Chromatography of Glutenin on Sepharose CL-4B in Dissociating Solvents: Molecular Weight Composition of Covalently Bonded Glutenin[1]

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Recently we reported that removal of noncovalently bonded protein from glutenin resulted in decreased viscoelasticity. In that study, two glutenin fractions were isolated by successive gel filtration of glutenin on Sephadex G-200 in the solvents acetic acid-guanidine hydrochloride (GuHCl)-cetyltrimethylammonium bromide (AGC) and sodium dodecyl sulfate (SDS). This note reports the $S_{20,w}$ values of these glutenin fractions, and the results of chromatography of glutenin on Sepharose CL-4B in the solvents AGC and SDS. Because of its higher fractionation range over Sephadex G-200, Sepharose CL-4B was used to obtain the information on the molecular weight composition of the covalently bonded glutenin.

MATERIALS AND METHODS

Preparation of AcOH-Glutenin

The source of the wheat variety K65 (strong) used in this study and the preparation of chromatographically pure glutenin (AcOH-glutenin) by gel filtration of crude glutenin (0.1*M* AcOH extract of gluten) on Sephadex G-200 using 0.1*M* AcOH as eluent was described earlier (Prasada Rao and Nigam 1987).

Preparation of AGC-glutenin and SDS-glutenin

AGC-glutenin is the void volume peak isolated by chromatographing AcOH-glutenin on Sephadex G-200, using the solvent mixture 0.1*M* acetic acid, 2.25*M* GuHCl, and 0.01*M* cetyltrimethylammonium bromide (AGC), pH 3.2, as eluent, SDS-glutenin is the void volume peak isolated by rechromatographing AGC-glutenin using the solvent 1% SDS in 0.1*M* acetic acid (pH 3.2). Details are given in an earlier paper (Prasada Rao and Nigam 1987). The fractions were monitored by measuring absorbance at 280 nm.

Determination of $S_{20,w}$ Values

Sedimentation velocity experiments were carried out at 25°C using 1% protein solutions. AcOH-glutenin was dissolved in 0.1*M* AcOH, AGC-glutenin was dissolved in solvent AGC, and SDS-glutenin was dissolved in solvent SDS. As different solvents have different dissociating effects on glutenin, and the aim was to determine $S_{20,w}$ values of the three glutenins (AcOH-, AGC-, and SDS-glutenin), they were determined in the solvents used for their respective isolations. Centrifugation was done at 59,720 rpm. From the photographs taken at different intervals $S_{20,w}$ values were calculated (Schachman 1959). Temperature, density, and viscosity corrections relative to water at 20°C were made.

Chromatography of AcOH-Glutenin on Sepharose CL-4B

Column chromatography was done on Sepharose CL-4B (1.6× 100 cm, volume 200 ml). First, AcOH-glutenin was chromatographed using solvent AGC as the eluent. This chromatography gave two peaks. Each peak was pooled separately, dialyzed against 0.1*M* acetic acid, and lyophilized. The lyophilized proteins were separately chromatographed using solvent SDS as eluent. The void volumes of Sepharose CL-4B column in solvents AGC and SDS were determined with blue dextran. Thyroglobulin (mol wt 660,000), ferritin (mol wt 440,000) and bovine serum albumin (mol wt 66,000) were used as molecular weight markers. Their elution volumes were determined in both AGC and SDS separately and are shown in Figure 1.

RESULTS AND DISCUSSION

$S_{20,w}$ Values of AcOH-, AGC-, and SDS-Glutenin

As AGC-glutenin is AcOH-glutenin minus 16% protein dissociated from it by solvent AGC, and SDS-glutenin is AGC-glutenin minus 11% protein dissociated from it by solvent SDS, the $S_{20,w}$ values of the glutenins can be expected to decline from AcOH-glutenin to AGC-glutenin to SDS-glutenin. In agreement with this, the $S_{20,w}$ values of AcOH-glutenin, AGC-glutenin, and SDS-glutenin were found to be 6.6 S ± 0.2, 5.8 S ± 0.15, and 4.7 S ± 0.1 respectively. The S values for glutenins reported in the literature range from 3.67 S to 6 S (Taylor and Cluskey 1962, Wu et al 1967, Kobrehel and Bushuk 1978). Our $S_{20,w}$ values as well as those reported in literature are low for proteins whose molecular

