DETERMINATION OF MOLECULAR-WEIGHT DISTRIBUTION IN WHEAT-FLOUR PROTEINS BY EXTRACTION AND GEL FILTRATION IN A DISSOCIATING MEDIUM[1]

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ABSTRACT

A solvent consisting of urea (3*M*), acetic acid (0.1*M*), and cetyltrimethylammonium bromide (0.01*M*) dissolved in water is useful for both extraction and chromatography of wheat-flour proteins. A clear extract, containing about 95% of the total proteins, is obtained by homogenization followed by centrifugation for 30 min. at 140,000 × *g* (max.). When the extract is chromatographed on a column of Sephadex G-200 an elution curve is obtained that appears to be a true profile of molecular-weight distribution; this can be calibrated with proteins of known molecular weight, isolated from other sources. Results of rechromatography and other evidence indicate that the elution curve does not reflect noncovalent aggregation of protein molecules. The elution curve contains four main peaks which are formed primarily, in order, by glutenins, gliadins, albumins, and nonproteins. Addition of mercaptoethanol to the extract nearly abolishes the glutenin peak and shifts the molecular-weight distribution to lower values. Experiments with sulfhydryl-blocking reagents indicate that neither the extraction method nor hand-milling of wheat induces a change in molecular-weight distribution by sulfhydryl-disulfide interchange.

It is now accepted (1–3) that the protein mixture of wheat flour is very complex and contains many molecular species having different sizes, structures, and configurations. Water-soluble albumins of molecular weight (MW) about 20,000, salt-soluble globulins of MW about 20,000–200,000, alcohol-soluble gliadins of MW about 25,000–100,000, and relatively insoluble glutenins of MW $>10^6$ are present. While the other molecular species are thought to consist of single polypeptide chains, some with intrachain disulfide bonds (4), glutenin molecules are now known to be disulfide-linked polymers or copolymers of numerous polypeptide chains (5).

The study of glutenins has been hampered by the lack of suitable solvents. Alkaline or strongly acidic solvents dissolve them quite readily but at the expense of cleaving some covalent bonds, particularly disulfide bonds. Dilute acetic acid is a milder and more useful solvent, from which a glutenin preparation can be recovered in good physical state (6), but it fails to dissolve all glutenins (7,8). Aqueous urea (9) is another mild solvent which is rather more effective. Since

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urea is well known for its ability to cause dissociation[2] and unfolding of protein molecules, it seems that association and conformation, as well as high molecular weight, may be factors contributing to the insolubility of glutenins. As yet, however, there seems to be no unequivocal evidence for association between glutenins alone, although association between the other flour proteins, and between the other proteins and glutenins, is well established (1,2,10–19).

The necessary task of isolating and characterizing individual proteins is being undertaken in several laboratories. In baking, however, the proteins are employed as a mixture, and their function must be determined at least as much by interactions within the mixture as by the properties shown by individual proteins when isolated. To explain function on a molecular basis it will ultimately be necessary to devise an analytical method that gives a complete, quantitative picture of the protein composition. A more modest objective is to attempt determination of the MW distribution by gel filtration.

Andrews *et al.* (20–22), Whitaker (23), and others (24–28) have recently shown that when a protein is subjected to gel filtration on Sephadex the elution volume can be correlated inversely with the molecular weight. Correlation is good if experiments are carried out under reproducible conditions and if certain side effects are avoided or suppressed, namely: (a) specific adsorption on Sephadex (20,23,24, 26,29), e.g. of cations, aromatic residues, or active sites; (b) repulsion from Sephadex, e.g. of electron-dense molecular surfaces (20,23,24); (c) formation or cleavage of covalent bonds, e.g. by oxidation or sulfhydryl-disulfide interchange; (d) association between proteins (30–32), or between proteins and lipids or polysaccharides; (e) conformational effects (20), e.g. exclusion of branched structures.

Gel filtration on Sephadexes G-75 (33–36), G-100 (16,17,35–40), and G-200 (35,39) has recently given several useful separations of wheat-flour proteins, but none of the solvents employed could overcome the association mentioned above; these systems might be useful for determining aggregate or particle weights but not MW's.

In this paper we describe the chromatography of wheat-flour proteins on Sephadex G-200 in a dissociating solvent (AUC) which contains acetic acid (0.1*M*), urea (3*M*), and cetyltrimethylammonium bromide (0.01*M*). The elution curves so obtained are interpreted as profiles of MW distribution in the total flour proteins. Side effects seem to be negligible. In the method described, the same solvent is used to extract the proteins from flour in nearly quantitative yield.

