

Biochemical Characterization and Quantification of the Storage Protein (Secalin) Types in Rye Flour

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ABSTRACT

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Kernels of the rye cultivars Danko and Halo were milled into white flour and compared with flour of the wheat cultivar Rektor. Flour proteins were extracted stepwise with a salt solution (albumins-globulins), 60% ethanol (prolamins), and 50% 2-propanol under reducing conditions (glutelins). The quantification by reversed-phase HPLC indicated that the extractable proteins of both rye flours consisted of $\approx 26\%$ albumins-globulins, 65% prolamins, and 9% glutelins. Compared with wheat flour, rye flours comprised significantly higher proportions of nonstorage proteins (albumins-globulins) and lower proportions of polymerized storage proteins (glutelins). SDS-PAGE revealed that the prolamin fractions of rye contained all four storage protein types (HMW, γ -75k, ω , and γ -40k secalins), whereas the glutelin fractions contained only HMW and γ -75k secalins. The quantification of secalin types by RP-HPLC showed a close relationship between the two cultivars. The γ -75k secalins contributed nearly half ($\approx 46\%$) of the total storage proteins, followed by γ -40k

secalins (24%) and ω secalins (17%); HMW secalins ($\approx 7\%$) were minor components, and 6% of eluted proteins were not identified. The amino acid composition of γ -40k secalins corresponded to those of γ -gliadins of wheat, whereas γ -75k secalins were characterized by higher contents of glutamine and proline. Matrix-assisted laser desorption/ionization and time of flight mass spectrometry (MALDI-TOF MS) indicated molecular masses of about 52,000 (γ -75k) and 32,000 (γ -40k), respectively. N-terminal amino acid sequences were homologous with those of wheat γ -gliadins except for position 5 (asparagine in γ -75k and glutamine in γ -40k secalins) and position 12 (cysteine in γ -75k secalins). The N-terminal amino acid sequences of HMW and ω -secalins were homologous with those of the corresponding protein types of wheat. Gel-permeation HPLC of prolamin fractions revealed that rye flours contained a significantly higher proportion of ethanol-soluble oligomeric proteins than wheat flour.

Rye (*Secale cereale* L.) and bread wheat (*Triticum aestivum* L.) are closely related by taxonomy and, accordingly, their kernel endosperms contain homologous storage proteins (Shewry et al 1984). In contrast to wheat, rye is unable to form gluten when flour is mixed with water, and differences in the structure and quantitative composition of storage proteins could be responsible. The storage proteins of rye (secalins) have been studied less intensively than those of wheat (gliadins and glutenins), and the lack of complete amino acid sequences of secalins and of detailed quantitative data make a comparison difficult.

The solvents applied to wheat flour have mostly been used for the extraction of rye storage proteins, 60 or 70% aqueous ethanol for prolamins (Charbonnier et al 1981; Kasarda et al 1983; Kubiczek et al 1993) and 50 or 55% 1-propanol or 2-propanol plus reducing agent for glutelins (Shewry et al 1983a). SDS-PAGE of different alcohol extracts from rye flour showed four groups of storage proteins: HMW secalins, γ -75k secalins, ω secalins, and γ -40k secalins (Shewry et al 1983a). HMW and γ -75k secalins were present only in an aggregated state stabilized by disulfide bonds (Shewry et al 1987). Studies of amino acid composition, partial amino acid sequences, and molecular weights indicate that HMW secalins are homologous with HMW subunits of wheat glutenin (Field et al 1982; Shewry et al 1988; Kipp et al 1996; Kipp and Wieser 1999; Köhler and Wieser 2000), and ω -secalins are homologous with the ω 1,2-type of wheat gliadins (Kasarda et al 1983). SDS-PAGE showed two types of γ -secalins with molecular weights of 40,000 (γ -40k) and 75,000 (γ -75k), sedimentation equilibrium ultracentrifugation showed 33,000 and 54,000, respectively (Shewry et al 1982). Amino acid analysis has also indicated some differences, notably more Glx and Pro in γ -75k secalins (Shewry et al 1982; Tatham and Shewry 1991). N-terminal sequences are identical at 17 of the first 20 positions (Shewry et al 1982; Rocher et al 1996). Altogether, the results demonstrated that γ -40k secalins are homologous with γ -gliadins of wheat, and the structural differences of γ -75k secalins are due to the presence of additional repetitive sequences rich in Gln and Pro (Shewry et al

1987). Three types of wheat storage proteins, ω 5 gliadins, α gliadins, and LMW subunits of glutenin, are missing in rye.

In contrast to wheat, little information is available about the quantity of the different storage protein types in rye flour. Quantification by scanning of electrophoresis gels showed that both γ -secalin types accounted for $>90\%$ of the total extractable storage proteins (Shewry et al 1982). The contents of HMW secalins in two rye flours is 0.33 and 0.34% of flour and 4.5 and 4.7% of total flour protein, respectively (Kipp et al 1996). The aim of the present work was to isolate, characterize, and quantify all secalin types present in two different rye flours (cultivars Danko and Halo) in comparison with a wheat flour (cultivar Rektor). The work includes a modified Osborne fractionation of flour proteins and the separation and quantification of the different protein types by means of reversed-phase and gel-permeation HPLC. SDS-PAGE and the determination of amino acid composition, N-terminal amino acid sequences, and molecular mass were used for the identification and characterization of secalins.

