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Haemagglutinin in *Triticale*

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Abstract. Saline extracts of several varieties of *Triticale* had haemagglutinin activity against rabbit, rat and fowl erythrocytes. In contrast to the wheat germ lectin the *Triticale* lectin was inactive against human B, O blood group type erythrocytes and rather high concentrations of the lectin are needed to agglutinate human A blood group type erythrocytes. The *Triticale* lectin was purified about 20–fold with a 10% recovery of activity from one of the varieties (DTS 138) by $(NH_4)_2SO_4$ fractionation followed sequentially by chromatography on DEAE-cellulose and sulphopropyl-Sephadex. Approximately 4 μg of the purified lectin caused visible agglutination with trypsinised rabbit erythrocytes. Among a variety of sugars tested D-glucose, D-mannose and N-acetyl-D-glucosamine (2·5—7·5mM) caused inhibition of agglutination.

Keywords. *Triticale;* haemagglutinin; animal erythrocytes; blood group specificity.

1. Introduction

Triticale is a man made cereal which is a product of the genomes of wheat (*Triticum*) and rye (*Secale*). Extensive *Triticale* breeding trials in Canada and Mexico have demonstrated the suitability of some varieties for commercial exploitation (Macintyre and Campbell 1974; Hulse and Laing 1974). In view of the possibilities of growing *Triticale* in some rain fed dry areas where wheat cannot be easily cultivated there have been some *Triticale* breeding programmes in India (Srivastava 1974; Sisodia 1974).

It has been known for some time that certain varieties of *Triticale* have anti-nutritional factors like alkyl resorcinols, phytates, tryptic and chymotryptic inhibitors (Tsen 1974). Although an extensive amount of work has been done with haemagglutinins (lectins) from legumes, similar information with cereals is rather limited. Current interest in the chemical nature and biological properties of haemagglutinins from cereals such as wheat (Allen *et al* 1973) and rice (Takahashi *et al* 1973) has prompted us to examine several *Triticale* varieties for lectin activity. The present paper deals with some of the properties of a partially purified haemagglutinin from *Triticale.*

2. Materials and methods

2.1. *Materials*

The varieties DTS 138 (Delhi *Triticale* strain—hybrid of IMJM × M 1010. IMJM is from Japan and M 1010 is a Mexican variety), M 1019 (Mexican variety

from CIMMYT), S 141 (Swedish strain) were obtained from M G Joshi, Genetics Division, Indian Agricultural Research Institute, New Delhi. The varieties JNK 6 To 90 (Jawaharlal Nehru Krishividyalaya strain, Jabalpur, originally from CIMMYT, Mexico) and Branco (CIMMYT, Mexico) were obtained from S R Shurpalekar of the Cereal Technology Division of our Institute. All varieties of *Triticale* were hexaploids. Human A, B and O type blood samples were obtained from the blood bank of the local hospital. Cow, sheep and buffalo blood were obtained from Government Veterinary Hospital, Mysore. Blood from other species of animals was obtained from the animal colony of our Institute. All chemicals such as sugars, buffer salts etc. were of the highest purity commercially available. Trypsin (about 2000 Anson units/g) was obtained from Merck A G, Darmstadt, W. Germany.

2.2. *Methods*

The *Triticale* samples were powdered (in a hand operated coffee grinder) and defatted thrice, each time intermittently shaken with five volumes (w/v) of petroleum ether (40-60°C) for 30 min and finally the powder was air dried. One g of the sample was suspended in ten volumes of 0.9% NaCl and kept for 18 hr at 5° C with occasional shaking. Later it was centrifuged and the clear or slightly opalescent supernatant was tested for haemagglutinin activity. Erythrocytes were trypsinised by the procedure of Liener (1955). Protein was estimated by the Folin-Ciocalteau procedure.

For the determination of haemagglutinin activity, 0.2 ml of isotonic saline containing various amounts of lectin (2-fold serial dilutions) were mixed with 0·2 ml of trypsinised rabbit erythrocytes (2% suspension) (Liener 1966) on plexiglass plates with concave receptors (haemagglutination slabs) and kept at 5° C for 18 hr. The plates were visually examined for agglutination and the positive results were occasionally confirmed by a microscopic examination. The highest dilution causing visible agglutination was regarded as the titre. This serological method was liable to a subjective error of the order of 10%. The amount of protein present at this dilution represents nearly the minimum quantity of protein necessary for agglutination, under the experimental conditions.

For determination of the agglutination activity against different species of erythrocytes the above procedure was used both with the trypsinised and the untrypsinised erythrocytes. Purified *Triticale* lectin from variety DTS 138 (after sulfopropyl Sephadex chromatography) was used at various concentrations ranging from 50μg/ml. For studying the inhibition of agglutination by different sugars, 25 μg of the purified lectin in 0·2 ml of saline was preincubated for 1 hr at 37°C with various concentrations (2·5 to 20 mM) of the different sugars in saline. Later 0·2 ml of trypsinised rabbit erythrocytes were added and kept overnight (18 hr) at 5°C. The plates were then observed for agglutination.

