NOTE

Electroendosmotic Preparative Electrophoresis as a One-Step Method for Purification of High Molecular Weight Subunits of Wheat Glutenin

A. CURIONI,[1] A. DAL BELIN PERUFFO,[1] and N. E. POGNA[2]

Wheat glutenin is a heterogeneous protein group of disulfide-linked high molecular weight (HMW) and low molecular weight (LMW) subunits (Huebner and Wall 1976, Payne and Corfield 1979). Preparation of purified glutenin polypeptides is difficult because of the high molecular weight of glutenin and its insolubility in most buffers commonly used for protein separation (Bietz and Wall 1975, Danno 1981). Several methods have been employed but, generally, they yielded fractions comprising several components or small quantities of single polypeptides. Huebner and Wall (1974) tried to separate glutenin subunits by gel-filtration and ion-exchange chromatography; Shewry et al (1984) purified nine glutenin subunits by gel filtration, ion-exchange chromatography, and preparative isoelectric focusing; and Matsumura et al (1984) and Moonen et al (1985) fractionated HMW glutenin subunits by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) and removed them from the gel by different elution methods.

In a previous work (Curioni et al 1988), a new preparative electrophoretic method permitting separation of protein molecules and their recovery in very small volumes was successfully used to purify native cellulolytic enzymes. This method, electroendosmotic preparative electrophoresis (EPE), uses the buffer flow that occurs between the electrodes (electroendosmosis) to elute electrophoresed molecules. In particular, proteins outflowing from the anodic end of a vertical, cylindrical gel enter a near-zero volume elution chamber at the interface between the gel surface and a semi-permeable membrane tightly held against it and are eluted through a capillary tube by the electroendosmotic flow from the lower buffer chamber towards the cathode.

In this communication, electroendosmotic preparative electrophoresis in the presence of SDS (SDS-EPE) was employed for one-step purification of HMW glutenin subunits of common wheat.

MATERIALS AND METHODS

Protein Extraction

The studies were conducted with flour of the Italian bread wheat cultivar Clara. Reduced total protein extracts were prepared by suspending 1 g of flour in 15 ml of a buffer solution containing 0.1*M* Tris-HCl, pH 8.0, 2.5% (w/v) SDS, 2.5% (v/v) 2-mercaptoethanol, and 6*M* urea in 18-ml capped Pyrex glass tubes. The flour-buffer mixture was shaken by hand for 2–3 min, heated for 3.5 min in a boiling water bath and then centrifuged for 15 min at 30,900 × *g* in a Sorvall RC-5B centrifuge. The supernatant was recentrifuged and 1.5 ml of the second supernatant was fractionated by SDS-EPE. The protein content of the supernatant was 22.7 mg/ml.

EPE

EPE was carried out with an ELFE electrophoresis unit (Genofit, Geneva, Switzerland). The protein exctract was fractionated on a SDS-polyacrylamide gel according to Laemmli (1970). The cylindrical separating gel (2 cm diameter and 3 cm long) was prepared with 10 ml of a solution containing 9.75% (w/v) acrylamide and 0.25% (w/v) bisacrylamide. Polymerization was achieved by adding 50 μl of 22% (w/v) ammonium persulfate and 3 μl of TEMED. This amount of catalyst gives a flat surface at the lower end of the gel, which greatly affects SDS-EPE resolution. The 3.9-cm stacking gel was prepared with 12 ml of a solution containing 3.66% (w/v) acrylamide and 0.094% (w/v) bisacrylamide. The gel was polymerized by adding 60 μl of 22% (w/v) ammonium persulfate and 6 μl of TEMED. The electrode buffer solution was 0.025*M* Tris, 0.192*M* glycine; 0.1% (w/v) SDS was added to the cathodic buffer. The electrophoretic unit was assembled as previously described (Curioni et al 1988); SDS-EPE was carried out at 25 mA and 15° C for 10–12 hr.

Proteins eluted from the gel were detected by absorbance at 280 nm with a Gilson Holochrome spectrophotometer and collected in 240-μl fractions. Protein contents of the fractions were determined according to Smith et al (1985) at 60° C for 30 min using bovine albumin as reference.

Analytical SDS-PAGE

Proteins in every third fraction were compared by SDS-PAGE on 8.5% gels according to Laemmli (1970) using a Dual Mini-Slab electrophoresis apparatus (Bio-Rad). Prior to electrophoresis, 70 μl of each fraction was placed in a 1.5-ml plastic test tube to which was added 30 μl of a buffer containing 50 m*M* dithiothreitol, 10% (w/v) SDS, 20% (w/v) sucrose, 0.025*M* Tris, and 0.192*M* glycine; 35 μl of this solution was electrophoresed at 100 V and 20° C until the tracking dye, bromophenol blue, ran off the gel (about 2 hr). The gels were stained overnight with Coomassie Brilliant Blue R-250 according to Koenig et al (1970), destained with 7.5% acetic acid, and photographed.