MATERIAL AND METHODS

Flours

The German rye cultivars Danko, harvested in 1997, and Halo, harvested in 1993, were grown in southern Germany. The kernels were frozen and stored at -18°C . For this study, portions of the kernels were defrosted and stored at 4°C for two weeks. Then, they were milled to flours using a laboratory mill (Quadrumat Jr, Brabender, Duisburg, Germany) and passed through a 0.2-mm sieve (Retsch, Haan, Germany). Flour from the German wheat cultivar Rektor harvested in 1991 and milled under the same conditions as the rye kernels was derived from a previous study (Antes and Wieser 2001). The water content of the flours was determined as weight loss after heating for 2 hr at 130°C , the ash content was determined according to ICC standard method 104/1, and the crude protein contents ($\text{N} \times 5.7$) according to Dumas (ICC standard method 167) using an FP-328 combustion instrument (Leco, Kirchheim, Germany). The content of free reduced glutathione and total glutathione (free reduced and oxidized glutathione plus protein-bound glutathione) was determined by an isotope dilution assay according to the method developed by Sarwin et al (1992). Danko flour had ranges of free reduced glutathione (59 nmol/g) and total glutathione (130 nmol/g) similar to those of wheat flours (Sarwin et al 1992).

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Extraction of Flour Proteins

Solvents. S1, 0.4 mol/L of NaCl + 0.067 mol/L of HKNaPO₄, pH 7.6 (albumins-globulins); S2, 60% (v/v) ethanol (prolamins); S3, 50% (v/v) 2-propanol + 0.08 mol/L of Tris-HCl, pH 8.0 + 1% (w/v) dithioerythritol (DTE) under nitrogen (glutelins).

Extraction. For an analytical approach, nondefatted flour (100 mg) was weighed into a centrifuge tube (10 mL) and extracted stepwise three times with 1 mL of solvent S1 at room temperature (RT ≈ 20°C), three times with 1 mL of solvent S2 at RT, and twice with 1.0 mL of solvent S3 at 60°C under nitrogen (Wieser et al 1998). Each extraction step was initiated with vortexing for 2 min at RT and continued with magnetic stirring for 13 min (S1, S2) and 30 min (S3), respectively. The suspensions were then centrifuged for 10 min at 7,000 × g and RT using a Z320 centrifuge (Hermle, Wehingen, Germany). The corresponding supernatants were combined and diluted to 5 mL (S1, S2) and 2 mL (S3), respectively. A portion of the prolamin extract (S2) was reduced with DTE (1%, w/v, 15 min, 60°C). The extracts were filtered through a 0.45-μm membrane before HPLC analysis. In preliminary extraction studies, nondefatted flour was extracted as described above, except that three extraction steps were performed for each solvent, and the corresponding supernatants were not combined but investigated separately. The supernatant of each extraction step was diluted to 1 mL, filtered through a 0.45-μm membrane and used for HPLC analysis. In a further study, the extraction procedure described above was applied to flour defatted with light petroleum (boiling range 40–60°C).

RP-HPLC

RP-HPLC of the filtered extracts was performed using an instrument with a solvent module 126, a System Gold software (Beckman, Munich, Germany) and a Nucleosil 300-5-C₁₈ column material (Macherey-Nagel, Düren, Germany) packed into a 4.6 × 240-mm

column by an HPLC packing instrument (Shandon, Pittsburg, PA) under a pressure of 450 bar (Wieser et al 1998). Column temperature was 50°C. Elution solvents A and B were 0.1% trifluoroacetic acid (TFA) and acetonitrile containing 0.1% TFA, respectively. The gradient applied for all extracts was 20% B at the beginning of the run rising up to 60% B after 30 min. The flow rate was 1.0 mL/min, and the sample loop was 1.5 mL. The volume of the analytical runs was 250 μL for albumins-globulins, 200 μL for rye prolamins, 100 μL for prolamins of Rektor, 300 μL for rye glutelins, and 50 μL for Rektor glutelins. In alcoholic extracts, TFA (500 μL) was injected before and after sample injection. The injection volumes for the preparative runs were 300–800 μL. Proteins were detected at 210 nm and quantified using the corresponding absorbance areas at 210 nm (Wieser et al 1998). For the integration of absorbance areas a blank run (injection of the sample solvent) was used as baseline. The eluates of the preparative runs corresponding to the peaks of the chromatograms were collected, concentrated under a stream of nitrogen, and freeze-dried. These subfractions were investigated by means of SDS-PAGE, N-terminal sequence analysis, and amino acid analysis to identify the secalin types within the chromatograms.

GP-HPLC

The unreduced prolamin extracts from the analytical extraction procedure were separated on a Superdex 200 HR column (Pharmacia Biotech, Freiburg, Germany), separation range $M_r = 10,000$ – $600,000$ at RT using 0.05 mol/L of sodium phosphate buffer (pH 6.9) + 0.5% (w/v) SDS as elution solvent. Injection volumes were 20 μL for the analytical runs and 100 μL for the preparative runs, respectively. The flow rate was 0.4 mL/min and the detection wavelength was 210 nm. The eluates of the preparative runs were collected in 1-mL intervals and analyzed by RP-HPLC before and after reduction of disulfide bonds.

TABLE I
Water-, Protein (N × 5.7), and Ash Contents (%) of Analyzed Flours^a

	Danko		Halo		Rektor	
	<i>n</i>	$\bar{x} \pm SD$	<i>n</i>	$\bar{x} \pm SD$	<i>n</i>	$\bar{x} \pm SD$
Water	2	14.1 ± 0.1	2	14.3 ± 0.1	2	11.4 ± 0.4
Protein	5	6.3 ± 0.1	5	5.1 ± 0.1	5	13.6 ± 0.1
Ash	4	0.38 ± 0.01	2	0.40 ± 0.00	2	0.57 ± 0.0

^a *n* = number of determinations; \bar{x} = mean value, SD = standard deviation.

