

Solubilizing Effect of Mercuric Chloride on the "Gel" Protein of Wheat Flour¹

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ABSTRACT

After repeated extraction of flours with 0.01M acetic acid, much of the residual "gel" protein could be extracted by 0.04 mM. mercuric chloride in 0.01M acetic acid.

With gel from a hard red winter wheat flour, 1 mmole of mercuric chloride solubilized more than 100 mmoles of protein N, or about 8 g. of gel protein. In seven flours, gel protein amounting to 12 to 28% of the total flour protein was solubilized; in general, the flours less stable to mixing yielded the smaller percentages of protein. The protein recovered by dialysis, concentration, and salt precipitation retained the rubbery cohesiveness typical of gluten; this would not be expected if significant rupture of disulfide bonds had occurred.

Acetic or lactic acid solutions and lactate buffers are the solvents that have been used most frequently in recent studies of the gluten proteins of wheat flour (1,2). A significant part of the protein of most flours is not extracted or solubilized by these solvents (3,4), however, so urea, guanidine hydrochloride, or other protein-dissociating agents often have been added to acetate and lactate solutions to increase the proportion of protein extracted. Sonication also has been employed (5,6).

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One of the more effective extractants is 0.01M cetyltrimethylammonium bromide and 3M urea in 0.01M acetic acid. With this solvent, Meredith and Wren (7,8) extracted about 95% of the protein from flour and up to 99% from dough, or from flour suspended in water for an hour. Phenol:acetic acid:water mixtures (1 or 2:1:1, v./v./v.) also have been used to extract nearly all flour nitrogen (5,9), and are reported to have marked dissociating power for wheat proteins (10).

We have used the term "gel protein"² to refer to protein in the insoluble but highly hydrated gelatinous material remaining after exhaustive extraction of flour with dilute acetic acid (3). In view of the relatively high concentrations of dissociating agents in aqueous solution usually needed to dissolve this material, it was surprising to observe a marked solubilizing effect of mercuric chloride at concentrations below 0.1 mM. Also, the effect was unexpected since mercuric chloride is commonly used to precipitate proteins in staining procedures.

Some general observations on the solubilizing effect are presented in this paper. More detailed characterization of the solubilized protein will be reported later.

MATERIALS AND METHODS

All flours were unbleached and unmalted. Laboratory millings were done on a Quadrumat Senior mill; wheats were tempered overnight to moisture levels of 13.5 to 15.5%, depending upon the type and protein content of the sample. Nitrogen (Kjeldahl), moisture, and ash were determined by standard methods.

Soluble proteins (i.e., those other than "gel proteins") were extracted in 0.01M acetic acid essentially as described earlier (3,11) by repeated suspension, settling, and decantation. The scale of operations was varied but the extractant-to-sample ratio (50:1, v./w.) was maintained as in the original procedure, with 250 ml. extractant per cycle and 5.0 g. flour. Protein in the extracts was determined by the Lowry method (12); the concentrations of mercuric chloride in extracts did not affect color development. Reference solutions were prepared from α -gliadin (13).

Crude gel material was obtained by centrifuging the flour residue at 30,000 \times g for 1 hr. at 4°C. A layer of compacted starch separated below a gelatinous upper layer. The latter was clearly separate and was easily removed for subsequent treatment.

RESULTS

Comparisons of Sulfhydryl-Blocking Reagents

As shown in earlier work on the increases in extractability of flour proteins brought about by dough mixing, changes occur much more rapidly and are larger when sulfhydryl-blocking reagents are added. The reagents must be present while the dough is being mixed; if the reagents are added to the 0.01M acetic acid extractant, the amount of protein extracted from flour or doughs is not increased. In that work (11), the reagents N-ethylmaleimide, *p*-chloromercuribenzoate, and iodoacetamide were used. Additional reagents known to react with sulfhydryl groups but considered less specific under the conditions present in a dough were tried later. These included silver nitrate, mercuric chloride, and cupric chloride.

