthermore, when fractionated in a 16% gel, peak II from the fourth run reveals two proteins of 14 kDa (arrowed

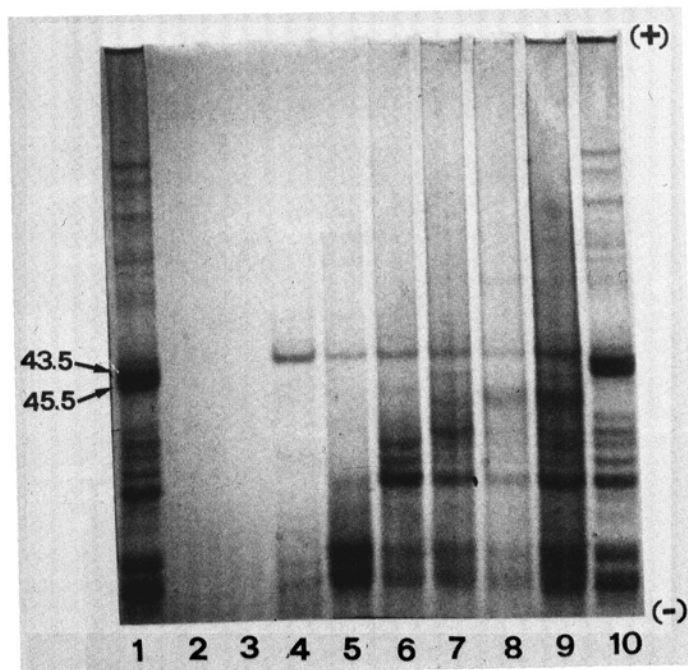


Fig. 3. Acid electrophoresis at pH 3.1 of proteins of peak II from the second (lane 2) to the eighth (lane 8) chromatographic run and of proteins of the peak of the ninth run (lane 9). Lanes 1 and 10, ethanolic gliadin extract; γ -gliadins 43.5 and 45.5 are arrowed.

in Fig. 5), which may correspond to the S protein fraction III described by Zawistowska et al (1986).

The glutenin polymers in peak II can be separated from the monomeric proteins by gel filtration on Sephadex G 150.

Chromatography of Peaks I and II on Sepharose CL-4B

Chromatography on Sepharose CL-4B demonstrated that peak I elutes at the column void volume. Therefore, as indicated by Huebner and Wall (1980), the molecular mass of the polymers in peak I can be estimated to be larger than 107 Da. In contrast, the elution volume of peak II is much higher than that of peak I, indicating a smaller molecular mass.

Desorption of Gluten Proteins from CPG Beads

Proteins desorbed from the CPG beads by the acetic acid-urea solutions and by the acetic acid-urea-SDS solution elute from the column as single peaks (Fig. 6). The urea-SDS solution appears to be the most effective eluent, since it desorbs about 41% of the total protein recovered by acidic eluents (Table II).

The urea and urea-SDS elutabilities of gluten proteins do not depend on the time elapsed between adsorption and elution; in fact, a three-day "residence time" did not result in any variation of the eluted protein quantities.

Electrophoretic analysis at pH 3.1 revealed that 2M urea desorbs γ -gliadins 43.5 and 45.5 (Peruffo et al 1981) (Fig. 7, lanes 2 and 3). Moreover, all the urea solutions desorb a γ -gliadin with an estimated electrophoretic mobility of 44.5. It is noteworthy that two-dimensional A-PAGE \times SDS-PAGE fractionation of total