TABLE II
Amino Acid Compositions of Flour, Osborne Fractions, and Single Secalins of Danko^a

Flour	Albumins/ Globulins	Prolamins	Glutelins	γ-Secalins ^b						
				Pr/e	Pr/f	Pr/g	Pr/h	Gl/e	Gl/f	
Asx	5.4	8.1	2.0	3.5	1.4	1.7	3.0	2.7	2.4	2.4
Thr	3.0	5.0	2.1	3.8	1.6	1.4	2.5	2.5	2.6	2.3
Ser	4.8	6.3	5.2	6.5	5.7	6.0	5.0	5.0	5.6	6.6
Glx	29.5	15.6	38.8	32.7	40.4	40.2	36.5	36.3	36.3	38.2
Pro	13.5	6.9	20.4	14.2	23.2	22.8	19.1	18.4	21.2	18.0
Gly	5.6	8.7	3.6	7.9	2.2	2.5	2.5	2.4	4.3	4.2
Ala	5.1	8.4	2.7	4.7	2.4	2.4	2.6	2.6	3.1	2.8
Cys ^c	3.2	5.1	2.6	2.3	2.9	2.8	3.4	3.4	2.9	2.6
Val	5.1	6.7	3.9	4.4	4.4	4.4	4.1	4.3	4.4	4.6
Met ^c	2.3	1.9	1.0	0.9	0.8	0.7	1.3	1.3	1.3	1.0
Ile	3.6	4.0	3.2	2.9	2.3	2.2	4.4	4.5	2.3	2.6
Leu	6.3	6.9	5.3	5.1	4.3	4.7	6.7	7.0	4.3	5.1
Tyr	1.9	2.6	1.4	2.2	1.3	1.2	0.8	0.7	1.7	1.4
Phe	3.8	3.0	4.5	3.3	4.5	4.2	4.5	4.8	3.8	4.1
His	1.4	1.9	1.0	1.6	1.2	1.1	1.3	1.5	1.4	1.5
Lys	2.5	3.8	0.8	1.9	0.3	0.6	0.6	0.7	1.1	1.2
Arg	3.0	5.1	1.5	2.1	1.1	1.1	1.7	1.9	1.3	1.4

^a Mol% (mean value of two determinations). Trp was not determined.

^b See Fig. 2A (Pr) and 2B (Gl).

^c Determined as cysteinic acid and methionine sulfon, respectively

Analytical Methods

Amino acid analysis was performed after hydrolysis of proteins with 6 mol/L of HCl + 0.01 mol/L of phenol (24 hr, 110°C, in vacuo) using an amino acid analyzer (LC 3000, Eppendorf-Biotronic, Hamburg, Germany). Cysteine and cystine were determined as cysteic acid and methionine as methionine sulfon after oxidation of proteins with performic acid and hydrolysis with HCl (Henschen 1986). SDS-PAGE was performed with a NuPAGE electrophoresis system (Novex) on 10% Bis-Tris gels (acrylamide and bisacrylamide; 8×8 cm, 10 wells) using Coomassie Brilliant Blue R 250 stain (Kasarda et al 1998). N-terminal sequences were determined using a protein sequencing system (Procise 492, Applied Biosystems, Weiterstadt, Germany). All proteins applied to N-terminal sequence analysis were reduced with DTE and alkylated with 4-vinylpyridine before analysis according to Friedman et al (1970). Cysteine residues were determined as phenylthiohydantoin derivative of 4-pyridylethylcysteine after Edman degradation. Mass spectrometry was performed on a matrix-assisted laser desorption/ionization and time of flight (MALDI-TOF) mass spectrometry (Biflex 3 Bruker, Bremen, Germany) with α -cyano-cinnamic acid as a matrix.

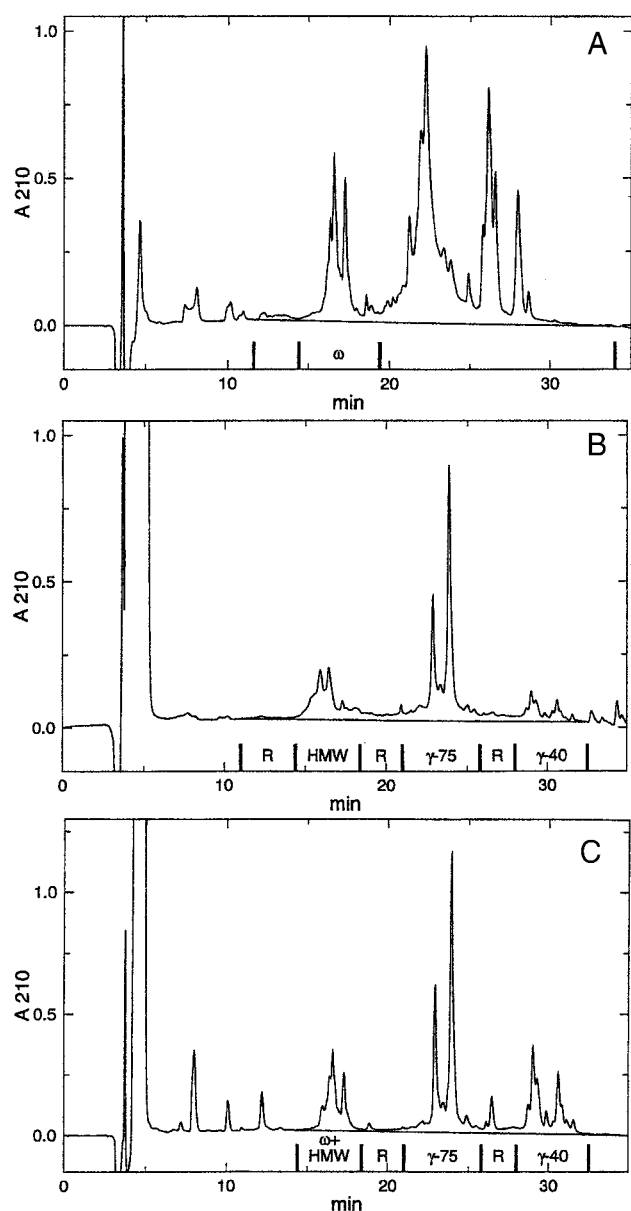


Fig. 1. Analytical RP-HPLC of storage proteins from Halo rye flour on C_{18} silica gel. **A**, Unreduced prolamin fraction; **B**, reduced glutelin fraction; **C**, reduced prolamin fraction.

