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# Lectins in Wheat Gluten Proteins

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The presence of heat-stable lectins in wheat gluten proteins was established by a modified hemagglutination assay using sheep erythrocytes. This was made possible by using 50% dimethyl sulfoxide in phosphate buffer (pH 6.81) to disperse the gluten proteins (which are otherwise insoluble in near neutral buffer without $Me_2SO$) without interfering with erythrocyte stability or the hemagglutination process. Lectin activity was found in both the gliadin and acid-soluble glutenin fractions of gluten, and this activity was not due to cross contamination from the wheat germ lectins. The quantitative distribution of lectin activity in a sample of a commercial blend flour differed markedly from that of a sample of soft winter wheat. These findings may have implications for the pathogenesis of gluten-sensitive enteropathy (celiac disease).

The mechanism of the pathogenesis of gluten-sensitive enteropathy (celiac disease), a genetic intolerance to gluten, remains unexplained. One theory proposed by Berger (1961) states that some product of gluten digestion is absorbed, whereupon antibodies are produced to these products, resulting in the symptoms of celiac disease. Another, by Weiser and Douglas (1976), proposed that wheat gluten may contain lectins that are toxic to celiac patients. Douglas (1976) isolated a wheat gluten protein ("gly-gli") that bound to the celiac intestinal mucosa but not to normal mucosa, giving support to the theory that gluten toxicity results from the binding of gluten lectin to oligosacchardies in the small intestines.

The theory of Weiser and Douglas (1976) can be interpreted to mean that the etiology of celiac disease could be similar to that of a lectin-induced enteropathy. Köttgen et al. (1982) provided additional support to this theory by demonstrating several lectin-like properties of gluten by means of laser nephelometry. However, a demonstration of the presence of lectins in wheat gluten using erythrocyte hemagglutination, the standard test for lectins (Jaffee, 1980; Goldstein and Hayes, 1978), has not been reported. The principal difficulty in testing gluten is the insolubility of its protein components in near neutral aqueous buffer solution, the medium originally prescribed for this assay, so that any lectins associated with this fraction would remain undetected. $Me_2SO$ was shown earlier by Krull et al. (1963) to be an excellent solvent for gluten proteins, even under acid conditions, and Seibert et al. (1967) showed that this solvent, at less than 25% concentration, does not cause hemolysis of normal erythrocytes. Thus, the use of $Me_2SO$ in the assay medium facilitated the dispersion of the wheat gluten proteins without interfering with erythrocyte stability or agglutination. This modification permitted definitive testing for lectins in wheat gluten, the results of which are reported herein.

## MATERIALS AND METHODS

**Preparation of Protein Fractions.** Whole kernels of soft winter wheat were washed successively 3 times with 95% ethanol and 4 times with acetone to remove surface contaminants. The air-dried sample was ground to an 80–100-mesh powder in a water-cooled micromill and defatted with 1-butanol. A 2-g portion was then extracted successively 5 times with 14-mL aliquots of 0.9% NaCl in 0.02 M phosphate buffer (pH 6.81), 70% ethanol, and 0.075 N NaOH solution; these extracts contained, respectively, the albumins and globulins, gliadins, and glutenins (Whitehouse, 1973). The fifth extract in each case showed little evidence of protein by the biuret test. This suggested that contamination of each fraction due to incomplete separation was minimal. The slurry following each extraction was centrifuged at 8000$g$ at 15 °C for 10 min, and the combined supernatants for each extractant were dialyzed against distilled water at 5 °C and lyophilized. The protein content of each fraction was calculated from a micro-Kjeldahl analysis (Concon and Soltess, 1973); 88% of the original protein was recovered in these fractions (Table I).

A sample of the identical flour (soft winter wheat) but without bran and germ (less than 50% extraction) was also defatted with 1-butanol and extracted similarly as above with buffered 0.9% NaCl (albumins and globulins), 70% ethanol (gliadins), 0.01 N acetic acid (acid-soluble glutenins), and 0.075 N NaOH (alkali-soluble glutenins). A commercial flour containing a blend of soft and hard red winter wheat (60/40 w/w; less than 70% extraction) was defatted and fractionated as above. Using this technique, protein recovery from both samples was greater than 95%.

**Hemagglutination Tests.** A modification of the method of Prigent and Bourrillon (1976) was carried out on Cook's microtiter plates. A sheep red blood cell (RBC) suspension was prepared according to the procedure of Hierholzer and Suggs (1969). Fifty microliters of 0.9% NaCl solution was pipetted into each well of the Cook's plate, followed by 50 $\mu$L of 4% RBC suspension and finally 50 $\mu$L of the protein solution. Each dilution of protein was tested in triplicate; serial dilutions of proteins were made with 50% $Me_2SO$ in 0.02 N (pH 6.81) phosphate buffer solution; blanks containing only this solvent mixture were included in each plate. The wells were sealed with acetate tape and the contents mixed gently but thoroughly and allowed to stand at room temperature. We found that gliadin and glutenin, as well as the albumin and globulin fractions of wheat flour, can be dispersed in a 50% (v/v) $Me_2SO$ buffer mixture, pH 6.81. In this case, the final concentration of $Me_2SO$ in the assay medium was less than 17% (v/v) and, as expected, did not cause hemolysis. However, to facilitate the dispersion of the gliadin and glutenin, these fractions were dispersed first in pure $Me_2SO$ whereupon the required amount of phosphate buffer was added to achieve the 50% concentration. For the albumin and globulin fractions, the sequence of addition of solvents was reversed.