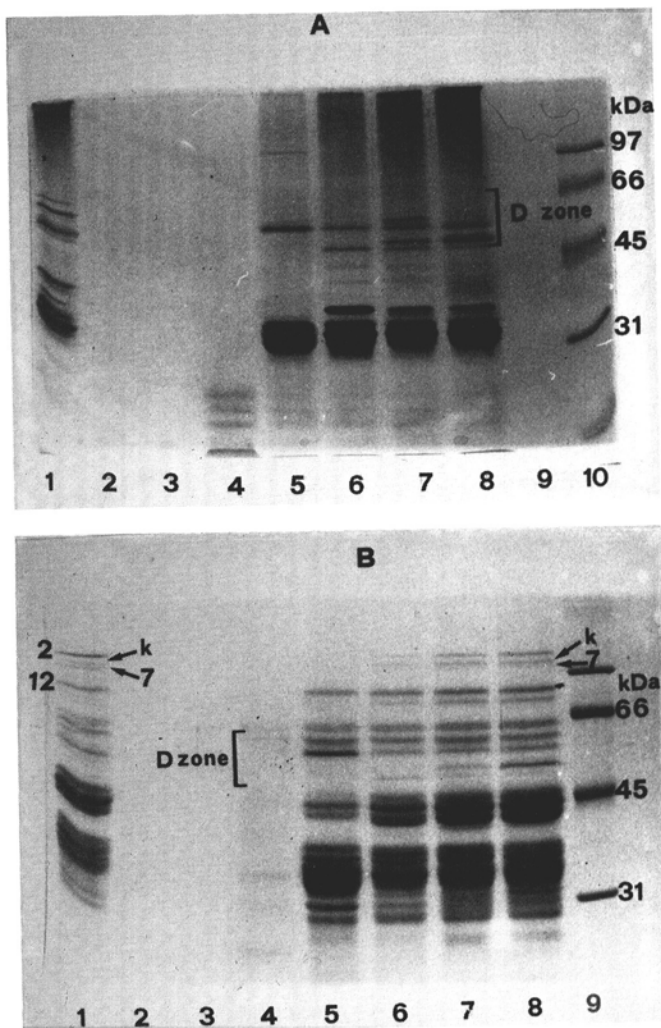


Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of unreduced (A) and reduced (B) proteins of peak II from the second (lane 2) to the eighth (lane 8) chromatographic run. Lane 1, unfractionated gluten extract; lane 10 (A) and lane 9 (B), molecular mass standards.

gliadins shows that band 45.5 is constituted of two components, one coded by chromosome 1A and the other by chromosome 1D (data not shown). It is assumed that, in the experimental conditions adopted here, these two components separate as distinct bands.

Pooled fractions A from 2M and 4M urea peaks (Fig. 6) contain glutenin aggregates, as demonstrated by SDS-PAGE analysis (Fig. 8B, lanes 2 and 4). These aggregates appear as a prominent streaking in both acidic (Fig. 7, lanes 2 and 4) and SDS unreduced (Fig. 8A, lanes 2 and 4) gels.

The proteins desorbed by the acetic acid-urea-SDS solution appear to be constituted of glutenin aggregates (Fig. 8B, lane 7), free from monomeric proteins (Fig. 8A, lane 7). About 68% of the adsorbed proteins was not eluted by the eluents used.

DISCUSSION

In this communication, we describe the fractionation of the acetic-acid-soluble gluten proteins by adsorption chromatography onto a column of CPG beads.

The protein adsorption-desorption phenomena that occur during each step of the frontal analysis make the state of the support matrix different from that of the previous runs. Therefore, the results obtained here can be thought of as being derived from eight distinct chromatographic runs.

The first run shows that adsorption of all the gluten proteins on the glass surface takes place. On the contrary, the results of the subsequent runs indicate that fractionation is governed by the adsorption-desorption phenomena and that the gel filtration effect is only subsidiary. Furthermore, the variation in protein composition of peak II in the different runs suggests that the adsorption process is sequential and competitive and that, at least

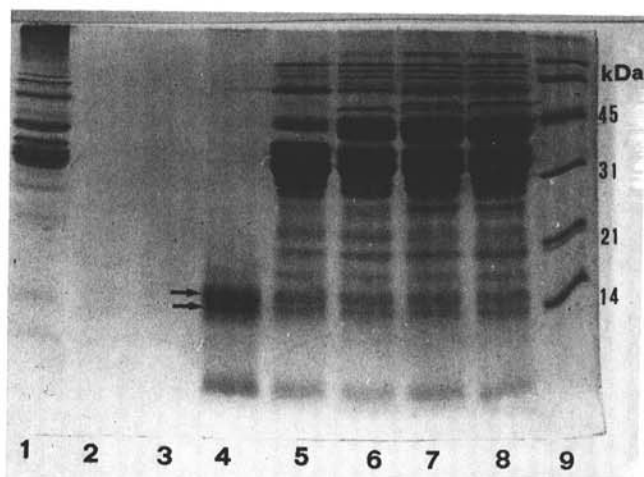


Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (16% acrylamide gel) of reduced proteins of peak II from the second (lane 2) to the eighth (lane 8) chromatographic run. Lane 1, unfractionated gluten extract; lane 9, molecular mass standards.

to some extent, reversible. Thus the adsorption-desorption process can be explained in terms of displacement of the adsorbed proteins by one or more proteins having a stronger tendency to adsorb (Norde 1986).