RESULTS AND DISCUSSION

Flour Samples

Kernels of the rye cultivars Danko and Halo and of the winter wheat Rektor were used for the present study. Because storage proteins of rye should be compared with those of wheat, the kernels were milled into white flour under conditions usually applied to wheat. Thus, outer pericarp and aleurone layer were removed and corresponding rye flours predominately consisted of the starchy endosperm comparable with white flour of wheat. The yield of the milling was 46.5% (Danko) and 48.2% (Halo), respectively. Water, protein, and ash contents of the flours are shown in Table I. Due to the milling conditions, ash contents (0.38 and 0.40%) and protein contents (6.3 and 5.1%) of the rye flours were much lower than those of flours commercially available.

Characterization of Protein Fractions

For the present study, a modified Osborne fractionation previously developed for wheat flour (Wieser et al 1998) was used on a microscale. Accordingly, flour proteins were separated stepwise into albumins and globulins (soluble in a diluted NaCl solution), prolamins (soluble in 60% ethanol without reduction of disulfide bonds), and glutelins (soluble in 50% 2-propanol under reducing conditions and increased temperature). Preliminary experiments with Danko flour and RP-HPLC analysis did not reveal significant differences between nondefatted and defatted flour, therefore, the defatting of flour before extraction was not necessary. Furthermore, analysis of subsequent extraction steps showed that 71–89% of each fraction protein was extracted by the first step. To ensure extraction without serious overlapping, three steps were necessary for albumins-globulins and prolamins, and two steps for glutelins. SDS-PAGE of the final extraction residue indicated that secalins had been extracted completely.

The amino acid composition of flour, albumins-globulins, prolamins, and glutelins (Table II) were in good agreement with data from literature (Preston et al 1975; Wieser et al 1980). The values

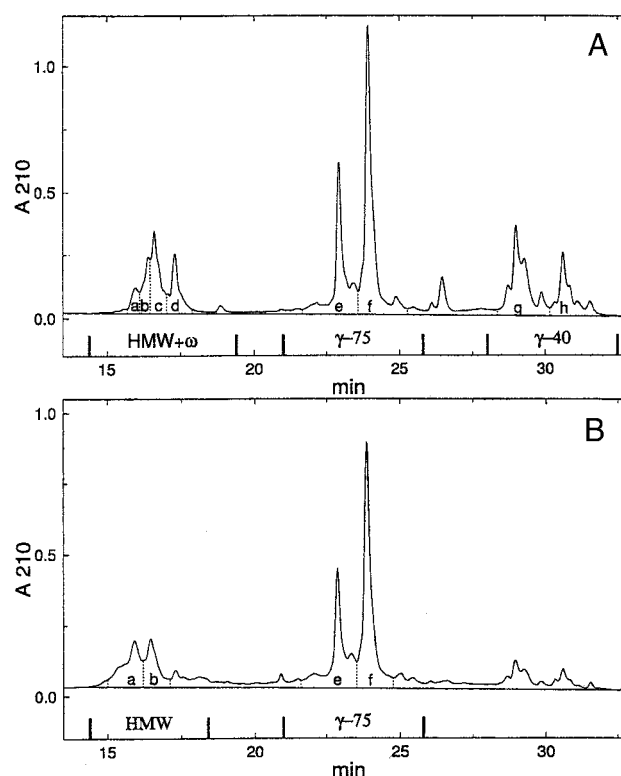


Fig. 2. Preparative RP-HPLC of storage protein fractions from Danko rye flour on C_{18} silica gel. **A**, Reduced prolamin fraction; **B**, reduced glutelin fraction.

of Glx, Pro, and Gly made the main differences between the prolamin and glutelin fractions.

Each of the three fractions was characterized by analytical RP-HPLC. The elution profiles of albumins-globulins from both rye cultivars were nearly identical to those of Rektor albumins-globulins (chromatograms not shown). The chromatograms of the prolamin and glutelin fractions from Halo flour (analytical runs) and those from Danko flour (preparative runs) are presented in Figs. 1 and 2, respectively. The chromatograms of Rektor were in agreement with those obtained in previous studies (Wieser et al 1998). The rye cultivars did not significantly differ in the elution profiles of prolamins and glutelin subunits, but they varied strongly from those of Rektor. For the quantification of the protein content of the different extracts, absorbance areas of the chromatograms measured at 210 nm were used; they highly correlated with the amount of protein, independent of protein type (Wieser et al 1998). The results for albumins-globulins, prolamins, and glutelin subunits of Danko, Halo, and Rektor (AU/mg of flour) are presented in Table III. The values for the albumin-globulin fractions of the three flours are in a relatively small range (AU 154–194) confirming previous results from different wheat cultivars showing that the amount of this protein group is scarcely dependent on the total protein content of flours (Wieser and Seilmeier 1998). In contrast, the amounts of prolamins were highly correlated ($r = 1,00$, $n = 9$) with the flour protein content and were, by far, highest for Rektor (870 AU), followed by Danko (469 AU), and Halo (351 AU). Both rye flours contained notable small amounts of glutelins (57 and 56 AU, respectively), when compared with Rektor glutelins (468 AU). The sum of extracted proteins also highly correlated ($r = 1,00$, $n = 9$) with flour protein content.