RESULTS AND DISCUSSION

The wheat cultivar Clara used in the experiments contains five HMW glutenin subunits designated 1, 5, 7, 9, and 12 according to the nomenclature of Payne and Lawrence (1983). A typical elution profile of 1.5 ml of protein extract from Clara wheat flour fractionated by SDS-EPE is shown in Figure 1. The proteins were separated into five peaks differing in molecular weight. The initial peak (I) and the area between the first two peaks (Fig. 1) were identified by electrophoresis as LMW (<70 kDa) gliadins, LMW

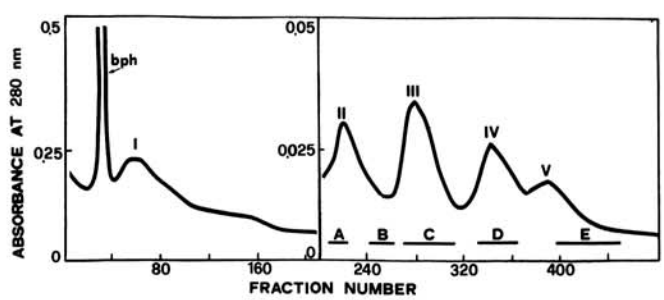

Fig. 1. Elution profile of reduced proteins from Clara wheat flour separated by electroendosmotic preparative electrophoresis in the presence of sodium dodecyl sulfate (SDS-EPE). Solid lines at the base indicate pooled fractions containing purified high molecular weight glutenin subunits. Chart speed, 1 mm/min; input range of recorder, 500 mV. Absorbance, 0.5 (first part of the chromatogram) and 0.05 (second part); bph = bromophenol blue.

[1]Dipartimento di Biotecnologie Agrarie, Università di Padova, Via Gradenigo 6, 35131 Padova, Italy.
[2]Istituto Sperimentale per la Cerealicoltura, Via Mulino 3, 20079 S. Angelo Lodigiano, Italy.

TABLE I
Volume and Protein Content of Pooled Fractions Containing Purified High Molecular Weight Glutenin Subunits Fractionated by Electroendosmotic Preparative Electrophoresis in the Presence of Sodium Dodecyl Sulfate on Gels of Different Concentrations

Pooled Fraction	High Molecular Weight Subunit	Gel I[a]		Gel II[b]		Gel III[c]	
		Fraction Volume (ml)	Protein Content (mg)	Fraction Volume (ml)	Protein Content (mg)	Fraction Volume (ml)	Protein Content (mg)
A	12	2.7	0.27	...	...	...	...
B	9	3.2	0.16	...	...	...	...
C	7	10.2	0.86	6.3	0.92	4.9	0.84
D	5	7.7	0.58	4.5	0.62	5.4	0.82
E	1	9.3	0.51	8.6	0.86	8.5	0.93

[a]Gel I = 9.75% acrylamide and 0.25% bisacrylamide (standard gel).
[b]Gel II = 8.78% acrylamide and 0.22% bisacrylamide.
[c]Gel III = 10% acrylamide and 0.013% bisacrylamide.

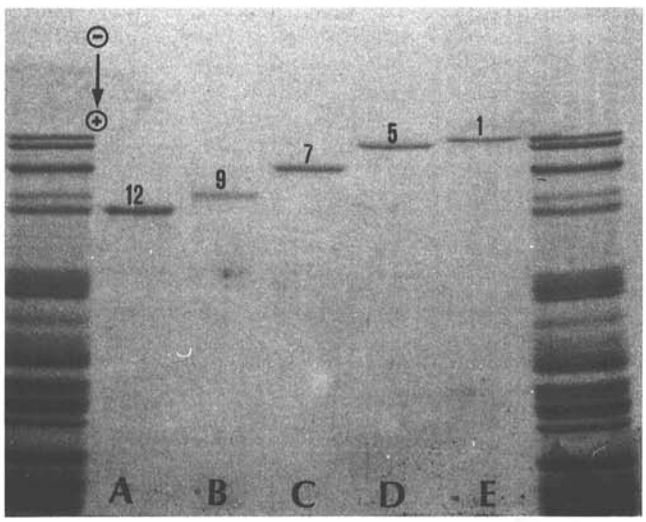

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the five pooled fractions from Fig. 1. High molecular weight subunits are numbered. On the left and right sides of the gel are the patterns of total reduced proteins from cultivar Clara.

glutenin subunits, albumins, globulins, and nonstorage proteins (data not shown).

Peak II contained HMW glutenin subunits 12 and 9; the pooled fractions, named A and B in Figure 1, contained pure subunits 12 and 9 respectively (Fig. 2, lanes A and B), whereas the remaining fractions of the peak all consisted of both subunits. The larger peak III comprised subunit 7 with traces of subunit 9; pure subunit 7 was present in the pooled fraction C (Fig. 2, lane C). The next peak IV consisted mainly of subunit 5, but subunits 7 and 1 were also present in the leading and trailing parts of the peak, respectively; pure subunit 5 was obtained in the pooled fraction D (Fig. 2, lane D). Finally, the trailing part of the smaller peak V contained pure subunit 1 (pooled fraction E; Fig. 2, lane E), whereas the leading half consisted of subunit 1 and trace amounts of subunit 5.