²The term "gel protein" is used in this paper to indicate material that is derived from flour rather than from a dough or gluten. It is not intended to imply that the "gel protein" is a specific protein or that glutenin or gliadin components are absent from the disaggregated or solubilized gel.

TABLE I. EFFECTS OF SULFHYDRYL-BLOCKING REAGENTS ON YIELD AND NITROGEN CONTENT OF GEL AND STARCH FRACTIONS^a

Flour	Reagent	Gel Layer		Starch Layer	
		Yield g.	N %	Yield g.	N %
Commercial spring wheat	None	9.8	4.7	52	0.35
	Mercuric chloride	0.0	... ^b	46	0.24
	N-ethylmaleimide	10.2	3.7	50	0.49
	Phenyl mercuric nitrate	9.0	3.6	51	0.24
	Silver nitrate	8.6	4.1	49	0.29
Lab-milled winter wheat	None	15.8	3.4	50	0.39
	Mercuric chloride	3.4	3.2	58	0.47

^aYields are expressed as g. per 100 g. flour; the experimental work was done with 50-g. samples. Reagents were 0.02 mM., in the 0.01M acetic acid extractant. The 50:1 ratio of extractant to sample provided one microeq. reagent per g. flour per extraction, which was repeated five times.

^bNo gel layer was present.

Mercuric chloride gave markedly different results than any other reagent, in that the gel fraction was much smaller and with some flours only a starch residue appeared to remain.

This is shown in Table I. Mercuric chloride was the only reagent to disperse the gel layer from the spring wheat flour. The yield and nitrogen content of the starch layer show that the gel protein was removed, rather than dehydrated or degraded and trapped in the starch layer. The winter wheat flour gel was not completely dispersed (about 80%), with a slight increase in the nitrogen content of the starch layer.

In the extraction procedure, the flour suspension was allowed to settle for an hour before the supernatant was decanted, but there was considerable loss of small particles, protein as well as starch, because of incomplete settling. The recovered residues (sum of gel and starch layers, Table I) thus totaled only about half the original flour weight. Consequently, the results cannot be taken to indicate that all the flour protein was solubilized by mercuric chloride; some of it may have been decanted without solubilization.

Treatment of Isolated Gel

Gel was prepared from winter wheat flour by extraction with 0.01M acetic acid only, and then portions were treated as shown in Table II. In this way, reagents and treatments could be compared without loss of protein from decantation of flour particles being a factor.

A suggestion of solubilization by enzymatic degradation was given by the "no reagent" samples. However, the rate or extent of solubilization shown by the mercuric-chloride sample was not approached. With 2.7 mg. mercuric chloride (0.01 mmole), more than 1 mmole of nitrogen was solubilized, or about 80 mg. of protein. The appearance of the sample also was markedly changed; it gave a clear solution with a dense sediment of starch. Potassium iodate had no effect under the conditions used.

TABLE II. SOLUBILIZATION OF ISOLATED GEL PROTEIN^a

Treatment	N in gel solubilized in	
	2 hr. % of total	24 hr. % of total
Refrigerated, no reagent added	... ^b	9
25°C., no reagent added	8	12
40°C., no reagent added	...	25
98°C., no reagent added	52	...
25°C., + 0.27 mg. mercuric chloride	...	28
25°C., + 2.7 mg. mercuric chloride	56	65
25°C., + 5.0 mg. potassium iodate	...	14

^aGel from winter wheat flour, Table I, was divided into portions of 75 ml., containing about 800 mg. solids and 27 mg. N. The reagents indicated and 0.01M acetic acid, to give total volume of 250 ml., were added and the mixture then centrifuged, after holding as shown, and protein in the supernatant determined.

^bThe value was not determined.

General Properties of Recovered Protein

Solutions obtained by mercuric-chloride treatment of gel material could be dialyzed against 0.01M acetic acid and concentrated several-fold without precipitation of protein. Addition of potassium chloride to about 1M gave a precipitate with rubbery, elastic properties similar to those of glutenin and gluten. Exploratory trials indicate that considerable carbohydrate accompanies the gel protein into solution and remains complexed to it through fractionation by gel filtration.