The differences between rye and wheat cultivars is very well reflected by the percental distribution of total extractable proteins on the three fractions (Table III). The proportions of Danko and Halo albumins-globulins (26 and 27%) were more than twice those of Rektor (13%). The prolamin fractions showed comparable high proportions (57–66%), whereas Danko and Halo glutelins were extremely low (8 and 10%) in comparison with Rektor (30%). Consequently, the ratio of prolamins to glutelins was much higher for Danko (8.2:1) and Halo (6.3:1) than for Rektor (1.9:1) and much higher than the ratio determined by Shewry et al (1983a) for the rye cultivar Rheidol by the Kjeldahl method. The quantity of glutelins, particularly of macropolymers, is an important factor for the dough and gluten properties of wheat (Weegels et al 1996; Southan and Mac Ritchie 1999). The data presented in Table III indicate that the storage proteins of rye are not able to develop

comparable amounts of polymers that are insoluble in aqueous alcohols.

The prolamin and glutelin fractions of Danko and Halo were further studied by SDS-PAGE under reducing conditions. The electrophoregrams shown in Fig. 3 are similar for both rye cultivars. In agreement with Shewry et al (1983a), the prolamin fractions contained all four secalin types (HMW, γ -75k, ω , and γ -40k secalins), whereas only HMW and γ -75k secalins appeared in the glutelin fractions. Based on the mobility of markers, the apparent molecular weights derived were 40,000 for γ -40k secalins, 50,000 for ω secalins, 70,000 for γ -75k secalins, and 100,000 for HMW secalins. Band intensities indicate a remarkable dominance of the two γ -secalin types in both prolamin and glutelin fractions.

Isolation and Characterization of Secalin Types

Preparative RP-HPLC of the reduced prolamin and glutelin fractions identified the protein type within the elution pattern (Fig. 2). Isolated proteins were alkylated with 4-vinylpyridine and analyzed by means of N-terminal sequence analysis. The results (Table IV) demonstrate that corresponding proteins from Danko and Halo had the same N-terminal sequences and that each protein fraction could be clearly assigned to the four secalin types. Within the reduced prolamin fraction (Fig. 2A), HMW secalins were eluted first

TABLE III
HPLC Absorbance Areas (AU/mg of flour) of Osborne Fractions of Danko, Halo, and Rektor

	AU \pm SD ^a	% ^b
Danko		
Albumins/globulins	186 \pm 15	26
Prolamins	469 \pm 6	66
Glutenins	57 \pm 3	8
Summation	712	100
Halo		
Albumins/globulins	154 \pm 11	27
Prolamins	351 \pm 2	63
Glutenins	56 \pm 5	10
Summation	561	100
Rektor		
Albumins/globulins	194 \pm 14	13
Prolamins	870 \pm 13	57
Glutenins	468 \pm 34	30
Summation	1,532	100

^a Mean value of three or four determinations in arbitrary units \pm standard deviation.

^b Based on total extractable protein.

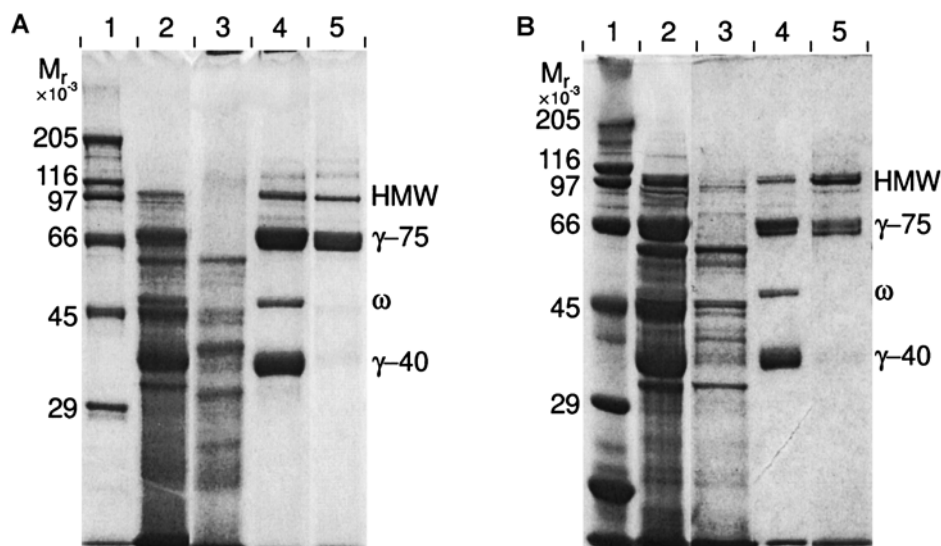


Fig. 3. SDS-PAGE of flours and Osborne fractions of rye cultivars Danko (A) and Halo (B). Lanes 1–5: marker proteins, flour proteins, albumins-globulins, prolamins, glutelins.

