Lectins in Grain Sorghum [*Sorghum bicolor* (L.) Moench]

Five varieties of grain sorghum [*Sorghum bicolor* (L.) Moench] with different chemical compositions and seed structural characteristics were analyzed for lectin activity by hemagglutination tests. Three varieties showed inhibition of hemagglutination by *N*-acetyl-D-glucosamine and D-maltose. Lectins in the three varieties did not differentiate the A, B, and O human blood groups; however, minor differences in agglutination titers were observed. For high tannin sorghum, extractability of lectin was increased after meals were treated with hexane or methanol. Divalent cations enhanced the hemagglutination titer of lectins in two of the varieties.

Lectins or agglutinins have been investigated extensively by diverse research institutions. These carbohydrate-specific proteins and glycoproteins are commonly present in animal tissues, microorganisms, and seeds of legumes and cereals. Because of their mitogenic properties, affinity for specific cell surfaces, possible adverse nutritional qualities, and possible involvement in disease resistance, lectins play a major role in many areas of biological and biochemical research (Sharon and Lis, 1972; Goldstein and Hayes, 1978; Sequeira, 1978). The binding of lectins to saccharides is similar to that of enzyme to substrate or of antigen to antibody. One report has attributed enzymic properties to lectins (Hankins and Shannon, 1978), and another has suggested that they might function as part of an "immune" system in plants (Ashwell and Morell, 1977).

The goal of this preliminary work was to determine the hemagglutinating properties, sugar specificities, and extractability of lectins in five varieties of grain sorghum—varieties that vary considerably in pericarp and endosperm structures and in chemical composition.

MATERIALS AND METHODS

Seed Samples. The five varieties were as follows: SC 301, all corneous ensosperm with thin pericarp; NSA 740, floury endosperm with thick pericarp; CK 60, intermediate floury/corneous endosperm with thick pericarp; TX 615, waxy endosperm with intermediate pericarp; GA 615, high tannin content with intermediate floury/corneous endosperm. The plants were grown in 1970 under identical agronomic conditions at College Station, TX, and seeds were stored under refrigeration (0 °C) after harvesting. The cultivars are further described elsewhere (Sullins, 1972; Sullins and Rooney, 1974; Neucere and Sumrell, 1980).

Extraction of the Lectin(s). Samples of whole grain were milled on a standard Wiley mill with a 40-mesh screen. Meals of each variety were extracted for lectins either directly or after removal of lipids with hexane. In each case, 500 mg of meal was extracted with 4.0 mL of phosphate-buffered saline (PBS), pH 7.2 (Hierholzer and Suggs, 1969), followed by centrifugation at 20000*g* for 30 min at 15 °C. Supernatants were analyzed for hemagglutination either directly or after storage at 2 °C for 2 days.

Preparation of Red Blood Cells. Human red blood cells (RBC) of the A, B, and O groups were obtained from a local blood bank. Five-milliliter portions of the suspended cells were washed 4 times with PBS buffer and centrifuged each time at 2000*g* for 20 min at 4 °C. The final packed cells were made to either 4% or 10% RBC suspensions in PBS for the agglutination assays.

Analytical Procedures. With microtitration plates (Cook's Engineering Corp., Alexandria, VA), hemagglutination assays were performed according to Prigent and Bourrillion (1976) by mixing samples with either 4% or 10% RBC suspensions. After agitation, the suspension was allowed to settle for several hours at room temperature, during which time relative titers were made on a visual scale. Inhibition of hemagglutination was performed with 4% solutions of saccharides in PBS as described earlier (Prigent and Bourrillion, 1976). Analysis of lectin(s) activation by Ca^{2+} and Mn^{2+} was performed according to Galbraith and Goldstein (1970). Lectin samples were dialyzed against PBS solutions containing 1 mM $MnCl_2$ and 1mM $CaCl_2$ and then against PBS buffer alone to remove unbound metals. Protein determinations were made by the method of Lowry et al. (1951).

Table I. Hemagglutination Activity[a] in PBS Extracts from Five Varieties of Grain Sorghum

blood group	variety GA 615	CK 60	NSA 740	SC 301	TX 615
A^+	–	+ (75)[b]	+ (130)	–	+ (225)
B^+	–	+ (150)	+ (260)	–	+ (550)
O^+	–	+	+	–	+
AB^+	–	+	+	–	+
A^-	–	+	+	–	+

[a] Hemagglutination activity determined with human RBC. [b] Parentheses denote specific titer defined as the minimum weight of protein (micrograms) required for visible agglutination of RBC.