This study demonstrated that γ -gliadins 43.5 and 45.5 and the two ω -gliadins coded by chromosome 1D remain adsorbed onto the glass at the end of the chromatographic experiment. Thus, their high affinity for the glass suggests that they can act as displacers. The variability in the electrophoretic patterns of the A and B glutenin subunits in peak II from different runs suggests that a selective desorption mechanism is effective also for the glutenin polymers. It is well known that, in a mixture of proteins, the preferential adsorption of the higher molecular mass proteins is caused by the high number of anchoring segments (Shirahama et al 1990 and references cited therein). Thus, the selective desorption of glutenin polymers in peak II is likely to be governed by those with the highest molecular mass. On the contrary, peak I polymers, which show molecular masses higher than those of peak II polymers, seem to be scarcely adsorbed onto glass beads, being eluted from the second chromatographic run. The peak I glutenin polymers are probably larger than the inner volume of the glass beads (2,000 Å average size), and therefore they can bind onto only a low number of sites placed on the edge of the glass pores or on the area between adjacent pores.

It is interesting to note that the elution order of adsorbed gliadins during the protein sequential loads is identical to that

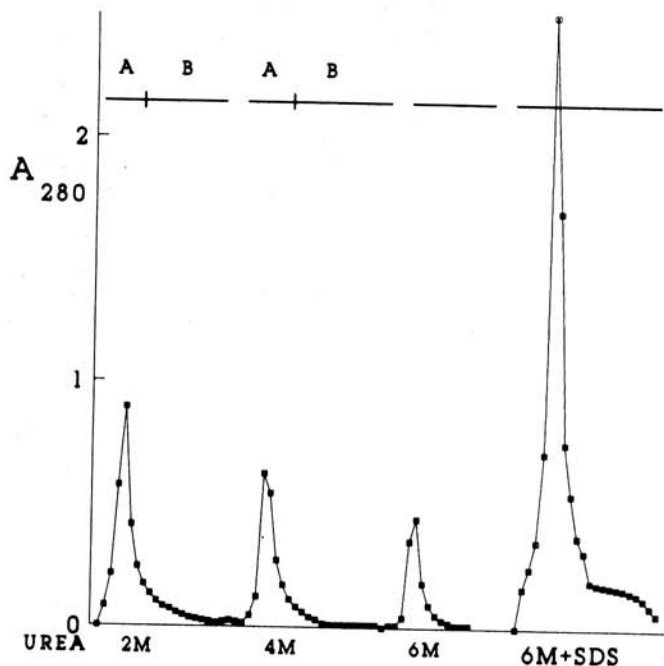


Fig. 6. Elution profiles obtained by washing the controlled-pore glass beads column sequentially with the solvents reported in Table II. The fractions were pooled as indicated. SDS = sodium dodecyl sulfate.

TABLE II
Desorption of the Gluten Proteins from the Controlled-Pore Glass Beads: Elution Scheme and Protein Recoveries

| Elution Step | Composition of Eluent | Elution Volume (ml) | Protein Recovery ^a (mg) |
|--------------|--|---------------------|-------------------------------------|
| 1 | 0.1M Acetic acid, 2M urea | 60 | Pooled fractions ^b A 6.6 |
| 2 | 0.1M Acetic acid, 4M urea | 60 | Pooled fractions B 2.6 |
| 3 | 0.1M Acetic acid, 6M urea | 60 | Pooled fractions A 5.1 |
| 4 | 0.1M Acetic acid, 6M urea, 0.5% (w/v) SDS ^c | 30 | Pooled fractions B 0.4 |
| 5 | 0.1M Acetic acid | 100 | Pooled fractions 3.9 |
| | | | Pooled fractions 12.8 |
| | | | |

^aProtein content determined at 280 nm.

^bSee Fig. 6. Total recovery was 31.4 mg.

^cSodium dodecyl sulfate.

obtained from a Phenyl-Sepharose column using a linear gradient of ethanol in 0.02M ammonia (Popineau 1985), with the exception of the two ω -gliadins and γ -gliadins 43.5 and 45.5, which remain tightly adsorbed onto the glass surface. Moreover, the S protein fraction III, which is less hydrophobic than gliadins as demonstrated by RP-HPLC (Zawistowska et al 1986), is eluted in advance of α -gliadins. These observations suggest that the adsorption-desorption process may be controlled to a great extent by hydrophobic interactions between the polypeptides and the glass surface. On the other hand, at pH values below 4, ionization of silanol groups is repressed, and therefore charge-charge interactions cannot be invoked to explain the protein adsorption onto the glass beads. Furthermore, if the model of a close-packed monolayer is adopted, the protein amount adsorbed should be between 0.21 and 1.57 $\mu\text{g}/\text{cm}^2$ (Schmitt et al 1983). In our experiments, sequential and competitive adsorption from flowing solution never led to adsorbed amounts that exceeded the above values. Moreover, since the pH of the environment is below the pI of the gluten proteins, positive charges of the polypeptide chains would prevent any mutual molecular attraction within the glass matrix, thus excluding an additional factor that can affect protein adsorption.