(elution region a and b), followed by ω secalins (c,d), γ -75-k secalins (e,f), and γ -40k secalins (g,h). The 25 positions of the N-terminal sequences determined for HMW secalin Pr/a were identical with corresponding sequences of a HMW secalin fraction from cultivar Gazelle (Shewry et al 1988) and closely related to the γ -type of wheat HMW subunits (Halford et al 1987). HMW secalin Pr/b was different in positions 17 (Arg) and 25 (Ile). The N-terminal sequences of ω secalins Pr/c and Pr/d were in agreement with those from rye cultivars Frontier (Kasarda et al 1983) and Petkus (Rocher et al 1996), and from wheat-rye hybrid Herzog (Seilmeier et al 2001). Beginning with position 2, the ω secalins were homologous with the basic variant ARQLNP of ω 1,2-type gliadins (Shewry et al 1984).

The γ -75k secalins (Pr/e and Pr/f) and γ -40k secalins (Pr/g and Pr/h) differed in the positions 5 (Asn or Gly), 11 (Gln or Glu), and 12 (Cys or Trp). Compared with γ -gliadins of wheat (Shewry et al 1982), typical differences in positions 5 (γ -75k, γ -40k), 11 (γ -40k), and 12 (γ -75k) could be detected (Table IV). The N-terminal sequences of γ -75k secalins were in agreement with those described in the literature (Shewry et al 1982; Rocher et al 1996) with one important exception: both γ -75k secalins Pr/e and Pr/f had a Cys residue at position 12 and not a Trp residue as described for the 75k-secalin fractions isolated from rye cultivars Rheidol and Petkus. The Cys residue in position 12 was unique to γ -75k secalins and could explain the aggregative nature of this protein type due to the formation of an intermolecular disulfide bond to another protein chain similar to LMW subunits of wheat glutenin (Müller and Wieser 1997). Within the glutelin fraction (Fig. 2B), HMW secalins were eluted first (elution region a,b), followed by γ -75k secalins (e,f). The N-terminal sequences were the same as those of the corresponding prolamins components (Table IV). SDS-PAGE of isolated secalins confirmed the assignment to the four secalin types (electrophoregrams not shown).

Protein subfractions obtained from the prolamins and glutelin fractions and identified as γ secalins were further characterized by amino acid analysis and, in part, by mass spectrometry. In agreement with studies of Shewry et al (1982), amino acid composition of γ -40k secalin subfractions Pr/g and Pr/h (Table II) were similar to those of γ -gliadins, whereas the composition of γ -75k secalin subfractions Pr/e and Pr/f had typically increased values for Glx (\approx 40 mol%) and Pro (\approx 23 mol%). The differences in the composition of γ -75k secalins from those of the prolamins and glutelin fractions could be due to larger portions of impurities in the subfractions Gl/e and Gl/f. MALDI-TOF mass spectrometry of γ -40k and γ -75k secalins from the prolamins fractions revealed significantly lower molecular masses compared with SDS-PAGE mobility, \approx 50,000–52,000 for γ -75k secalins and 32,000 for γ -40k secalins. The overestimation of the molecular weights by SDS-PAGE is consistent with previous studies of wheat and barley storage pro-

teins and is due to a stretched conformation of the protein regions with a higher Pro content (Shewry et al 1982; Seilmeier et al 2001). The values obtained by mass spectrometry agreed with those determined by sedimentation equilibration ultracentrifugation (Shewry et al 1982).

Corresponding γ -75k secalins isolated from the prolamins and glutelin fractions (Pr/e and Gl/e, Pr/f and Gl/f) agreed with regard to the mobility on SDS-PAGE, retention time and elution profile of RP-HPLC, N-terminal sequences, and aggregative nature. As mentioned, the small differences in the amino acid composition could be due to different amounts of impurities. Most probably, they have the same primary structures; the major portion is integrated in alcohol-soluble aggregates with relatively low molecular weights (prolamins) and the minor portion is integrated in insoluble aggregates with higher molecular weights (glutelins).

Quantitative Distribution of Secalin Types

HPLC absorbance areas beneath the corresponding peaks (Fig. 1) were used to determine the proportion of secalin types in the prolamins and glutelin fractions of Danko and Halo (Table V). As already indicated by SDS-PAGE, γ -75k secalins were dominating in the glutelin fractions with proportions of 53% (Danko) and 52% (Halo), respectively. The proportion of HMW secalins of Halo (30%) was significantly higher than that of Danko (22%). Traces of γ -40k secalins amounted to 13% (Danko) and 10% (Halo). The residual absorbance areas between the major protein groups (R) with nonidentified components (Fig. 1B) was of 12 and 8%, respectively.

The elution profiles of the unreduced prolamins fractions (Fig. 1A) and the comparison with the reduced prolamins fractions (Fig. 1C) indicated that a considerable amount of oligomers, in addition to monomers, was present that were mainly coeluted in the region of γ -75k secalins and possibly of γ -40k secalins. Under reducing conditions, SDS-PAGE indicated that the oligomers consisted of γ -75k secalins and HMW secalins. Therefore, only the monomeric ω secalins could be quantified; they had proportions of 18% (Danko) and 19% (Halo), respectively (Table V). After reduction of disulfide bonds and corresponding cleavage of oligomers into monomers (Fig. 1C), all four secalin types could be quantified. The resulting proportions were 46% (Danko) and 45% (Halo) for γ -75k secalins, 27% (Danko), and 26% (Halo) for γ -40k secalins; 5% were unidentified proteins (R). The absorbance areas in the region of ω secalins were increased by 4% (Danko) and 5% (Halo) when compared with the unreduced fractions (Fig. 1A). These proportions correlated with the HMW secalins released from oligomers by the reduction of disulfide bonds.

