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Human erythrocyte specific lectin from the seeds of Indian coral tree, *Erythrina variegata* Linn, var. *orientali* Linn, Merrill

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Abstract. Human erythrocyte specific lectin was isolated from the seeds of *Erythrina variegata* Linn. var. *orientalis* Linn. Merrill. The lectin preferentially agglutinated erythrocytes in the sequence of O>B>A = AB. The lectin was purified 19-fold by affinity chromatography on acid treated sepharose 4B with an yield of 81%. The purified lectin was found homogeneous on polyacrylamide gel electrophoresis. The erythroagglutination reaction was inhibited by N-acetyl-D-galactosamine, D-galactose and lactose at very low concentration. The haemagglutination by the purified lectin was not inhibited by different hexose and pentose sugars even at high concentration. The purified lectin was a glycoprotein and agglutinated leucocytes at 3 µg protein concentration. The lectin induced transformation of peripheral blood lymphocytes in cultures.

Keywords. *Erythrina variegata;* lectin purification; human erythrocyte specific; mitogen.

Introduction

It has been known for many years that extracts of a variety plant seeds contain haemagglutinins which are now called lectins. Plant lectins are carbohydrate binding proteins which recognize and interact with specific monosaccharide or oligosaccharide units and they agglutinate cells and/or precipitate glycoconjugates (Goldstein *et al.,* 1980). Lectins are used extensively in immunohaematology as blood typing reagents and in testing erythrocyte polyagglutinable states (Prokop and Uhlenbruck, 1969; Levine, 1974; Gold and Balding, 1975; Brown and Hunt, 1978; Judd, 1979). They have proven to be useful tools for studying cell surface architecture and related membrane functions. The effect of plant lectins on eukaryotic cells is quite varied. Some are toxic, others are mitogenic for cells such as lymphocytes, and still others bind to the cell surface and apparently do not perturb cellular metabolism (Rapin and Burger, 1974; Nicolson, 1974; Lis and Sharon, 1977; Goldstein and Hayes, 1978; Hart, 1980; Weir, 1980).

Erythrina Linn (Leguminosae), a genus of trees or shrubs, rarely herbs, is widely distributed in tropical and subtropical regions. About eight indigenous species and ten introduced ones occur in India (The wealth of India, 1952). Within the genus *Erythrina,* erythroagglutinating activities of various specificities in crude extracts of the seeds had been demonstrated (Boyd, 1950; Cazal and Lalaurie, 1952; Tiggel-

Abbreviation used: PBS, Phosphate buffered saline.

man-van Krugten *et al.,* 1956; Makela, 1957; Boyd *et al.,* 1961; Martin and Bomchill, 1966; Bird and Wingham, 1973; Lee *et al.,* 1977).

The present work describes a new human erythrocyte specific lectin, a glycoprotein, isolated from the seeds of the Indian coral tree *Erythrina variegata* (Linn.) var. *Orientalis* (Linn.) Merrill and some of its properties.

Materials and methods

Dry seeds, after the removal of the testa, were finely ground in a hand operated grinder avoiding generation of heat. The following steps were carried out at 4-6° C. Finely ground beans (10 g) were stirred overnight in 500 ml of 0.15 M phosphate buffered saline pH 7.2 (PBS). The resulting mixture was filtered through cheese cloth and centrifuged at 20,000*g* for 60 min to remove the seed debris. The supernatant (crude extract) was subjected to $(NH_4)_2\ SO_4$ fractionation of proteins. The precipitates obtained at different $(NH_4)_2\ SO_4$ saturations were dissolved in PBS contaning 0.1 mM each of $CaCl_2$, $MnCl_2$ and $MgCl_2$, and dialysed extensively against the same buffer. An aliquot (100 mg protein) of the most active fraction 30-70% $(NH_4)_2\ SO_4$ saturation was applied to a column of acid treated sepharose 4B (Ersson *et al.,* 1973) through washing of the column (30 × 1.2 cm) with PBS containing metal ions and elution of the lectin from it with 0.05 M glycine HCl buffer, pH 3.0 in 0.5 M NaCl. The active fractions were pooled together, neutralized by saturated solution of $NaHCO_3$, dialysed against PBS containing the metal ions and lyophilised.