[2] Throughout this paper the terms *association* and *dissociation* refer to intermolecular bonding by noncovalent forces, namely, by ionic and dipole-dipole interactions, by hydrogen bonding, and by Van der Waals-London interactions (apolar and possible hydrophobic bonding).

Materials

The flour used was an untreated bread flour, milled commercially from a mixed grist originating in Canada, Russia, England, Australia, and Argentina. Its protein content as received was 12.8% (Kjeldahl N × 5.70) and its moisture content 14.3%. It was stored at 2°C. between experiments.

Solvent AUC. This was an aqueous solution, 0.1*M* in acetic acid, 3*M* in urea, and 0.01*M* in cetyltrimethylammonium bromide ($CH_3 \cdot (CH_2)_{15} \cdot N(CH_3)_3 + Br^-$).

Solvent AU. This was an aqueous solution, 0.1*M* in acetic acid and 3*M* in urea.

Methods

Preparation of Flour Extract in AUC. Flour (4.00 g.) was blended into stirred AUC (69 ml.) and the mixture was placed in a top-drive homogenizer for 2 min. After standing at room temperature for 2 hr. (during which time foam subsided) it was centrifuged at 140,000 × *g* (max.) for 30 min. Each centrifuge tube then contained a sediment of partly swollen starch granules and insoluble gums, a surface scum of lipid material, and a main layer of clear solution. In general, a fresh extract was prepared for each chromatographic run; when storage was necessary it was done at 2°C.

Preparation of Chromatographic Column in AUC. Beads of Sephadex G-200 (140- to 400-mesh) from a single batch were used in all experiments. Beads (6 g.) were suspended in AUC (380 ml.) and left, with occasional stirring, for at least 72 hr. The suspension was then poured into a column tube, of internal diameter 2.3 cm., which was fitted with a jacket thermostatted at 25.0°C. and plugged with glass wool and a layer of glass ballottini. The column so formed was percolated with AUC overnight before use, and its height was made exactly 45 cm. by removal of a little gel from the top. Between runs the gel was poured out, reslurried, and repacked.

Chromatography of Flour Extract in AUC. The column was mounted on a direct-volume-measuring fraction collector set to take 3-ml. fractions. Clear, centrifuged extract (3.00 ml., containing about 22 mg. proteins) was run on and development was carried out with AUC, for 24–36 hr., while 75 fractions were collected.

Experiments with Solvent AU. Essentially the same techniques were used.

Determination of Protein Content by Ultraviolet Measurement. E_{280} was measured in a 1-cm. cell against solvent in a Unicam SP-500

spectrophotometer. (The absorption maximum of flour extracts was actually at 276 mμ.) A haze correction (41) was applied for extracts but not for column fractions. The Beer-Lambert law was obeyed in AUC and AU at all the concentrations tested. Read against water, E_{280} for AUC was 0.015.

Determination of Protein Contents of Extraction Residues by Biuret Method. The sediment from an extraction was re-extracted twice, both times with enough solvent to restore the original volume. (The purpose of re-extraction was to remove protein solution trapped in the sediment; in experiments with AUC, ultraviolet measurements gave no evidence that re-extraction increased the solubilization of proteins.) It was then placed in a prewashed Visking bag and continuously dialyzed against toluene-saturated, distilled water at 2°C. for 5 days. The whole suspension in the bag was then lyophilized, and the residue analyzed by a modification of Jennings's (42) biuret method, in which carbon tetrachloride was omitted and color measurement was preceded by centrifugation at 140,000 × *g* (max.).

When the biuret method was tested on highly purified (43) granular wheat starch (N × 5.70 = 0.19%), the result obtained was zero. However, for 1 mg. of "conventional" gliadin it gave a result 13% lower when starch (75 mg.) was present than when it was absent. To overcome such interference in extraction residues, the method was calibrated by adding known quantities (0–0.6 mg.) of gliadin to aliquots (75 mg.) of the residues. When plotted, the results gave straight lines (different for AU and AUC residues) which by extrapolation indicated the protein contents. In quantities up to 5 mg., gliadin gave the same response (with respect to N × 5.70) as flour in the biuret method.

Extraction of Iodoacetamide-Treated Flour. Flour (4.00 g.) was dispersed under nitrogen in 68 ml. of nitrogen-purged 3*M* aqueous urea solution containing 370 mg. of iodoacetamide. Stirring was continued and, after 10 min., cetyltrimethylammonium bromide was added to 0.01*M* concentration; after a further period of 10 min., glacial acetic acid was added to 0.1*M* concentration. The suspension was then homogenized and centrifuged for chromatography in the usual way (the elution curve is shown in Fig. 2). In another experiment 250 mg. of N-ethylmaleimide was used in place of the iodoacetamide (see Table III).