The selective desorption of peak II components reflects differences in their behavior at the interfaces. These differences may play a key role in determining the functional properties of gluten.

Glutenin polymers in peaks I and II coagulate in an elastic mass that closely resembles the original gluten after addition of salts. This means that glutenin polymers retain their ability to aggregate after contact with the glass, indicating that the possible structural changes occurring during adsorption do not influence their functional properties. Since acetic acid does not affect the rheological properties of the gluten proteins (MacRitchie 1985), the chromatographic fractions obtained here can be used in reconstitution studies.

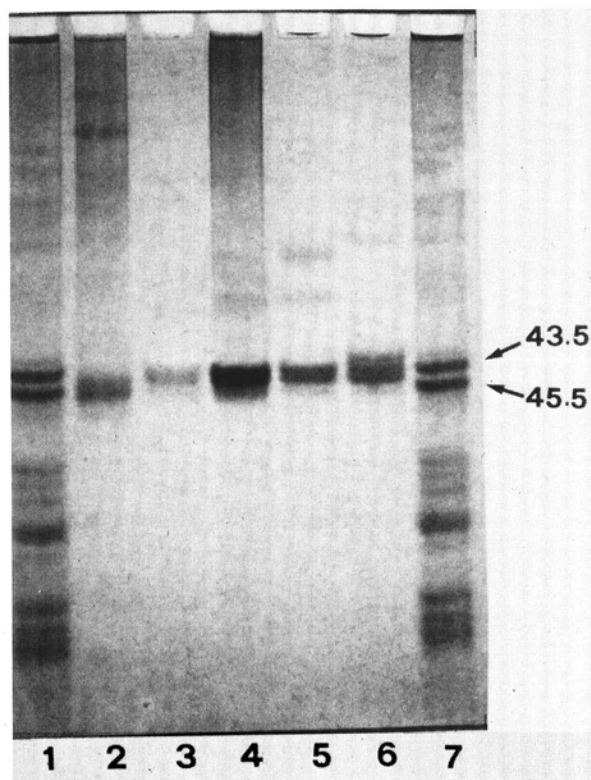


Fig. 7. Acid polyacrylamide gel electrophoresis at pH 3.1 of protein desorbed from controlled-pore glass beads by 0.1N acetic acid containing 2M urea (lane 2, pooled fractions A from Fig. 6; lane 3, pooled fractions B from Fig. 6), 4M urea (lane 4, pooled fractions A from Fig. 6; lane 5, pooled fractions B from Fig. 6), and 6M urea (lane 6). Lanes 1 and 7 are ethanolic gliadin extract.

Gluten proteins not elutable by frontal analysis cannot be desorbed from the glass by increasing the pH of the eluent or its ionic strength (data not shown), indicating that interactions between gluten proteins and glass are not electrostatic. On the contrary, sequential washings of the column at acidic pH with urea solutions of increasing concentration and urea-SDS solution allow the selective recovery of different types of proteins. However, the protein recovery calculations indicate that desorption is still incomplete. This is consistent with the findings of Bohnert and Horbett (1986) and with those of Mizutani (1981), who respectively demonstrated that the use of SDS and urea does not elute all the adsorbed proteins from solid surfaces.

Selective desorption of gliadins induced by urea solutions could reflect differences, at least to some extent, in their hydrophobicity, since urea has been found to weaken hydrophobic interactions (Dill and Shortle 1991). Acidic urea solutions desorb mostly γ -gliadins 43.5, 44.5, and 45.5. Unlike α -, β -, and the remaining γ -gliadins, they remained tightly adsorbed during the frontal chromatographic analysis of acetic acid-soluble gluten proteins, indicating that their hydrophobicity is higher than those of the other fast-migrating gliadins, as already observed by reversed-phase HPLC (Popineau 1985).