Normal typed human erythrocytes were obtained from S.S.K.M. Hospital, Calcutta. Blood from different species of animals used in this study were procured either from the Insitute Animal House or from other local sources. Erythrocytes were washed in PBS and a 2% cell suspension in PBS was used to assay for activity. Haemagglutination was determined in Plastic microlitre dishes by serially diluting 50 µl samples into 50 µl PBS. Blood cells (50 µl) were added to each well and haemagglutination was usually determined after 60 min. Control wells containing only PBS and red cells were always included. Inhibition of haemagglutination was examined by using several sugars at mM concentrations in PBS. Proteins were determined by Lowry's method (Lowry *et al.,* 1951) using bovine serum albumin as Standard. Polyacrylamide gel electrophoresis was carried out on 7.5% gels according to Davis (1964). Total neutral sugar was determined by anthrone method (Spiro, 1966). Leucoagglutination was carried out on a microscope slide using 50 µl leucocytes suspension isolated from 2 ml whole blood and suspended in 2 ml PBS by the method of Boyum (1968). The mitogenic activities of the lectin preparation were measured by assessing their effect on the incorporation of $[^3H]$-thymidine by the cultured human lymphocytes.

Results

Table 1 presents the results of haemagglutination test conducted on the erythrocytes of several species of animals using the crude extract of the seed. No agglutination was observed with erythrocytes of rabbit, guinea-pig, rat, mouse, goat, sheep, cow, buffalow, horse, chicken, pigeon and frog even at a concentration of 500 µg protein. Interestingly, the extract selectively agglutinated human erythrocytes belonging to O, B, A and AB groups. Further, the extract showed highest

Table 1. Haemagglutination test by crude extract of seeds of *Erythrina variegata* Linn. var. *orientalis* Linn. Merrill.

Agglutination	Erythrocytes	Minimum amount of protein required for agglutination (μg)
Positive	Human O	65
	B	140
	A, AB	285

A 2% suspension of washed RBC in PBS was used. Incubation time was 60 min at 37°C. No agglutination was observed with the 2% washed erythrocytes of rat, guinea-pig, mouse, goat, rabbit, dog, cow, sheep, horse, buffalow, chicken, pigeon and frog using 500 μg of protein.

affinity for human 'O' group erythrocytes, at a much lower protein concentration. The purification procedure (table 2) indicates the isolation of the lectin with higher specific activity. The activity as measured by haemagglutination indicated the recovery of 81% of the original activity with a 19-fold purification. Leuco-agglutination phenomenon was observed with 3μg purified lectin in 50 μl cell suspension within 2-5 min at 37° C suggesting the presence of a strong leucoagglutination property in the lectin along with the haemagglutinating activity. Polyacrylamide disc gel electrophoresis (figure 1) revealed a sharp single protein band. It can be seen that the purified lectin band corresponded to the fastest moving band amongst other proteins of 30-70% $(NH_4)_2$ SO_4 saturation fraction under analogous experimental condition. The purified lectin was a glycoprotein containing

Table 2. Summary of purification of the lectin from the seeds of *Erythrina variegata* Linn. var. *orientalis* Linn. Merrill.

Fractions	Total Protein (mg)	Total haemagglutination activity with 'O' group erythrocyte (titre)	Specific* activity	Yield (%)	Purification fold
Crude Extract	2200	7240	3.3	100	1
$(NH_4)_2SO_4$ saturation					
0–30%	800	nil	—	—	—
30–70%	394	6516	16.5	90	5
70–90%	620	200	0.3	—	—
Supernatant	215	nil	—	—	—
Affinity purified fraction	92	5888	64	81	19

★ Specific activity was expressed as titre per mg of protein and the titre was defined as the highest dilution showing visible agglutination.
Minimum concentrations of the purified lectin for erythro-agglutination of O, B and A group erythrocytes were in the range of 3-4 μg, 5-7 μg and 10-14 μg respectively.

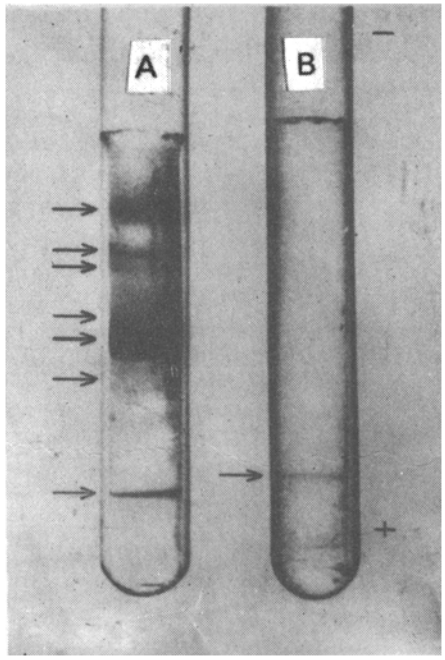

Figure 1. Polyacrylamide gel electrophoresis at pH 8.3 on 7.5% gels, current 5 mA/gel, time 45 min, amount of protein applied 50 µg in each case. Stain: Coomassie brilliant blue in methanol acetic acid mixture. **A.** 30-70% $(NH_4)_2$ SO_4 precipitated protein. **B.** Purified lectin.