Treatment of Flour with Ethylene Oxide. A tray of flour, 2 mm. in depth, was kept in ethylene oxide vapor at room temperature for 18 hr., then exposed for 2 hr., and raked occasionally to remove occluded reagent.

Results and Discussion

1. Extraction

After centrifugation, the maximum volume of extract that could be poured from above the sediment was 86% of the volume of solvent employed. Replacing the extract with fresh solvent and repeating the extraction procedure gave an extract in which the protein content (measured by E_{280}) was 6% of that in the first extract: this could be more than accounted for by the 14% of solvent trapped in the residue in the first extraction.

The discrepancy between the proportions of solvent and proteins trapped in the residue is interesting because it indicates that two-thirds or more of the trapped solvent was inaccessible to proteins. Discrepancies of this kind were also found in model experiments that were made on granular starch, carefully purified (43) from the same flour. The starch produced a sediment containing about five times its weight of solvent; when commercial gliadin was dissolved initially in the solvent the starch caused a rise in E_{280}, indicating that about one-third of the trapped solvent was inaccessible. Presumably, all the solvent that occupied interstices between the starch granules was accessible to proteins, and at least part of the solvent inside the granules was inaccessible. However, these observations could not be interpreted fully because granule-size distribution, selective uptake of detergent and/or urea by the granules, diffusion of proteins into them (44), and the presence of pentosans etc. in the flour sediments were all possible contributing factors. Moreover, the extent of granule swelling rose with time of immersion in AUC and fell with increasing starch or protein concentration. Suffice it to say that these factors appeared to be negligible when the flour-solvent ratio was low and when the extraction with AUC was carried out as described.

It is not important that the whole of the extract should be removable from the sediment, but it matters that what is removed should contain a representative sample. Hence it must be confirmed whether all of the proteins are dissolved by AUC. This requires accuracy in determination, against which several difficulties arise: (a) Kjeldahl-N determination cannot be applied directly. (b) Starch-granule swelling elevates the protein concentration in the extract by a factor varying with, among other things, the flour-solvent ratio. (c) Calibration factors for N, ultraviolet, and biuret analyses differ for the total proteins and fractions of them. (d) Nonprotein flour constitutents may interfere in analyses, e.g. nucleotides in the ultraviolet analysis.

In Table I the results of our attempts to measure the complete-

TABLE I

COMPLETENESS OF PROTEIN EXTRACTION, AS DETERMINED BY SEVERAL METHODS

SOLVENT	ULTRAVIOLET [a]	ULTRAVIOLET, TRIPLE EXTRACT [a,b]	ULTRAVIOLET,[a] FLOUR-SOLVENT RATIO × 0.13 [c]	BIURET ON RESIDUE
	%	%	%	%
Acetic acid (0.1*M*)	66	68		
Acetic acid (0.1*M*) + urea (3*M*) (AU)	83	80	70	88
Acetic acid (0.1*M*) + urea (3*M*) + cetyltrimethylammonium bromide (0.01*M*) (AUC)	101	91	84	95
Acetic acid (0.1*M*) + urea (3*M*) + Tween 80 (0.01*M*)	85		75	
Acetic acid (0.1*M*) + urea (6*M*)	135		85	

[a] E_{280} × N in acetic acid triple extract × 100 ÷ E_{280} in acetic acid triple extract × N in flour.
[b] Samples were extracted three times and extracts combined.
[c] In the procedure described, 0.53 g. flour was used instead of 4.00 g.

ness of extraction are summarized. The ultraviolet results clearly show the effect of the flour-solvent ratio. However, this effect is opposed by an error in the calibration, which was based upon $E^{1\%}_{280}$ measured for protein content (N × 5.70) extractable by acetic acid. $E^{1\%}_{280}$ was 6.58 in the urea-containing solvents, considerably higher than the value (5.21) found for a "conventional" gliadin preparation. In fact, 6.58 is likely to be higher than the true value for total proteins, because the contributions of nonproteins and albumins (with a relatively high content of aromatic amino acid residues (1)) in material extracted by acetic acid must be disproportionately high. The results of analyzing the extraction residues by the biuret method are considered to be the most reliable, and 95% for AUC (Table I) seems acceptable.