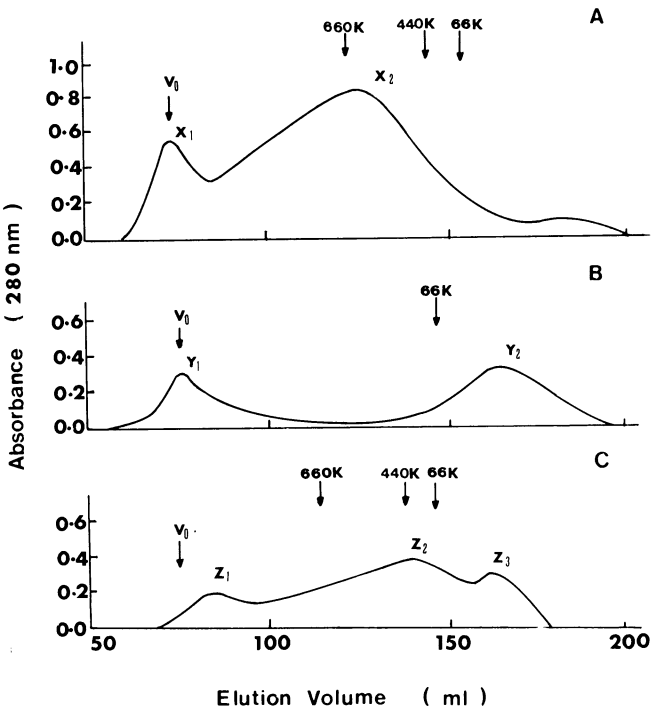

Fig. 1. A, Chromatogram of AcOH-glutenin on Sepharose CL-4B using AGC as eluent. **B,** Chromatogram of peak X1 on Sepharose CL-4B using sodium dodecyl sulfate as eluent. **C,** Chromatogram of peak X2 on Sepharose CL-4B using sodium dodecyl sulfate as eluent. Elution positions of three standard molecular weight marker proteins are shown by the three arrows, respectively: thyroglobulin 660,000, ferritin 440,000, and bovine serum albumin 66,000. Void volumes were determined with blue dextran.

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weights are in millions. At present no convincing explanation for their low S values is available, but glutenin is an extended asymmetric molecule with relatively high surface area (Bietz and Huebner 1980) and this may have something to do with it.

Chromatography of AcOH-glutenin on Sepharose CL-4B

Glutenin is a mixture of many high molecular weight proteins, and chromatography on wide pore gels in dissociating solvents such as urea, GuHCl, SDS, cetyltrimethylammonium bromide, sodium thiocyanate, and their combinations has been used to study its composition (Huebner and Wall 1976, 1980; Preston 1982, 1984; Bottomley et al 1982; Graveland et al 1982, 1985). In all these studies, each solvent was used separately, and the elution profiles are different for different solvents. This may be due to incomplete dissociation of noncovalently bonded protein by any one solute-solvent system. In the present study, solvents AGC and SDS were used in succession to dissociate noncovalently bonded protein from the same glutenin sample. This method dissociates more noncovalently bonded protein from glutenin than either AGC or SDS alone, and, therefore, the glutenins obtained after chromatography in the second solvent (SDS) are likely to have only covalently bonded (interpolypeptide disulfide bond) proteins (Prasada Rao and Nigam 1987).

Figure 1A gives the gel filtration profile of the AcOH-glutenin in solvent AGC. This chromatography gives a void volume peak X1 and a bed volume peak X2 extending over the entire bed volume range. Figure 1B and 1C give the gel filtration profiles of the peaks X1 and X2 in solvent SDS. The data from these chromatograms is summarized in Table I. The last column of the table gives recoveries calculated for no experimental losses during chromatography. These values will be used in the discussion.

The glutenin in peak X1 is likely to have a molecular weight of over 10 million, and it constitutes about 24% of AcOH-glutenin (Table I). This glutenin, on rechromatography in solvent SDS, gave a void volume peak Y1, and a bed volume peak Y2 of low molecular weight proteins dissociated from X1 by SDS. The proportions of Y1 and Y2 in X1 are 38% and 62%, respectively (Table I). Thus, in glutenin X1 more protein was bound by noncovalent bonds than by interpolypeptide disulfide bonds.

Peak X2, which constitutes about 76% AcOH-glutenin (Table I), on rechromatography in solvent SDS gave three peaks Z1, Z2, and Z3 (Fig. 1C). Peak Z1 (about 18% of X2) elutes immediately after void volume, and its glutenin may have a molecular weight of a few millions. Peak Z2 (about 64% of X2) represents relatively low molecular weight glutenins eluting over a wide range. The molecular weights of its glutenins may range from over 200,000 to less than a million. The estimate for the lower limit is based on earlier results, where it was shown that all glutenins are excluded from Sephadex G-200 (Prasada Rao and Nigam 1987). Peak Z3 (about 18% of X2) is of low molecular weight proteins dissociated from AcOH-glutenin by solvent AGC (as peak X2 extends up to the end of the bed volume, these are isolated along with X2 glutenin) and the proteins dissociated from X2 glutenin by SDS.