The reversibility of agglutination with sugars (specifically N-acetyl D-glucosamine) was studied by the tube procedure (Lis *et al* 1970). Polyacrylamide gel electrophoresis using Tris-glycine buffer at pH 8·3 was carried out according to the method of Davis (1964).

Table 1. Haemagglutinin activity of different varieties of *Triticale*.

Variety of *Triticale*	MCA* (μg/400 μl)	
	(a)	(b)
DTS 138	90	25
S 141	100	35
M 1019	85	20
JNK 6 TO90	96	45
Branco	135	34

*MCA—Minimum concentration of protein needed for visible agglutination of trypsinised rabbit erythrocytes under assay conditions described in text.

(a) The crude saline extract was used as such. Other methods of extraction at pH 4 gave similar results.

(b) The protein was precipitated from the crude saline extract [about 60% saturation of $(NH_4)_2SO_4$] and dissolved in 6 mM potassium sodium phosphate buffer pH 7·4. It was dialysed extensively against the same buffer containing 0·01% sodium azide and then tested for activity.

3. Results and discussion

Aqueous or saline extracts of several varieties of *Triticale* agglutinated trypsinised rabbit erythrocytes (table 1). The lectin was partially purified from aqueous extracts by $(NH_4)_2SO_4$ fractionation (39 g of $(NH_4)_2SO_4$ per 100 ml at 4°C). Approximately 45% of the inactive protein was retained on a DEAE-cellulose column previously equilibriated with 6 mM sodium-potassium phosphate buffer pH 7·4. The effluent containing the lectin was dialysed and then absorbed on a sulfopropyl Sephadex C-50 column equilibrated with 0·1 M sodium acetate buffer pH 3·8. The column was washed successively with the same buffer containing increasing concentrations (0·10; 0·25 and 0·50 M) of NaCl. Most of the lectin (about 20% of the total protein applied to the column) was eluted in the buffer containing 0·5 M NaCl. The overall purification was about 20×and approximately 10% of total activity was recovered. The purified lectin showed two major components and a few minor components on polyacrylamide gel electrophoresis (7·5 gel) at pH 8·3. Microheterogeneity of purified wheat germ lectin was recently reported (Ewart 1975). The *Tritiacle* lectin was not held on carboxymethyl-cellulose columns at pH 5·2 and differed in this respect from the wheat germ lectin (Ewart 1975). It was stable to acetone fractionation [60% (v/v) acetone at 5° for 30 min] but was inactivated by a heat treatment (50% loss of activity when heated at 60° C for 10 min in 6 mM phosphate buffered saline at pH 7·4). It was not adsorbed on Con A-Sepharose 6B columns (Bessler and Goldstein 1973). Attempts to demonstrate the requirement of a metal ion (Ca^{2+} or Mg^{2+}) for agglutination after treatment with EDTA (Pavlova *et al* 1971) were unsuccessful.

The purified lectin (from variety DTS 138) readily agglutinates trypsinised erythrocytes from rabbit, rat and fowl. The agglutination against trypsinised human blood group A erythrocytes was sluggish and needed high concentrations of the lectin (table 2). It did not aglutinate either normal or trypsinised human B and O type blood group erythrocytes or erythrocytes from other species such as cow, buffalo, sheep and guinea pig. The agglutination of trypsinised rabbit erythocytes was inhibited by low concentrations (2·5 mM to 7·5 mM) of D-glucose, D-mannose and

Table 2. Specificity of agglutination of the *Triticale* lectin towards erythrocytes from different species.

Species	MCA (μg/400 μl)*	
	Untrypsinised erythrocytes	Trypsinised erythrocytes
Human A	0	600
B	0	0
O	0	0
Rabbit	19	4
Rat	38	5
Fowl	38	10

Highly purified lectin from *Triticale* variety DTS 138 (after sulfoprophyl Sephadex Chromatography) was used in these experiments. The values given represent the minimum concentration of the lectin required for visible agglutination under the experimental conditions described in the text.

N-acetyl-D-glucosamine. A variety of other sugars such as D-galactose, D-fructose, D-arabinose, D-xylose, lactose, L-fucose, maltose and L-rhamnose did not inhibit agglutination even at 20 mM.

The partially purified wheat germ lectin agglutinated human A, B, AB and O group erythrocytes (Liske and Franks 1968) while haemagglutination activity with A_1 and B group erythrocytes was reported with the crystalline lectin (Nagata and Burger 1972). Our preliminary studies indicated that the *Triticale* lectin differed from wheat germ lectin in some molecular properties (such as size and net charge at different pH values) and specificity against different erythrocytes. Further detailed work on the *Triticale* lectin is necessary to elucidate the fundamental chemical and biological differences with the wheat germ lectin.

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