Table I demonstrates the specific improvement in extraction made possible by the cationic detergent, cetyltrimethylammonium bromide. At the same molarity a nonionic detergent, Tween 80, gave less improvement. An anionic detergent, sodium dodecyl sulfate, actually precipitated proteins from an AU extract (which is consistent with Sullivan's findings (45)).

2. Chromatography

We determined the elution volumes (V_e) for some proteins of which the MW's are known with reasonable certainty (Table II), and plotted (20–23,28) $\log_{10}$ MW against V_e. Apparently this relation applies as well (Fig. 1) to the present system as it does to the systems used previously. The largest deviations from the relation are seen at the

TABLE II

PROTEINS USED FOR THE CALIBRATION SHOWN IN FIGURE 1

PROTEIN[a]	V_e	MOLECULAR WEIGHT	REFERENCE
	ml.		
(Haze	60	>10[6])	
Serum albumin dimer (bovine)	79	129,800	25,46
γ-Pseudoglobulin (bovine)	85[b]	147,000	47
Conalbumin	98	76,600	48
Serum albumin (bovine)	104	64,900	46
κ-Casein	110	56,000	49
Ovalbumin	121	45,200	50
Desoxyribonuclease (bovine pancreatic)	123[c]	40,000	51
α_s-Casein	141	27,500	52
Ovomucoid	143	28,000	53
Trypsin	149	23,800	54
β-Lactoglobulin monomer	151	18,900	55
Trypsin inhibitor (soybean)	158[d]	21,500	56
Cytochrome *c* (horse heart)	158	12,400	57
Papain	159	20,900	58
Lysozyme (hen's egg)	172	14,400	59
(N-Ethylmaleimide	193	125)	
(L-Tryptophan	197	190)	

[a] Samples 0.5–5 mg. chromatographed, usually in the presence of NEMI.
[b] Shoulders at 68 and 78 ml.
[c] Second peak at 144 ml.: see refs. 60, 61.
[d] Shoulder at 141 ml.

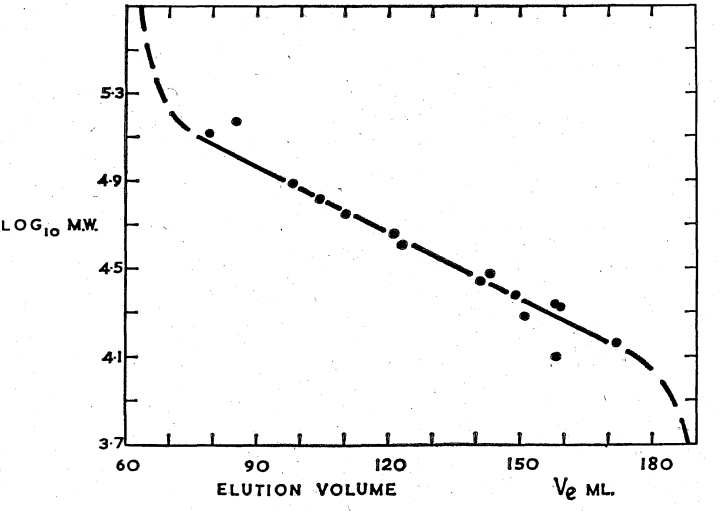

Fig. 1. Calibration of the column. See Table II for list of proteins used.

extremes of MW, but in general the precision of any MW reading from V_e is probably about ± 12%, as suggested by Andrews *et al.* (20,21). Pepsin gave V_e = 147 ml., corresponding to a MW only two-thirds of the true value; in view of Perlmann's findings (62) this is

attributable to autolysis promoted by the urea and acid in AUC, and so no point for pepsin is shown in Fig. 1. Proteins that are dissociable into subunits cannot be used for calibrating a system such as this unless (as in the case of beta-lactoglobulin) the MW's of the subunits are known. Calibration in the high-MW range was therefore difficult. For example, the accepted MW of catalase is 225,000, but in this system it gave two peaks for which Fig. 1 indicated MW's about 90,000 (colorless) and 20,000 (colored).