Although agglutination was evident in 1–2 h, the differences in hemagglutinating activity were most pronounced after 8–12 h. The increased time apparently is necessary for the erythrocyte to completely settle from this medium whose viscosity and density are increased by the presence of $Me_2SO$. Lack of agglutination is indicated by the complete settling of the erythrocytes into the vertex

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Table I. Hemagglutinating Activity of Protein Fractions of Whole Wheat and Navy Bean

| source (% protein) | fractions | recovery, % of total protein[a] | units/g of protein[b] |
|---|---|---|---|
| wheat (9.05)[a] | buffered saline (albumins and globulins) | 32.8 | 5 700 |
| | 70% ethanol (gliadins) | 22.0 | 8 100 |
| | 0.075 N NaOH (glutenins) | 33.0 | 30 300 |
| navy bean (18.55)[a] | buffer | 71.4 | 44 800 |
| | 70% ethanol | 0.6 | 44 600 |
| | 0.075 N NaOH | 5.5 | 97 100 |
| milk proteins | buffer | 85.0 | 0 |

[a] Average of duplicates. [b] Average of triplicates rounded to the nearest hundred.

of the conical well. Hemagglutination is indicated by the presence of a suspension of aggregated erythrocytes that slowly settles as a diffuse mass. The minimum protein concentration showing lectin activity is considered to contain 1 unit (Prigent and Bourrillon, 1976), and lectin activity is expressed as units per gram of protein (specific activity).

For degermed, bran-free, soft winter wheat flour and commercial wheat flour, we tested agglutination with the above procedure using non-trypsinized and trypsinized RBC's (Liener, 1955). In our assay system, the wheat gluten lectins displayed the same activity with both trypsinized and non-trypsinized RBC; however, trypsinization resulted in faster settling of agglutinate with a more distinctive pattern evident within 4–6 h.

Navy bean proteins, which are known to contain potent lectins (Jaffe, 1980; Kakade and Evans, 1965), were separated by the same procedure as whole wheat into buffered saline, 70% ethanol, and 0.075 N NaOH fractions, to serve as positive controls. All of these fractions caused hemagglutination, and no difference in hemagglutinating activity was noted when the assays were conducted with or without $Me_2SO$, demonstrating that known lectins produce a positive response in this assay and that 17% $Me_2SO$ does not interfere with the hemagglutination assay.

Dialyzed and lyophilized milk proteins that have not been shown to contain lectins, but nevertheless are known to cause an enteropathy in sensitive individuals, were used for negative control. These proteins did not cause any hemagglutination even at a level of 500 $\mu$g (150 $\mu L^{-1}$ well$^{-1}$, demonstrating that $Me_2SO$ in this assay system does not cause these non-lectin proteins to have nonspecific hemagglutinating effects.

Portions of all fractions described above were autoclaved for 30 min at 110 °C (total time 45 min; 15-min cooling and depressurization). Ten milligrams of each lyophilized sample was prepared for autoclaving as follows: saline fractions were dissolved in 2.0 mL of water; the ethanol and acid-soluble fractions were dispersed in 0.3 mL of 70% ethanol and 0.3 mL of 0.01 N acetic acid, respectively, and sonicated at 60 °C until completely dispersed (3–4 h) and brought to a total volume of 2.0 mL with distilled water. The alkali-soluble glutenins were added to 2 mL of distilled water and sonicated 3–4 h until dispersed. After being autoclaved, samples were freeze-dried and the resulting powder was dispersed in $Me_2SO$ for testing in the hemagglutination assay. The effect of autoclaving gluten fractions of refined soft winter wheat and commercial flours are reported herein.

## RESULTS AND DISCUSSION

Heretofore, the standard test for lectins excluded the possible detection of these substances in those protein fractions that are insoluble in the assay medium at near neutral pH. To test the theory of Weiser and Douglas (1976), the presence of these substances in the insoluble protein fractions required investigation. Initially, whole wheat was tested so as not to exclude any possible source of insoluble lectins. Table I demonstrates the presence of lectins in the three fractions of whole wheat protein.

Activity was expected in the buffered saline fraction because this wheat sample included the germ. Wheat germ lectins have been extensively studied (Goldstein and Hayes, 1978), and several forms have been identified from various strains of wheat (Rice, 1976; Thomas et al., 1977). These proteins are extractable with saline or near neutral buffer solution.

