

Identification, isolation and some properties of lectin from the seeds of Indian coral tree [*Erythrina variegata* (Linn.) var. *orientalis* (Linn.) Merrill]

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A D-galactose-binding lectin agglutinating human erythrocytes has been purified from the seeds of the Indian coral tree [*Erythrina variegata* (Linn.) var. *orientalis* (Linn.) Merrill] by affinity chromatography on acid-treated Sepharose-6B gel. It has a higher reactivity for O-group erythrocytes. The lectin is a glycoprotein having a leucoagglutinating property.

Lectins, sugar-binding proteins or glycoproteins with the capacity to agglutinate cells, are widely dispersed in plants, vertebrates and invertebrates (Goldstein *et al.*, 1980). Some of these substances are highly specific in that they agglutinate erythrocytes of the human ABO or MN blood groups, whereas others agglutinate independently of these blood groups (Prokop & Uhlenbruck, 1969; Goldstein & Hayes, 1978; Brown & Hunt, 1978). Seed extracts of several species of the genus *Erythrina*, belonging to the family Leguminosae, display haemagglutinating activity (Toms & Western, 1971; Gold & Balding, 1975). Lee *et al.* (1977) documented, in a survey of lectins among south-east-Asian members of the Leguminosae, that the seed extracts of *Erythrina indica* Lamk and *Erythrina variegata* Linn. agglutinated human erythrocytes of A, B, O and AB blood groups, whereas *Erythrina guineensis* G. Don seed extract was guinea-pig-erythrocyte-specific. The latter two species were listed for the first time; however, no particular attention was given by the authors to this property of the seed extracts.

Many of the species of *Erythrina* are cultivated in India, and several varieties or forms are known (Council of Scientific and Industrial Research, 1952). We present herewith the identification, purification and partial characterization of the lectin in the seeds of the Indian coral tree [*Erythrina variegata* (Linn.) var. *orientalis* (Linn.) Merrill].

Part of this work was presented in abstract form at the International Symposium on Lectins as Tools in Biology and Medicine, held at the Indian Institute of Experimental Medicine, Calcutta 700 032, India, on January 7-9, 1981.

Experimental

Indian-coral-tree [*Erythrina variegata* (Linn.) var. *orientalis* (Linn.) Merrill] seeds were obtained from

Botanical Survey of India, Indian Botanic Garden, Howrah, India.

Normal typed human erythrocytes were obtained from Central Blood Bank, Calcutta, India. Blood samples from the other species used in the present study were procured from the Institute Animal House and other local sources.

Sepharose-6B, Coomassie Brilliant Blue R, Porcine thyroglobulin, *N*-acetylneuraminic acid, fetuin and the sugars used were from Sigma. All other chemicals required in the present study were either of analytical grade or of the best quality available.

Acid-treated Sepharose-6B was prepared as described by Ersson *et al.* (1973) by washing the gel with cold 0.2 M-HCl and adding the acid-washed gel (25 ml) to 0.2 M-HCl (50 ml). This suspension was placed in a gently shaking water bath at 50°C for 2 h. The gel was washed with 0.15 M-phosphate-buffered saline [composition (g/litre): NaCl, 8; Na₂HPO₄ (anhydr.), 1.15; KCl, 0.2; KH₂PO₄ (anhydr.), 0.2], pH 7.2, and was ready for use.

The haemagglutination assay was performed by standard serial-dilution technique of Salk (1944), by using a 2% erythrocyte suspension in phosphate-buffered saline. The agglutination was assessed after 60-min incubation at 37°C. The activity was expressed as the titre, the reciprocal of the greatest dilution at which agglutination could be detected. Leucocytes were isolated (Boyum, 1968) from blood, and leucoagglutination was performed on a microscope slide after incubation as stated above. Inhibitory activities of carbohydrates, sialic acid, fetuin and porcine thyroglobulin were determined in a similar system with serial dilutions of the compounds.

Protein was determined by the method of Lowry *et al.* (1951), and those in column eluate fractions were also monitored spectrophotometrically at 280 nm.