As shown in Fig. 2, the elution curve of a flour extract in AUC has four main peaks. When "conventional" preparations of glutenin,

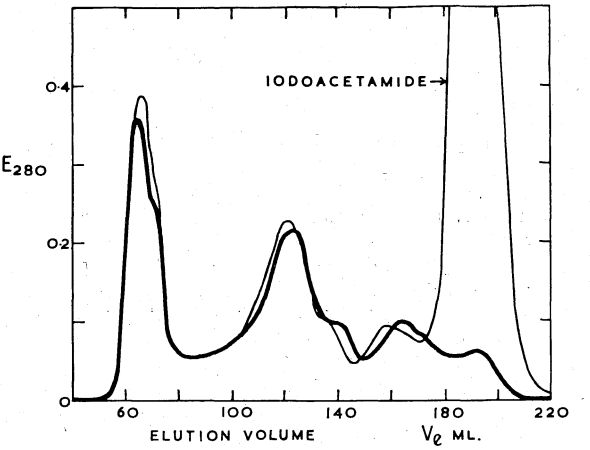

Fig. 2. Elution curves for flour and for flour treated with iodoacetamide. Solvent, AUC.

gliadin, and albumin, and nonproteins of low molecular weight, were chromatographed they formed main peaks at virtually the same elution volumes as those in Fig. 2, respectively. For convenience, therefore, although it is clearly not rigorous, we use the following designations for parts of the elution curve:

Between beginning and minimum at V_e about 85 ml., *glutenin peak.*

Between minima at V_e about 85 ml. and 145 ml., *gliadin peak.*

Between minima at V_e about 145 ml. and 180 ml., *albumin peak.*

Between minimum at V_e about 180 ml. and end, *nonprotein peak.*

The quantitative distribution between these four peaks (Table III) and the shape of the elution curve were highly reproducible, and total recovery was quantitative within limits of experimental error. It was

TABLE III

QUANTITATIVE DISTRIBUTION AMONG THE FLOUR PROTEIN PEAKS

Modifications of Described Methods	Peak Designations							
	Glutenin		Gliadin		Albumin		Non-protein	
	ΣE[a]	%	ΣE	%	ΣE	%	ΣE	%
None	1.78	33	2.45	45	0.85	16	0.32	6
	1.71	32	2.42	46	0.86	16	0.33	6
	1.74	34	2.26	44	0.77	15	0.40	8
Flour treated with NEMI (2 μeq./g.) in aqueous suspension	1.81		2.30		0.77		[b]	
Flour treated with NEMI (500 μeq./g.) in aqueous suspension	1.73		2.21		[b]		[b]	
Flour treated with gaseous ethylene oxide	1.87	35	2.34	43	0.82	15	0.37	7
Flour left for 24 hr. at 25°C. in aqueous suspension	1.99	36	2.21	40	0.81	14	0.55	10
Flour suspended in 3*M* aqueous urea and air bubbled through for 20 min.	1.74	32	2.41	45	0.76	14	0.48	9
AU used for extraction and chromatography[c]	2.51	47	1.68	31	0.80	15	0.40	7
	2.47	46	1.71	32	0.91	17	0.37	7

[a] Sum of E_{280} for 3-ml. fractions.
[b] NEMI contributes to E_{280}.
[c] Volume of extract chromatographed was raised to give the usual load.

necessary to fulfill the following practical requirements: (a) Sephadex should be allowed to swell in the solvent for several days before its first use (20–22). (b) The column should be thermostatted during packing as well as running (27,28,63). (c) The column should be repacked between runs, because the flow rate falls by roughly one-third during a run. (d) The sample should be applied in AUC of the correct composition, to avoid changing the porosity of the Sephadex by osmotic shock. (Doubling the urea concentration in a sample solution greatly impaired resolution on the column.) (e) The load should be minimized to an extent consistent with the accurate measurement of E_{280}. (f) Microbiological attack on Sephadex and samples should be prevented (as it is by cetyltrimethylammonium bromide).

Because some proteins readily undergo sulfhydryl-disulfide interchange when dissolved in urea solutions (64), tests were made using SH-blocking reagents. In one test, the flour was treated with a large excess of iodoacetamide before extraction and chromatography, but this caused no significant change (see Fig. 2) apart from a large contribution of reagent to the nonprotein peak. (The glutenin peak changed in shape but not size: as explained below, this was probably

caused by a time interval between extraction and chromatography.) Including N-ethylmaleimide (NEMI) in the AUC used for extraction, or treating the flour with aqueous NEMI or gaseous ethylene oxide (65) before extraction, likewise caused no significant changes (see Table III). Hence it appears that the MW distribution is not changed by sulfhydryl-disulfide interchange under the conditions employed. However, since NEMI is not potentially harmful in the determination of MW distribution, and could conceivably be beneficial in some circumstances, we generally included it in the AUC used for extraction. Moreover, it provided a useful marker for column volume because its MW is negligibly small, it has an appreciable E_{280}, and (unlike tryptophan — see Table II) is does not appear to be adsorbed by Sephadex.