Comparison of Flours

Work in several laboratories has related the proportion of less- and more-soluble proteins in flours to the mixing time and stability values shown by their mixing curves (3,14,15,16,17). The amounts of mercuric chloride-dispersible gel protein of a series of flours therefore was determined to see whether this fraction of the insoluble proteins might show a trend. Results with seven flours are given in Table III. Their farinogram characteristics are consistently related to the amounts of mercuric chloride-solubilized gel protein obtained, with the latter decreasing as less mixing was required or tolerated by a flour. A more extensive series of samples and a better definition of extraction conditions are required, however, before any relationship can be considered to be established.

DISCUSSION

Mercuric chloride exerted its solubilizing effect on flour proteins at much lower concentrations than urea, ethyl alcohol, sodium salicylate, or even acetic acid. The well-known reactivity of mercury salts with sulfhydryl groups and the stability of the mercaptides that are formed would suggest that such reactions account for the solubilization, except that no other sulfhydryl-blocking reagent approached as large an effect. The possibility that sulfhydryl-blocking reagents may rupture disulfide bonds in wheat proteins during dough mixing has been suggested (18). Slow destruction of disulfide compounds has been demonstrated with small molecules,

TABLE III. COMPARISONS OF FARINOGRAM VALUES AND SOLUBILIZATION OF GEL PROTEIN BY MERCURIC CHLORIDE^a FOR SEVEN FLOURS

Flour	Protein Content %	Farinogram		Gel Protein Solubilized by Mercuric Chloride	
		Peak Time min.	Stability min.	g./100 g. flour	% of total flour protein
Red River 68, Calif.	12.0	10.0	26.5	3.40	28
Winalta, Montana	14.3	7.5	12.0	3.48	24
Ceres, Montana	13.6	7.0	16.0	2.92	21
Spring, long patent ^b	14.7	7.0	12.0	2.60	18
Marquis, Montana	13.7	5.0	8.5	2.04	15
Yogo-Rushmore, Montana	14.2	5.0	4.5	2.16	15
Lemhi, Idaho ^b	7.9	1.5	1.5	0.92	12

^aGel was separated from 2.5 g. flour by extraction with 0.01N acetic acid, then treated with 0.02 mM. mercuric chloride in 0.01M acetic acid for 1 hr. at about 25°C., repeated until 1 liters of extractant were used.

^bCommercially milled flour.

i.e., oxidized glutathione, held in solution with N-ethylmaleimide (19). We are not aware of evidence that similar disulfide-bond rupture occurs in proteins, particularly at normal dough temperatures and without mechanical stress, which are the conditions for isolating the gel fraction. Furthermore, the recovered gel protein was apparently little changed in physical properties. A marked change would be expected if disulfide bonds were ruptured, in view of the well-known marked effects of reagents such as thioglycol and cysteine on gluten properties, especially on elasticity.

At present, it seems more likely that mercuric chloride (or HgCl⁺ ion) is complexed to sulfur or nitrogen atoms in the proteins, or in some way held on the surface of protein molecules so that ionic and hydration effects increase their solubility. This suggests that only certain protein components are involved, or that specific protein-carbohydrate complexes make up the gel fraction and are dissociated by binding of the mercury salt to one or the other. In any case, mercuric chloride appears to provide an alternative solvent for use with part of the more insoluble flour proteins.

Relatively few flours were examined in the present work, but the comparison of mixing characteristics with the amount of protein solubilized by mercuric chloride seems consistent with other work (3,14,15,16,17) that relates mixing time and stability of doughs to the proportions of easily soluble and more insoluble fractions of flour proteins. The mercuric chloride-solubilization may provide a sharper fractionation of the more insoluble proteins than previously used systems; therefore, examination of more samples to explore possible correlations with mixing properties is suggested by the present results.

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