The electrophoretic procedure used was essentially that described by Ornstein & Davis (1962). A 50 μ g sample of protein was applied on top of the spacer gel. The spacer and the running gels contained 2.5% (w/v) and 7.5% (w/v) polyacrylamide respectively. The gels were run at 20°C for 30 min with a current of 5 mA/gel tube. Protein staining was done by the method of Chrambach *et al.* (1967).

Total neutral sugar was determined by the phenol/ H_2SO_4 method described by Dubois *et al.* (1956), with glucose as a standard.

Results and discussion

Dehusked seeds (10g) of the Indian coral tree were crushed and mixed with 300 ml of phosphate-buffered saline containing 0.1 mM-CaCl₂, -MnCl₂ and -MgCl₂ at 4°C and stirred overnight. The resulting mixture was filtered through cheese-cloth and filtrate cold-centrifuged at 20000g for 60 min. The clear supernatant (crude extract) was used for agglutination of erythrocytes from various species, namely human, rat, guinea pig, mouse, goat, rabbit, dog, cow, sheep, horse, buffalo, chicken, pigeon and frog. Only erythrocytes from human O, A and B blood groups were agglutinated. In all other cases, no agglutination could be observed. This indicated the human-blood-group-specificity of the lectin in the crude extract. Partial purification (Table 1) was achieved by (NH₄)₂SO₄ fractionation of proteins. The fractions precipitated by 0–20%, 20–40%, 40–60%, 60–80% satd. (NH₄)₂SO₄ were dissolved in phosphate-buffered saline containing 0.1 mM-CaCl₂, -MnCl₂ and -MgCl₂. These fractions and the supernatant after 60–80% saturation were dialysed with several changes against the same buffer. About 60% of the total haemagglutinating activity was recovered in the material precipitated at 40–60% saturation. Further purification

of this fraction was achieved by affinity chromatography on a column (22 cm \times 1.2 cm) of acid-treated Sepharose 6B. This was washed and equilibrated with phosphate-buffered saline containing 0.1 mM-CaCl₂, -MnCl₂ and -MgCl₂, and a portion of the fraction (40 mg of protein) was applied on to this column. The unabsorbed proteins were washed out by the same buffer. Some haemagglutinating activity was detected in the non-absorbed protein peak. The lectin was eluted from the column by 0.05 M-glycine/HCl buffer, pH 3.0, containing 0.5 M-NaCl. Most-active fractions were pooled, neutralized with a saturated solution of NaHCO₃, dialysed against phosphate-buffered saline containing the metal ions and freeze-dried. The activity as measured by haemagglutination demonstrated the recovery of 43% of the original activity with a 22-fold purification. In Table 1 this affinity-purified fraction was expressed in terms of total protein (600 mg) obtained from 40–60%-(NH₄)₂SO₄ saturation. The purified lectin thus obtained gave a single protein band on disc-gel electrophoresis, indicating the homogeneity of the lectin. It was further observed that this band corresponded to the fastest-moving band among others obtained under similar experimental conditions with the proteins of crude extract and 40–60% satd.-(NH₄)₂SO₄ fractionation respectively.

Some characteristic properties of the lectin are shown in Table 2. The crude extract demonstrated a selectivity in agglutinating human erythrocytes belonging to O, B and A blood groups in the order of O > B > A. This pattern was maintained through all the purification steps. The purified lectin agglutinated blood-group-O erythrocytes at a minimum concentration of 3 μ g/ml, B-group erythrocytes at 6 μ g/ml and A-group erythrocytes at 12 μ g/ml.

Sugar analysis demonstrated that lectin was a glycoprotein composed of between 9 and 12% neutral sugars.

Table 1. Summary of purification of the lectin

The haemagglutination test was done with 2% washed erythrocytes of human O-group blood in phosphate-buffered saline. Haemagglutination activity was expressed as titre (see the text). Specific activity was expressed as titre/mg of protein.

Fraction	Total protein (mg)	Total haemagglutination activity (titre)	Specific activity (titre/mg)	Yield (%)	Purification (fold)
Crude extract	2850	7980	2.8	100	1
Precipitates from (NH ₄) ₂ SO ₄ fractionation at the following saturations:					
0–20%	67.2	0	0	—	—
20–40%	800.0	0	0	—	—
40–60%	600.0	4800	8	60	3
60–80%	625.0	1000	1.6	12	—
Supernatant	33.0	0	0	—	—
Affinity-purified fraction	54.0	3456	64	43	22

Table 2. *Some characteristic properties of the purified lectin*

Assays were done as described in the text, with human erythrocytes. The amount of lectin used in each case for the inhibition study was 25 μ g.