Deliberately aerating a flour suspension caused no appreciable change in the glutenin and gliadin peaks, and only a small net transfer from the albumin to the nonprotein peak (Table III). Leaving flour

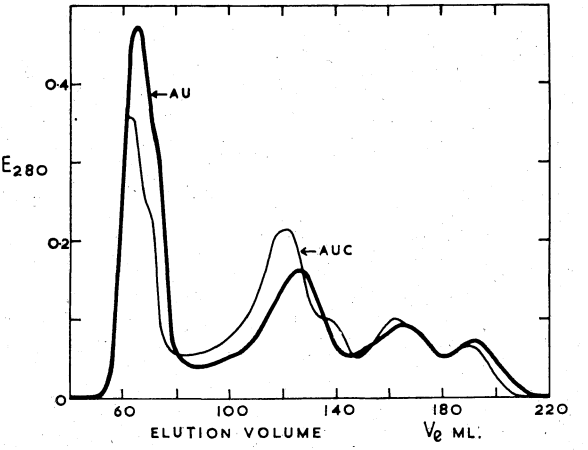

Fig. 3. Elution curves for flour proteins extracted in AU and chromatographed in AU and AUC.

suspended in water changed the MW distribution only a little (Table III), raising the glutenin and nonprotein peaks at the expense of the others.

Before we established that the detergent was needed in extraction we had carried out both extraction and chromatography in AU, and had, even then, obtained four main peaks (Fig. 3). However, the "glutenin" peak accounted for a much larger proportion of the total area (see Table III). When detergent was incorporated (0.01*M*) in the sample

solution and chromatography carried out in AU, the elution curve was not significantly changed. In contrast, when chromatography was carried out in AUC (Fig. 3), nearly a third of the area under the "glutenin" peak was transferred to the gliadin peak. This was interpreted to mean that the detergent dissociated proteins of lower MW from the glutenins.

Whether the detergent *completely* overcame dissociation was still to be decided. When a flour extract in AUC containing NEMI was "shocked," by adjusting the urea concentration to 9.2*M* temporarily (for 1 hr.) before chromatography, the elution curve was not significantly changed. Nor were the relative areas under the peaks changed when the urea concentrations in the extract *and* on the column were made 5*M*, although the shape of the curve changed slightly (an expected result of changing the gel system).

Rechromatography provided perhaps the best evidence that dissociation was complete. If a fraction represents a specific part of the MW distribution, and if association and the other side effects (a–e) mentioned at the beginning of this paper are negligible, when rechromatographed it should give a peak at precisely the same V_e, and no subsidiary peaks at higher V_e. This should be true whether the fraction comes from a peak, a shoulder, or a featureless part of the elution curve. An extract was prepared containing NEMI (50 μeq./g. flour) and its elution curve obtained as usual. Then certain fractions (indicated by hatched areas: see top of Fig. 4) were, without concentration or other treatment, rechromatographed under the usual conditions. The curves thus obtained are shown in Fig. 4 (superimposed on frames of the hatched areas). All but one fulfill the requirements mentioned. The one exception is for the shoulder of the glutenin peak (V_e about 70 ml.) which on rechromatography behaved like the main peak (V_e about 60 ml.), shifting in the direction opposite to that expected for dissociation. As already mentioned, the shoulder did not always appear, and it was found in other experiments that it never appeared when an extract was stored, for 1 day at room temperature or 2 days at 0°C., before chromatography.[3] These phenomena may be caused by a slow conformational change in the glutenins.

By means of the calibration graph (Fig. 1), certain peaks and shoulders in the flour elution curve can be assigned MW's that approximate to some reported for isolated flour proteins, as shown in Table IV.

[3] Storage of an extract sharpens the glutenin peak and so tends to improve its resolution from the gliadin peak. We now make use of this to compensate for what appears to be reduced porosity in recent batches of Sephadex G-200.