approximately 10% neutral sugars. A number of mono- and disaccharides were tested for their ability to inhibit the agglutination reaction between the erythrocytes and the lectin (table 3). The haemagglutinating activity of this lectin was completely inhibited by N-acetyl-D-galactosamine, D-galactose and lactose respectively, and the rest of the sugars tested did not elicit any inhibition of the reaction under identical experimental conditions.

Table 3. Effect of various sugars on erythro-agglutination.

Inhibition of agglutination	Sugar	Concentration (mM)
Positive	D-Galactose	3
	Lactose	1.3
	N-Acetyl-D-galactosamine	2

Human O group erythrocytes were used for the haemagglutination assay. 30 min preincubation of 25 µg purified lectin with the test sugar was carried out in each case. Rest of the procedure is same as described in the text.

No inhibitory effect even at 120 mM concentration of D-glucose, D-mannose, fructose, L-fucose, D-arabinose, L-xylose, L-rhamnose, glucopyranoside, methyl-α-D-mannopyranoside, D-glucosamine, D-mannosamine, L-sorbose, sorbitol, D-mannitol, D-glucuromic acid, maltose, sucrose, cellobiose.

Discussion

Some seeds of the genus *Erythrina,* like several others belonging to leguminosae, contained erythroagglutinating activity. Indian coral tree has been attributed with diverse medicinal values in Ayurveda and ancient Hindu medicine (Chopra *et al.,* 1956). In a chemical and pharmacological evaluation of the genus *Erythrina,* the alkaloids of *Erythrina variegata* L. were studied by Ghosal *et al.* (1972). Recently a similar study on *Erythrina mulungu,* which is a medicinal plant used in Folk medicine, has been reported (Sarragiotto *et al.,* 1981). Lee *et al.* (1977) in a survey of lectin in south-east Asian Leguminosae recorded for the first time that *Erythrina variegata* L. agglutinated specifically human erythrocytes of A, B, O and AB blood groups. However, no particular attention was given to this property of the seed extracts. A D-galactose binding lectin, agglutinating human erythrocytes of blood groups O, B, A and AB, was purified from the seeds of Indian coral tree, *Erythrina variegata* L. var. *orientalis* Linn. Merril. by affinity chromatography on acid-treated sepharose 4B gel. The extract exhibited a high affinity for O group erythrocytes and in suitable dilutions specifically identified this blood group. The lectin preferentially agglutinated human erythrocytes, and exhibited a preference in the sequence of O>B>A=AB. The lectin is a glycoprotein having leucoagglutinating property (Datta and Basu, 1981). The purified lectin showed mitogenic activity with human peripheral lymphocytes in preliminary observations. Gilboa-Garber and Mizrahi (1981) isolated a new mitogenic D-galactosephilic lectin from the seeds of coral tree, *Erythrina corallodendron.* The lectin was shown to be a glycolprotein of molecular weight around 110,000-120,000, and exhibited a considerable mitogenic activity towards human lymphocytes (predominantly *T* cells) only after their treatment with neuraminidase. However, in regard to the intensity of their agglutinating activity towards erythrocytes, obtained from different animals, and human donors of diverse ABO blood groups, the lectin strongly agglutinated only untreated human erythrocytes and exhibited a preference for O and B compared with A red blood cells. In a study of D-galactose binding lectins isolated from the seeds *Butea frondosa, Erythrina indica* and *Mamordica charantia,* Horejsi *et al.* (1980) showed that the purified *E. indica* lectin agglutinated human group O erythrocytes slightly better than those from B and A groups respectively. The purified lectin was shown to be a metallo-glycoprotein having a molecular weight, 66,200 ± 3000. Bhattacharyya *et al.* (1981) purified non-specific D-galactose binding lectins from some *Erythrina* species. A D-galactose/N-acetyl-D-galactosamine–specific lectin was isolated from *Erythrina crista galli* and it agglutinated human erythrocytes of all blood types as well as rabbit erythrocytes (Iglesias *et al.,* 1982), and the immunochemical studies on the combining site of the lectin were done by Kaiadas *et al.* (1982).

Therefore, the finding of this new lectin with erythro-agglutinating, leucoagglutinating and mitogenic property would be of importance in the field of immunohaematology and both structural and cellular immunology.

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