Erythrocyte blood group	Minimum amount of protein for agglutination (μ g)	Lowest concentration of sugar for complete inhibition of agglutination (mM)		
		Lactose	D-(–)-Galactose	N-Acetyl-galactosamine
O	3	1.5	2.8	1.1
B	6	1.5	2.8	1.1
A	12	1.5	2.8	2.2

Leucocytes from human blood were agglutinated by the lectin within 5 min at 37°C. This indicates the presence of a strong leucoagglutination property of the lectin in addition to its haemagglutinating activity.

Agglutination of human blood-group-O, -A and -B erythrocytes was inhibited by lactose, D-galactose and N-acetyl-D-galactosamine (Table 2). The sugars D-glucose (92 mM), D-mannose (92 mM), D-arabinose (111 mM), xylose (111 mM), N-acetyl-D-glucosamine (75 mM) and L-fucose (101 mM) were ineffective under the same experimental conditions. In addition, no interaction of the lectin with fetuin (25 mM), porcine thyroglobulin (25 mM) or sialic acid (25 mM) could be observed.

Horejsi *et al.* (1980) recently described the isolation of D-galactose-binding lectins from the seeds of *Butea frondosa* (flame of the garden), *Erythrina indica* and *Momordica charantia* (bitter gourd) by affinity chromatography on α -D-galactose-polyacrylamide gels. The purified lectins agglutinated human blood-group-O erythrocytes slightly more than those belonging to B and A blood groups. In each case the haemagglutination was inhibited to various extents by several carbohydrates.

The purified lectin from the seeds of *Erythrina variegata* (Linn.) var. *orientalis* (Linn.) Merrill, besides being specific for human erythrocytes, with a definite higher affinity for blood-group-O erythrocytes, is also an addition to the list of purified galactose-binding lectins.

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References

- Boyum, A. (1968) *Scand. J. Clin. Lab. Invest.* **21**, Suppl. 97, 77–89
- Brown, J. C. & Hunt, R. C. (1978) *Int. Rev. Cytol.* **52**, 277–348
- Chrambach, A., Reisfield, R. A. & Wyckoff, M. (1967) *Anal. Biochem.* **20**, 150–154
- Council of Scientific and Industrial Research (1952) *The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products*, vol. 3, pp. 195–199, Council of Scientific and Industrial Research, New Delhi
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956) *Anal. Chem.* **28**, 350–356
- Ersson, B., Asberg, K. & Porath, J. (1973) *Biochim. Biophys. Acta* **310**, 446–452
- Gold, E. R. & Balding, P. (1975) *Receptor Specific Proteins*, pp. 151–236, Excerpta Medica, Amsterdam
- Goldstein, I. J. & Hayes, C. E. (1978) *Adv. Carbohydr. Chem. Biochem.* **35**, 127–340
- Goldstein, I. J., Hughes, R. C., Monsigny, M., Osawa, T. & Sharon, N. (1980) *Nature (London)* **285**, 66
- Horejsi, V., Ticha, M., Novotny, J. & Kocourek, J. (1980) *Biochim. Biophys. Acta* **623**, 439–448
- Lee, D. W., Tan, G. S. & Lew, F. Y. (1977) *Planta Medica* **31**, 83–93
- Lowry, O., Rosebrough, N., Farr, A. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Ornstein, L. & Davis, B. J. (1962) *Disc Electrophoresis*, Distillation Products Industrial Division, Eastman Kodak, Rochester, NY
- Prokop, O. & Uhlenbruck, G. (1969) *Human Blood and Serum Groups*, pp. 72–83, Maclaren and Sons, London
- Salk, J. (1944) *J. Immunol.* **49**, 87–89
- Toms, G. C. & Western, A. (1971) in *Chemotaxonomy of the Leguminosae* (Harborne, J. B., Boulter, D. & Turner, B. L., eds.), pp. 367–462, Academic Press, New York