The proportion of prolamins and glutelins (Table II) and of single protein types (Table V) were combined to calculate the propor-

TABLE IV
N-Terminal Sequences of Secalins of Danko and Halo

Peak ^a	Type	Sequence ^b
Pr/a	HMW secalin	EGEASGQLQCERELQESSLEACRQV...
Pr/b	HMW secalin	EGEASGQLQCERELQERXLEACRQ...
Pr/c	ω secalin	RQLNPSEQELQSPQQPVP...
Pr/d	ω secalin	RQLNPSEQELQS...
Pr/e	γ -75k secalin	NMQVNPSGQVQCPQQQFPQPQQSS...
Pr/f	γ -75k secalin	NMQVNPSGQVQCPQQQFPQPQQSS...
Pr/g	γ -40k secalin	NMQVGPSTGQVEXPQQQLPQXQQXV...
Pr/h	γ -40k secalin	NMQVGPSTGQVQEWVQQQLPQXQQXV...
Gl/a	HMW secalin	EGEASGQLQCERELQESSLEACRQV...
Gl/b	HMW secalin	EGEASGQLQCERELQERXLEACRQI
Gl/e	γ -75k secalin	NMQVNPSGQVQCPQQQFPQPQQSS...
Gl/f	γ -75k secalin	NMQVNPSGQVQCPQQQFPQPQQSS...

^a See Fig. 2A (Pr) and 2B (Gl).

^b One letter code for amino acids, x = not identified.

tions of storage proteins in the flours. The values in Table VI demonstrate a good agreement of Danko and Halo. Almost half of the total storage proteins were γ -75k secalins (47 and 46%, respectively). Although this type forms aggregates like LMW subunits of wheat (Shewry et al 1987), the major portion (38–41% of total storage proteins) was present in the prolamins and only 6–7% in the glutelin fraction. The second major type were γ -40k secalins (25 and 24%, respectively), which appeared mainly in the prolamins due to their monomeric state. Similarly, ω secalins appeared exclusively in the prolamins and accounted for \approx 16% of total storage proteins. HMW secalins amounted to 6.1 and 8.5%, respectively; in contrast to wheat HMW subunits, the larger portion of HMW secalins occurred in the prolamins fractions, which was demonstrated by the reduction of disulfide bonds (Fig. 1C). Similar to γ -75k secalins, they obviously form aggregates of low molecular weights that are soluble in 60% ethanol. In both flours about 6% of total extractable storage proteins was not identified.

Comparing the two rye flours with an international assortment of bread wheat (Wieser and Kieffer 2001), the proportions of the homologous γ -40k secalins and γ -gliadins (\approx 27%) were in a similar range. On the other hand, the proportions of ω secalins were much higher, and those of HMW secalins were lower than the proportions of ω -gliadins (\approx 9%) and HMW subunits (\approx 10%), respectively, in wheat flour. With respect to the second aggregative protein type, γ -75k secalins had proportions more than twice compared with LMW subunits of glutenin (\approx 21%). α -Gliadins absent in rye flour

TABLE V
Distribution (%) of Secalin Types in Prolamin and Glutelin Fractions^a

Type	Prolamin		Glutelin	
	Danko	Halo	Danko	Halo
HMW	4.1 ± 0.3	5.1 ± 1.6	22.5 ± 1.1	29.9 ± 0.9
γ -75k	46.0 ± 2.1	44.6 ± 1.3	52.7 ± 1.2	52.2 ± 2.0
ω	18.3 ± 0.2	19.2 ± 1.2	0	0
γ -40k	26.4 ± 0.8	25.8 ± 0.2	13.1 ± 0.7	9.8 ± 0.0
Residual protein ^b	5.2 ± 0.8	5.3 ± 0.7	11.7 ± 0.6	8.1 ± 1.1

^a Mean value of two determinations ± standard deviation.

^b Absorbance area outside secalin types (R in Fig. 1B and 1C).

TABLE VI
Distribution (%) of Secalin Types in Danko and Halo Flours

Type ^a	Danko	Halo
HMW _{pr}	3.7	4.4
HMW _{gl}	2.4	4.1
HMW _{Σ}	6.1	8.5
γ -75k _{pr}	41.0	38.4
γ -75k _{gl}	5.7	7.2
γ -75k _{Σ}	46.7	45.6
ω _{pr}	16.3	16.6
ω _{gl}	0.0	0.0
ω _{Σ}	16.3	16.6
γ -40k _{pr}	23.6	22.3
γ -40k _{gl}	1.4	1.3
γ -40k _{Σ}	25.0	23.6
R _{pr}	4.6	4.6
R _{gl}	1.3	1.1
R _{Σ}	5.9	5.7

^a Fractions: pr, prolamins, gl, glutelin fraction, R, not identified.

TABLE VII
Proportions (% ± SD) of Subfractions Obtained by GP-HPLC of Unreduced Prolamin Fractions of Danko, Halo, and Rektor^a

Fraction ^b	Danko	Halo	Rektor
A	40.9 ± 0.6	39.9 ± 1.1	22.1 ± 0.1
B	9.5 ± 0.5	10.1 ± 0.4	16.8 ± 0.1
C	49.6 ± 1.0	50.0 ± 0.8	61.1 ± 0.0

^a Mean value of two or three determinations ± standard deviation.

^b See Fig. 4.

were the dominating protein type in wheat flour (\approx 32%). Altogether, rye flours presented a higher percentage of aggregative proteins (53–54%) than wheat flours (\approx 31%). Nevertheless, only small amounts of polymeric proteins (glutelins) were present in rye flour. Consequently, the aggregative behavior of rye storage proteins must be quite different from the behavior of wheat storage proteins.