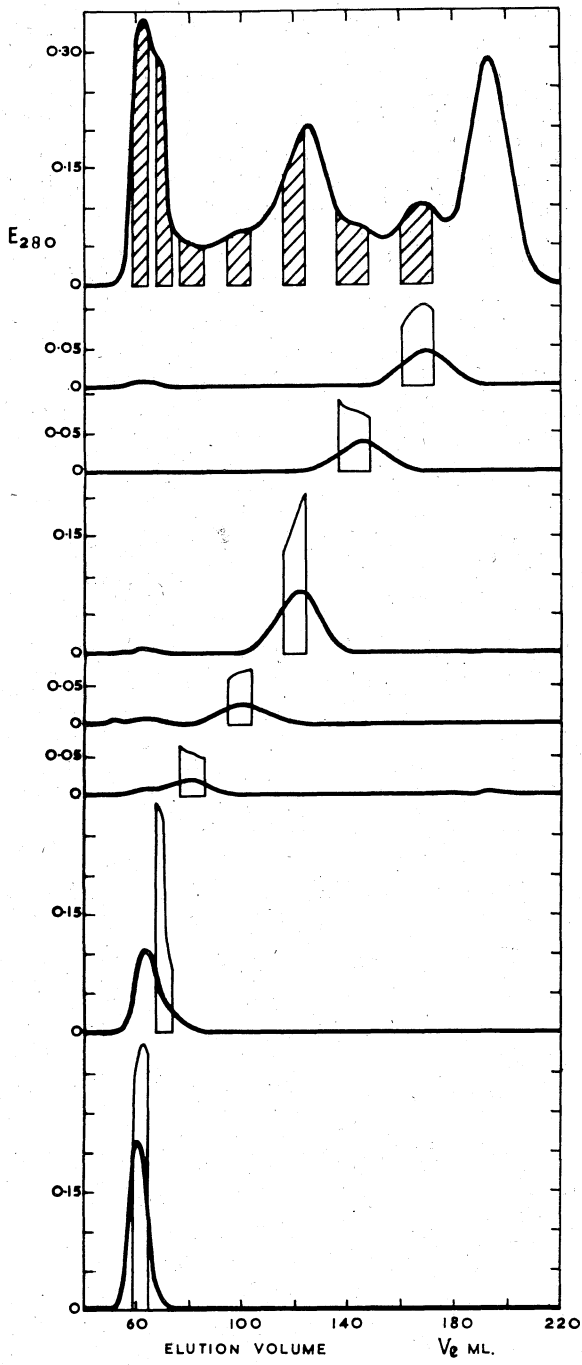

Fig. 4. Elution curves for flour proteins and for fractions subjected to re-chromatography. Solvent, AUC.

TABLE IV

CORRESPONDENCE OF MOLECULAR WEIGHTS WITH SELECTED LITERATURE VALUES

From Elution Curve			From Literature			
V_e ml.		M.W.	M.W.	Protein	Method	Reference
ml.						
102	(shoulder)	69,000	75,000	A globulin		18
			59,000	β-Amylase	Sedimentation velocity	66,66a
123	(peak)	43,000	45,000	$\alpha_{1,2}$-Gliadin	Light scattering	36
			41,000–44,000	Gliadin	Osmotic pressure in urea	67
139	(shoulder)	30,000	31,000	β-Gliadin	Sedimentation equilibrium in aluminum lactate	68
			31,000	γ-Gliadin	Sedimentation equilibrium in guanidinium chloride	69
			26,000	γ-Gliadin	Sedimentation equilibrium in guanidinium chloride	15
165	(peak)	16,000	17,000	Albumins	Sedimentation velocity in urea	70

3. Applications

Results given above indicate that extraction or mere wetting of flour does not induce a change in MW distribution through sulfhydryl-disulfide interchange. We tested further whether hand-milling induces

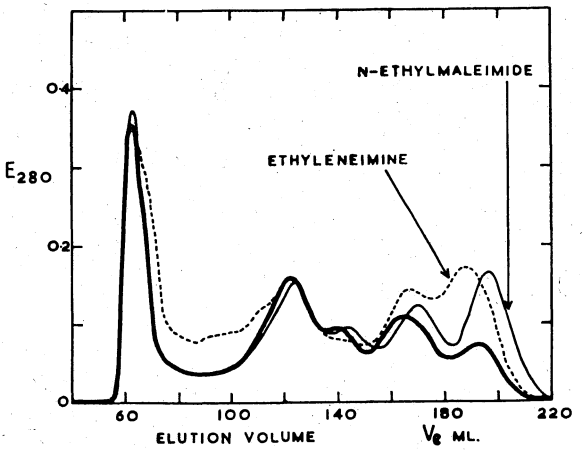

Fig. 5. Elution curves for flours obtained from wheat treated with N-ethylmaleimide and ethyleneimine. Heavy curve, control. Solvent, AUC.

such a change. A small sample of English wheat was kibbled and treated with ethereal NEMI or gaseous ethyleneimine (71) before milling to 70% extraction by means of a pestle and mortar, and the MW distributions in the flours obtained were compared with that in a flour prepared as a control (Fig. 5). NEMI caused no significant change, apart from itself contributing to the nonprotein peak. Ethyleneimine gave a less clear-cut result because (probably by polymerizing) it contributed to all parts of the distribution, but it supported the same conclusion.