Both protein content and volume of the five pooled fractions were greatly affected by acrylamide concentration and acrylamide/bisacrylamide ratio in the resolving gel (Table I). The recoveries of purified subunits 1 and 5 were higher on a gel of 10% acrylamide and 0.013% bisacrylamide (gel III); however, subunits 9 and 12 could not be separated on this gel or on gels of lower acrylamide concentration such as gel II. Subunit 7 could be quantitatively purified by using all three gel formulations. Because HMW glutenin subunits account for less than 10% of wheat flour proteins (Payne et al 1984), the recoveries of purified subunits after SDS-EPE fractionation were rather high. Furthermore, the fractionation pattern was stable from one experiment to another.

The procedure presented here permitted one-step preparation of all HMW glutenin subunits contained in the wheat flour in only 10–12 hr. Using SDS-EPE it was possible to prepare, in several separations, large amounts (10–20 mg) of each subunit. This is important in studies of the relationship between subunit structure (amino acid composition, sequence, secondary structure) and functionality of glutenin in the breadmaking process.

This technique also permits separation of low-molecular weight polypeptides when appropriate conditions of gel concentration and relative amounts of stacking and separating gels are used. For example, the two major bands (14 and 16 kDa) of gluten S protein (Zawistowska and Bushuk 1986) were purified using 5 ml of stacking gel solution and 15 ml of a separating gel solution containing 20% (w/v) acrylamide and 0.066% (w/v) bisacrylamide (data not shown).

LITERATURE CITED

BIETZ, J. A., and WALL, J. S. 1975. The effect of various extractants on the subunit composition and associations of wheat glutenin. Cereal Chem. 52:145-155.

CURIONI, A., DAL BELIN PERUFFO, A., and NUTI, M. P. 1988. Purification of cellulases from *Streptomyces* strain A20 by electroendosmotic preparative electrophoresis. Electrophoresis 9:327-330.

DANNO, G. 1981. Extraction of unreduced glutenin from wheat flour with sodium dodecyl sulfate. Cereal Chem. 58:311-313.

HUEBNER, F. R., and WALL, J. S. 1974. Wheat glutenin subunits. I. Preparative separation by gel-filtration and ion-exchange chromatography. Cereal Chem. 51:228-240.

HUEBNER, F. R., and WALL, J. S. 1976. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. Cereal Chem. 53:258-269.

KOENIG. R., STEGEMANN, H., FRANCKSEN, H., and PAUL, H. L. 1970. Protein subunits in the potato virus X group. Determination of the molecular weights by polyacrylamide electrophoresis. Biochim. Biophys. Acta 207:184-189.

LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophase T4. Nature 227:680-685.

MATSUMURA, Y., KAWAMURA, Y., MATOBA, T., and YONEZAWA, D. 1984. Separation of subunit polypeptides of glutenin by SDS-PAGE and determination of their cysteine contents. Agric. Biol. Chem. 48:1281-1285.

MOONEN, J. H. E., SCHEEPSTRA, A., and GRAVELAND, A. 1985. Biochemical properties of some high molecular weight subunits of wheat glutenin. J. Cereal Sci. 3:17-27.

PAYNE, P. I., and CORFIELD, K. G. 1979. Subunit composition of wheat glutenin proteins, isolated by gel filtration in a dissociating medium. Planta 145:83-88.

PAYNE, P. I., and LAWRENCE, G. J. 1983. Catalogue of alleles for the complex gene loci, *Glu-A1, Glu-B1* and *Glu-D1* which code for the high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res. Commun. 11:29-35.

PAYNE, P. I., HOLT, L. M., JACKSON, E. A., and LAW, C. N. 1984. Wheat storage proteins: Their genetics and their potential for manipulation by plant breeding. Phil Trans. R. Soc. Lond. B 304:359-371.

SHEWRY, P. R., FIELD, J. M., FAULKS, A. J., PARMAR, S., MIFLIN, B. J., DIETLER, M. D., LEW, E. J. L., and KASARDA, D. D. 1984. The purification and N-terminal amino acid sequence analysis of the high molecular weight gluten polypeptides of wheat. Biochim. Biophys. Acta 788:23-34.

SMITH, P. K., KROHN, R. I., HERMANSON, G. T., MALLIA, A. K., GARTNER, F. H., PROVENZANO, M. D., FUJIMOTO, E. K., GOEKE, N. M., OLSON, B. J., AND KLENK, D. C. 1985. Measurement of protein using bicinchoninic acid. Anal Biochem. 150:76-85.

ZAWISTOWSKA, U. and BUSHUK, W. 1986. Electrophoretic characterisation of low-molecular weight wheat protein of variable solubility. J. Sci. Food Agric. 37: 409-417.

[Received May 31, 1988. Revision received November 15, 1988. Accepted November 21, 1988.]