The highest activity was found in the NaOH fraction, followed by the ethanol fraction. The question remained, however, whether these substances were intrinsic to the endosperm. To answer this question, identical wheat as above was scrupulously freed of all germ and bran (less than 50% extraction), and the proteins separated into four fractions. Table II shows that the lectin activity in these samples is confined to their gliadin and acid-soluble glutenin fractions. The lack of lectin activity in the buffered saline fraction confirms that the germ was completely removed from this sample, and therefore the activity in the gliadin and acid-soluble glutenin fractions were derived exclusively from insoluble proteins intrinsic to the endosperm. No lectin activity was seen in the residual alkali-soluble glutenin fraction.

Douglas (1976) isolated a fraction from wheat gluten designated "gly-gli" that bound more tightly to the celiac intestinal mucosa than any other gluten protein. In our hemagglutination assay, this protein also had a very high lectin activity, i.e., 59 000 units/g of protein. Gly-gli is contained in our acid-soluble glutenin fraction and probably accounts for a significant amount of the activity of this fraction. However, lectin activity per se is not synonymous with toxicity (Jaffee, 1980), and further studies

Table II. Hemagglutinating Activity of Protein Fractions of Degermed Soft Winter Wheat and of Commerical Wheat Fractions

| source (% protein) | fractions | recovery, % of total protein | units/g of protein: raw | units/g of protein: autoclaved[a] |
|---|---|---|---|---|
| degermed soft winter wheat (7.1%) | buffered saline (albumins and globulins) | 15.9 | 0.0 | 0.0 |
| | 70% ethanol (gliadins) | 44.1 | 8 600 | 8 800 |
| | 0.01 N acetic acid (glutenins, acid soluble) | 8.1 | 29 000 | 27 000 |
| | 0.075 N NaOH (glutenins, alkali soluble) | 27.3 | 0.0 | 0.0 |
| commercial blend of 60% soft winter wheat and 40% hard winter wheat (9.9%) | buffered saline | 18.3 | 0.0 | 0.0 |
| | 70% ethanol | 49.8 | 18 000 | 17 000 |
| | 0.01 N acetic acid | 6.6 | 23 600 | 19 700 |
| | 0.075 N NaOH | 21.3 | 0.0 | 0.0 |

[a] Autoclaved for 30 min at 110 °C.

are necessary to correlate celiac toxicity with the type of lectin activity observed in our assay system.

Commercial flour (less than 70% extraction) that is a 60/40 mixture of soft and hard red winter wheat was similarly fractionated and tested for lectin activity. Table II indicates no lectin activity was detectable in the buffered saline and NaOH fraction, again demonstrating a lack of germ in this highly refined flour. As in our soft winter wheat sample, the activity was also confined to the gliadin and acid-soluble glutenin fraction. Note, however, the altered distribution of lectin activities between the gliadin and glutenin fractions. The gliadin fraction has more than twice the activity found in the corresponding fraction from our soft winter wheat sample. The activity in the glutenin fraction from the commercial flour is lower than that found in the soft winter wheat. These results suggest that lectin activity may vary with different wheat varieties.

Of greater relevance to celiac disease is the high heat stability of the gluten lectins. Note that in both soft winter wheat and commercial flour, the gliadin lectins are essentially unaffected by autoclaving (Table II). The acid-soluble glutenin lectins are only slightly affected by autoclaving under these conditions. In contrast, the lectins isolated from whole wheat by buffered saline were totally inactivated by autoclaving, as were all fractions isolated from navy beans. These may explain the fact that raw navy beans are highly toxic to rats but the cooked beans are not (Jaffee, 1980).

The presence of heat-stable lectins in the gluten fraction of wheat supports the theory of Weiser and Douglas (1976) regarding the pathogenesis of celiac disease. Most studies on celiac disease implicate the gliadin fraction as the toxic component (Frazer, 1962; Kasarda, 1980). However, some toxicity has been observed in the glutenin fraction as well (Jos et al., 1977; Van de Kamer, 1953). Although this toxicity may be the result of gliadin contamination, the demonstration of the presence of lectin activity intrinsic to this fraction underscores the possible contribution of this fraction to the overall gluten toxicity. Although the specific activity of the gliadin fraction in our soft winter wheat sample is lower than that in the acid-soluble gluten fraction, the total lectin activity in the former is almost 40% higher than in the latter. In the commercial wheat flour, which contains 40% hard red winter wheat, the total lectin activity in the gliadin fraction is almost 6-fold greater than that in the glutenin fraction. These findings are consistent with the observation that toxicity and antigenicity of gliadin is greater than for glutenin in many celiac patients (Beckwith and Heiner, 1966). Thus, although the specific activity of the gliadin fraction is lower than the acid-soluble glutenin fraction, our results showing greater total activity in this gliadin fraction are also consistent with the view that gliadin plays a major role in the pathogenesis of celiac disease.

The presence of lectins in gluten proteins suggests a mechanism for the binding of these proteins, or their digestion fragments, to the celiac intestinal mucosa. Lectin binding to cell surfaces is well established (Jaffee, 1980; Goldstein and Hayes, 1978) and is compatible with the immunological theory regarding the pathogenesis of celiac disease (Falchuk, 1979). The binding of gluten lectins to the celiac intestinal mucosa could cause direct damage, or subsequent absorption could trigger immunologic reactions that then cause damage to the intestinal mucosal cells.

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