All the urea solutions used in this study elute γ -gliadins 43.5 and 45.5, but the quantitative ratio of 43.5 to 45.5 increases with

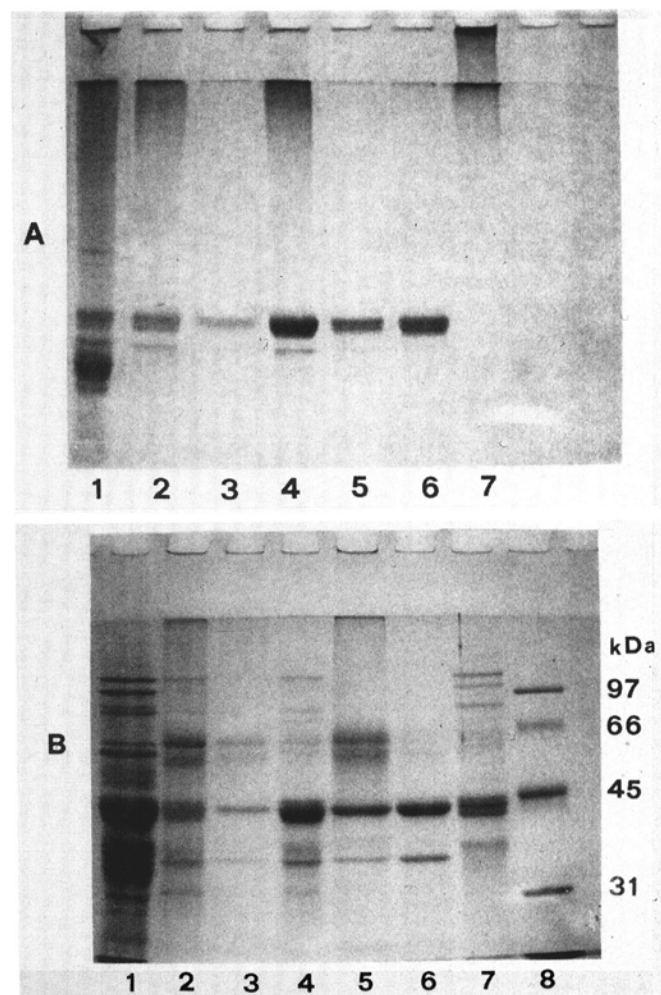


Fig. 8. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of unreduced (A) and reduced (B) proteins desorbed from controlled-pore glass beads by 0.1N acetic acid containing 2M urea (lanes 2, pooled fractions A from Fig. 6; lanes 3, pooled fractions B from Fig. 6), 4M urea (lanes 4, pooled fractions A from Fig. 6; lanes 5: pooled fractions B from Fig. 6), 6M urea (lanes 6), and 6M urea-0.5% SDS (lanes 7). Lane 1, total gluten extract. Lane 8 (B), molecular mass standards.

increasing urea concentration in the eluent, indicating that the hydrophobicity of γ -gliadin 43.5 is higher than that of 45.5.

The elutability of the same γ -gliadin by acidic solutions at different urea concentrations evidently reflects the existence of at least three protein populations with different "states" of adsorption. These states can be ascribed to the fact that a protein attaches to the sorbent surface via several segments characterized by a spectrum of binding strengths (Bohnert and Horbett 1986). Moreover, a protein adsorbed from the solution at the beginning of an adsorption experiment may assume a greater degree of spreading (structural change) than later-arriving molecules, allowing for a greater number of contacts between protein and surface (Slack and Horbett 1989).

The addition of the surfactant SDS to the acidic 6M urea solution allows the elution of significantly more protein than was eluted by each of the urea solutions considered. The elutability of the proteins by SDS can be explained by assuming that SDS binding to adsorbed proteins may induce conformational changes that break protein-surface interactions. SDS can also diffuse between bound proteins to the protein-surface interface, where it can replace protein-surface contacts (Bohnert and Horbett 1986).

SDS is more effective than urea in disrupting hydrophobic interactions; accordingly, it could be inferred that some glutenin aggregates show stronger hydrophobic interactions than γ -gliadins, as suggested by their elution behavior.

CONCLUSIONS

Adsorption chromatography on CPG beads appears to be an extremely valuable addition to the available techniques for characterizing and separating wheat proteins. In fact, a high proportion of the acetic-acid-soluble gluten proteins can be fractionated in the absence of any detergent or organic solvent.

Two different molecular weight classes of glutenin polymers free from both starch and monomeric proteins can be obtained. Furthermore, the class with the lowest molecular weight is constituted of a number of glutenin polymer groups different in subunit composition and, possibly, in hydrophobicity.

γ -Gliadins encoded by group 1 chromosomes showed high hydrophobicity compared to α - β -, and other γ -gliadins.

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