RESULTS AND DISCUSSION

Qualitative hemagglutination analysis of lectin activity in the five varieties of sorghum is shown in Table I. With equal quantities of protein applied to each well, only CK 60, NSA 740, and TX 615 showed hemagglutination. No differentiation could be made among the blood groups or Rh factors from visual inspection of the plates. GA 615 and SC 301 showed no agglutination activity. Relative titers of agglutination in the three varieties that showed activity were determined after serial dilution with A^+ and B^+ RBC. Values in parentheses show that varieties CK 60 and NSA 740 had slightly higher titers than did TX 615. These values are merely approximate, however, because the reading of the end point is subjective and might change somewhat depending on the individual cell sample.

The carbohydrate-binding specificity of lectins varies with simple sugars and macromolecules such as glycoproteins and lipopolysaccharides. Sugar–lectin complementarity in this study was tested for inhibition of agglutination with the following sugars: L-arabinose, *N*-acetyl-D-glucosamine, D-fucose, D-galactose, D-glucose, D-mannose, D-maltose, and D-xylose. As noted in Table II, none of these sugars except *N*-acetyl-D-glucosamine and D-maltose inhibited the agglutination reaction. Other plant lectins were reported to have complex sugar specificities; these include those isolated from kidney beans (Kornfield and Kornfield, 1970) and from the scarlet runner bean (Nowakova and Kocourek, 1974). The configuration of the hydroxyl groups on the simple sugars and polysaccharides

Table II. Sugar Specificity for Inhibition of Hemagglutination[a] in PBS Extracts of Three Varieties of Grain Sorghum

sugar[b]	inhibitory act.[c]
L-arabinose	-
N-acetyl-D-glucosamine	+
D-fucose	-
D-galactose	-
D-glucose	-
D-mannose	-
D-maltose	+
D-xylose	-

[a] Hemagglutination activity determined with human A^+ RBC. [b] Final concentration of sugars was 20 mg/mL. [c] Qualitatively the same for varieties CK 60, NSA 740, and TX 615.

is critical for binding to lectins. Generally speaking, lectins interact with the nonreducing terminal glycosyl groups of polysaccharides and chain ends of glycoproteins. The complex interactions involved for different sugar–lectin systems have been reported by Goldstein and Hayes (1978) and by Allen et al. (1973).

For further investigation, lectins in varieties GA 615 and SC 301, 40-mesh samples, were defatted with hexane followed by extraction with PBS. Results of hemagglutinating tests showed that lectins in GA 615 were extractable after hexane treatment but not those in SC 301. Other experiments showed that lectins were readily extracted from GA 615 after removal of tannins with methanol. These two varieties were also tested for possible enhancement of agglutination induced by divalent cations. Lima bean lectins, for example, showed reduction in hemagglutination titers by 75% after removal of manganese ions (Galbraith and Goldstein, 1970). Other studies showed that the manganese ion is required for activity of soybean lectin (Jaffe et al., 1977). In the present study full-fat meals of GA 615 and SC 301 were extracted with PBS under normal conditions and then treated with $CaCl_2$ and $MnCl_2$ followed by hemagglutinating analyses. Both Ca^{2+} and Mn^{2+} activated the lectins in SC 301 with human blood groups A^+, A^-, O^+, and B^+. GA 615, however, showed trace activity only with cells from blood groups O^+ and A^+. Extensive research involving concanavalin A has shown that metal coordination (Ca^{2+} and Mn^{2+}) in that lectin consisted of four protein ligands and two water molecules (Gold and Balding, 1975). The resultant octahedral complex formed is undoubtedly one step in the chain of reactions leading to hemagglutination.

Undoubtedly, the carbohydrate binding sites on lectins are much more complex than may appear from inhibition studies with simple sugars and from cation activation. In this preliminary study, at least two simple sugars did bind to lectin(s) in grain sorghum. Also, divalent cations did enhance agglutination reactivities in two of the varieties. No differences existed in sugar specificities of the lectins among the varieties, and the A, B, and O human blood groups were not differentiated. The results indicate differences in extractability of lectins among the five varieties.

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Selective Toxicity of *Ocimum canum* Extract against *Cyperus rotundus* L. 1

An aqueous extract of *Ocimum canum* (hoary basil) has shown toxic activity against purple nut sedge, *Cyperus rotundus* L. In laboratory tests, the extract has no toxic effect on seed germination and growth of plants of *Vigna mungo* (L.) Hepper, *Triticum aestivum*, and *Oryza sativa*. The extract can be applied to moist or flooded soil. Characterization of the active ingredient is in progress.

Many workers (Pandey, 1980; Anon, 1977; Turner, 1977; Wills, 1976, 1977) have tested various synthetic chemicals against *Cyperus rotundus* (purple nut sedge), a noxious weed, and reported effective control with their repeated applications. However, plant products might provide an alternative source of weed control (Rizvi et al., 1980, 1981)

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