Gel Chromatography

The RP-HPLC patterns of the prolamins fraction of the rye flours changed significantly after the reduction of disulfide bonds (Fig. 1A and C). This indicated the presence of oligomeric proteins equivalent to the so-called HMW gliadins (or aggregated gliadins or ethanol-soluble glutenins) of wheat (Shewry et al 1983b). For quantification, oligomers and monomers of the prolamins fractions were separated by GP-HPLC on Superdex 200 (separation range $M_r = 10,000$ – $600,000$). The elution range of oligomers and the type of secalins were identified by collecting the eluates in 1-min intervals and by subsequent RP-HPLC before and after reduction of disulfide bonds. The chromatograms of GP-HPLC for the Danko and Halo prolamins fractions were almost identical (Fig. 4A and B). RP-HPLC of the eluates revealed that oligomers formed by HMW secalins and γ -75k secalins were eluted first (subfraction A), followed by oligomers, possibly dimers, consisting of γ -75k

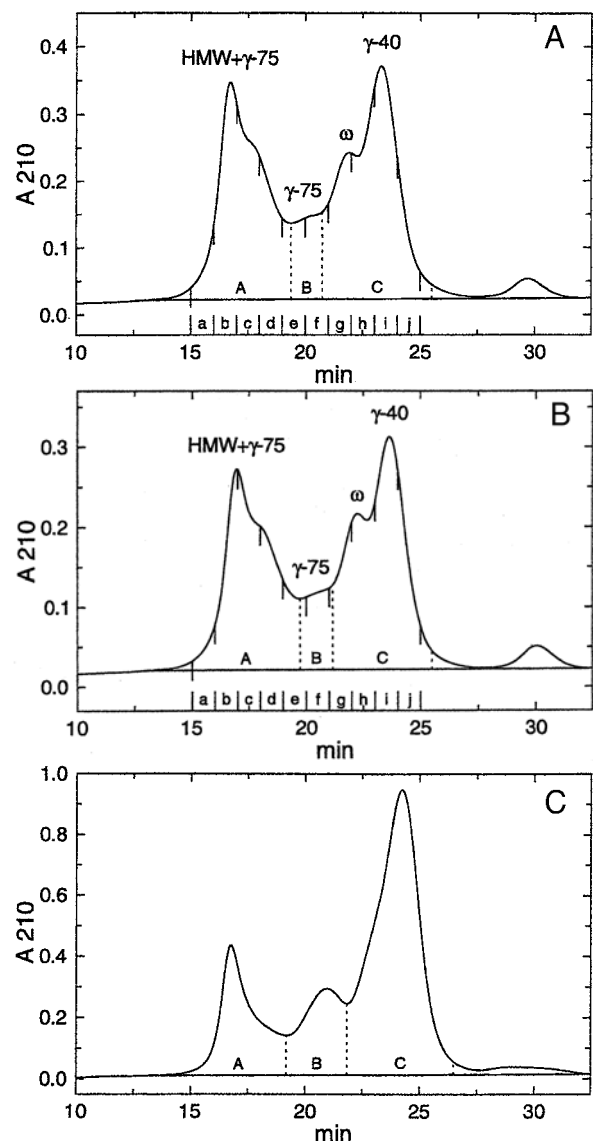


Fig. 4. Gel-permeation HPLC of unreduced prolamins fractions of rye cultivars Danko (A), Halo (B) and wheat cultivar Rektor (C).

secalins (subfraction B). After an elution time of 21 min, ω - and γ -40k secalins were eluted as monomers (subfraction C).

With reference to previous GP-HPLC of prolamins from different wheat cultivars (Wieser et al 1987), subfraction A of Reaktor prolamins (Fig. 4C) could be assigned to oligomers, subfraction B to monomeric ω -gliadins, and subfraction C to monomeric α - and γ -gliadins. Due to the presence of ω 5 gliadins with molecular masses of \approx 50,000 (Seilmeier et al 2001), wheat monomers covered a somewhat higher range of molecular weights than rye monomers. The proportions of the subfractions A, B and C are presented in Table VII. It is clearly demonstrated that the two rye prolamins fractions contained much more oligomers (A + B = 50%) than the Reaktor prolamins fraction (A = 22%). RP-HPLC analysis after reduction of disulfide bonds showed that the oligomeric subfractions A and B contained HMW secalins and γ -75k secalins. The ω and γ -40k secalins were present exclusively in monomeric subfraction C. The literature (Shewry et al 1983b) reports that the oligomeric subfraction A (HMW gliadins) of wheat prolamins is completely different from the corresponding subfraction in rye. It contains LMW subunits as the major protein type but also variants of α - and γ -type gliadins, possibly with an odd number of Cys residues. In contrast to rye, HMW subunits are absent in subfraction A. These results again indicate that rye and wheat are significantly different in the ability of aggregative storage proteins to polymerize. This is due to structural differences between γ -75k secalins and LMW subunits of glutenin and probably between HMW secalins and HMW subunits of glutenin.

CONCLUSIONS

Rye flour contains four different types of storage proteins. Three of them, γ -40k, ω , and HMW secalins, are homologous with the corresponding protein types of wheat. The fourth type, γ -75k secalins, is unique to rye, and contributes nearly half of the storage proteins. The γ -75k and HMW secalins are in an aggregated state and the γ -40k and ω secalins are in a monomeric state. Despite partial homology, storage proteins of rye differ significantly from those of wheat with respect to structural and quantitative parameters that are important for the formation and properties of wheat gluten. Typical for rye are the low content of storage proteins and the high ratio of alcohol-soluble proteins to insoluble proteins. In spite of the high proportion of aggregative proteins and in contrast to the gluten proteins of wheat, secalins are not able to create comparable amounts of polymeric proteins due to structural characteristics of γ -75k and HMW secalins. Because of the low amounts of polymerized proteins, it is reasonable that a gluten-like protein network with viscoelastic properties is not formed when rye flour is mixed with water.

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