From the recoveries given in the last column of Table I, the proportions of various glutenin peaks in AcOH-glutenin can be calculated. These are the void volume peak Y1 (mol wt over 10 million) 9%; peak Z1 (mol wt a few million) 13.5%; peak Z2 (mol wt ranging from over 200,000 to less than a million) 49%. Finally, the low molecular weight proteins dissociated from AcOH-glutenin by solvents AGC and SDS (peaks Y2 and Z3) together constitute about 28.5% of AcOH-glutenin. It may be noted that in the earlier study with Sephadex G-200, the proportion of low molecular weight dissociable proteins in AcOH-glutenin was 27% (Prasada Rao and Nigam 1987). In that study, it was said that glutenin remaining after successive gel filtration in solvents AGC and SDS is not likely to contain any noncovalently bonded protein. This was suggested because a third dissociating solvent phenol-acetic acid-water (1:1:2) did not dissociate any low molecular weight protein from the residual glutenin obtained by successive gel filtration of glutenin on Sephadex G-200 in solvents AGC and SDS. Accepting this, it can be said that peaks Y1, Z1, and Z2 are composed only of glutenins containing interpolypeptide disulfide bonds. The results also show that the highest molecular weight glutenin X1 contains more noncovalently bonded protein (Fig. 1B) than the lower molecular weight glutenins of peak X2 (Fig. 1C).

TABLE I
Data from the Chromatograms Given in Figure 1

Peak	Eluent[a]	Elution Volume (ml)	Yield[b] (%)	% Adjusted for 100% Recovery
X1	AGC	73	22	24
X2		124	71	76
Y1	SDS	76	36	38
Y2		166	60	62
Z1	SDS	83	17	18
Z2		141	62	64
Z3		163	17	18

[a] AGC = 0.1*M* acetic acid-2.25*M* GuHCl, and 0.01*M* cetyltrimethylammonium bromide. SDS = 1% sodium dodecyl sulfate in 0.1*M* acetic acid.
[b] Percent of the absorbance loaded recovered in each peak.

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LITERATURE CITED

BIETZ, J. A., and HUEBNER, F. R. 1980. Structure of glutenin: Achievements at the Northern Regional Research Center. Ann. Technol. Agric. 29:249.

BOTTOMLEY, R. C., KEARNS, H. F., and SCHOFIELD, J. D. 1982. Characterization of wheat flour and gluten proteins using buffers containng sodium dodecyl sulfate. J. Sci. Food Agric. 33:481.

GRAVELAND, A., BOSVELD, P., LICHTENDONK, W. J., MOONEN, H. H. E., and SCHEEPSTRA, A. 1982. Extraction and fractionation of wheat flour proteins. J. Sci. Food Agric. 33:1117.

GRAVELAND, A., BOSVELD, P., LICHTENDONK, W. J., MARSIELLE, J. P., MOONEN, H. H. E., and SCHEEPSTRA, A. 1985. A model for the molecular structure of the glutenin from wheat flour. J. Cereal Sci. 3:1.

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.

HUEBNER, F. R., and WALL, J. S. 1980. Wheat glutenin: Effect of dissociating agents on molecular weight and composition as determined by gel filtration chromatography. J. Agric. Food Chem. 28:433.

KOBREHEL, K., and BUSHUK, W. 1978. Studies of glutenin. XI. Note on glutenin solubilization with surfactants in water. Cereal Chem. 55:1060.

PRASADA RAO, U. J. S., and NIGAM, S. N. 1987. Gel filtration chromatography of glutenin in dissociating solvents: Effect of removing noncovalently bonded protein components on the viscoelastic character of glutenin. Cereal Chem. 64:168.

PRESTON, K. R. 1982. Gel filtration of wheat gluten proteins on Sephacryl S-300. Cereal Chem. 59:73.

PRESTON, K. R. 1984. Gel filtration and characterization of neutral salt extracted wheat gluten proteins varying in hydrophobic properties. Cereal Chem. 61:76.

SCHACHMAN, H. K. 1959. Ultracentrifugation in Biochemistry. Academic Press: London.

TAYLOR, N. W., and CLUSKEY, J. E. 1962. Wheat gluten and its glutenin component: Viscosity, diffusion and sedimentation studies. Arch. Biochem. Biophys. 97:399.

WU, Y. V., CLUSKEY, J. E., and SEXSON, K. R. 1967. Effect of ionic strength on the molecular weight and conformation of wheat gluten proteins in 3*M* urea solutions. Biochim. Biophys. Acta 133:83.

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