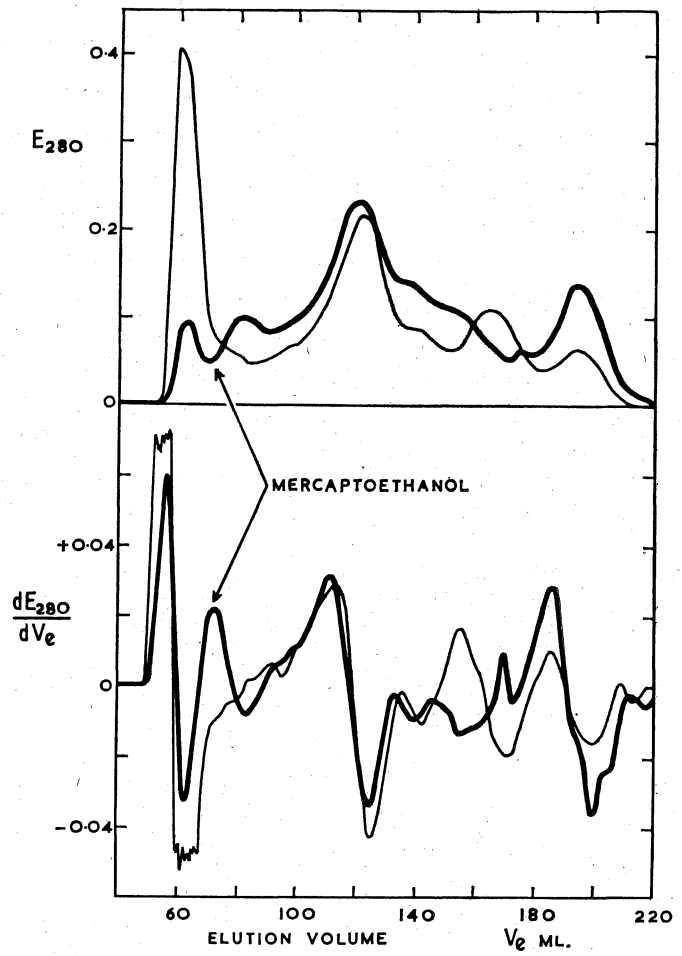

Fig. 6. Elution curves (upper) and differential elution curves (lower) for flour proteins, with and without the addition of mercaptoethanol (30 μeq./ml.) to the extract. dE_{280}/dV_e = (E_{280} for fraction) − (E_{280} for preceding fraction).

Figure 6 shows the effect of adding a small proportion of mercaptoethanol to an extract before chromatography. Although reduction of protein disulfide groups was not complete in this experiment, the elution curves demonstrate clearly the depolymerization of glutenins. Tracey (37) recently showed that plotting the derivative of a Sephadex elution curve improves the revelation of complex features. In Fig. 6 the derivative curves thus illustrate the pronounced changes in MW distribution brought about by mercaptoethanol.

4. Concluding Remarks

It is theoretically advantageous and practically convenient to use the same solvent for both extraction and chromatography, and our results appear to justify the use of cetyltrimethylammonium bromide in both methods. We used the detergent in extraction hoping that it would disrupt apolar (e.g. hydrophobic) bonds which, hypothetically, might be responsible for the insolubility of some proteins in AU. Whether this truly is its effect (and in chromatography also) requires more investigation.

Sephadex G-200 was chosen because it has the widest pores of the grades available and, under favorable circumstances, can resolve proteins with MW's up to 10^6 or more (21,22). However, it is the grade that is most likely to give low and falling flow rates, particularly when urea is present in the solvent. We found that flow rates depended critically upon avoiding thermal and osmotic changes. Scope remains for improving the resolution by using longer columns and Sephadex particles of carefully graded size (72), to maintain adequate flow rates. Recycling, with automatic monitoring, should also be valuable (26).

High-speed centrifugation gives an extract that is virtually free from haze; perhaps because a dissociating solvent is used, this does not diminish the yield. If haze is not removed it passes through Sephadex in the void volume and makes E_{280} of the glutenin peak extremely high (16,17,37).

The methods described appear to give a precise and reproducible profile of MW distribution in flour proteins. The results of chromatography and rechromatography indicate that flour contains many protein components of different MW's. In the flour studied the high-MW glutenins accounted for 35% of the total proteins, as estimated by the ultraviolet method.

Two applications of the methods have been described. Further applications to various flours, and to studies of doughs and batters, are in progress.

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