Properties and N-terminal amino acid sequences of three *Erythrina* lectins from Costa Rica (Leguminosae)

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Abstract: Three *Erythrina* lectins, from *E. fusca, E. globocaliz* and *E. costaricensis* were isolated by affinity chromatography. The lectins are glycoproteins with a monomeric molecular weight of 28 000. They agglutinate human erythrocytes irrespective of blood type and the activity was inhibited by N-acetyl-galactosamine, galactose and lactose. The N-terminal amino acid sequences of the three lectins were determined by automated Edman degradation of the native proteins. Comparison with the sequences of ten other legume lectins revealed extensive homology. The N-terminal amino acid for *E. fusca* is Val, and Ala for *E. globocaliz* and *E. costaricensis* .

Key words: *Erythrina,* legume, lectin, agglutination, N-terminal amino acid sequence.

Erythrina is a family of deciduous leguminous trees and shrubs widely spread in the tropics and subtropics. Six species have been reported from Costa Rica: *E. costaricensis, E. fusca, E. globocaliz, E. cristagalli, E. lanceolata,* and *E. poeppigiana* (Flores and Rivera 1984). Recently, a lectin from *E. costaricensis* was purified and characterized (Nanne-Echandi and Aragón-Ortiz 1991). Here we report the isolation of lectins from *E. fusca* and *E. globocaliz* by using affinity chromatography on lactose-agarose column. The N-terminal amino acid sequences of these, and of the *E. costaricensis* lectin, were determined. Comparison with the sequence of *E. corallodendron* lectin (Adar *et al.* 1989) reveals a high percentage of homology.

MATERIAL AND METHODS

Lactose-Agarose, $CaCl_2$, Tris-HCl, NaCl, lactose, glycine, sodium dodecylsulfate (SDS), Coomassie brilliant blue, polyacrylamide, acetone, carbohydrates, alcohols and proteins used as molecular weight markers were from Sigma (USA).

Fresh mature seeds from *E. fusca* and *E. globocaliz* were obtained during the dry season of the year and crude extracts were prepared: 80 g of dry seeds in each case were soaked in distilled water for 24 h at 5 °C and homogenized in 1/2 L of 0.9% NaCl in a blender. The suspension was filtered and centrifuged at 11000g. The clear supernatant was precipitated with four volumes of acetone, centrifuged and left at room temperature for 12 h. The acetone free precipitate was dissolved in three volumes of saline, centrifuged and the clear supernatant lyophilized and kept at -20 °C.

One gram of each extract was suspended in 8 mL of 0.01 M Tris/HCl +0.02 M $CaCl_2$, pH 7.2, centrifuged and the supernatant, adjusted to pH 7.2, applied to a 2 cm bed lactose-Agarose column (1.2 x 10 cm). The column was eluted with the same buffer until the absorbance decreased to 0.03 units (OD=280 nm). Volumes of 2.5 mL per tube were collect-

ed and tested for agglutination of human RBC. Elution was continued with 0.2 M lactose containing 0.15 M NaCl in the starting buffer. The lectin was pooled and thoroughly dialyzed against distilled water, concentrated by ultrafiltration using UM2 membrane and lyophilized. Polyacrylamide gel electrophoresis in Tris-glycine, pH 8.4 was performed according to the method of Davis (1964). Staining was done with 0.05% Coomassie brilliant blue and with Schiff reagent for the detection of glycoproteins. PAGE in the presence of SDS was performed according to the method of Laemmli (1970) in a 4-30% (W/V) gradient gel. Automated N-terminal amino acid sequences analysis, was determined by Edman degradation (Edman and Begg1967) of the native proteins. A gas phase sequencer (Applied Biosystems, model 477A) connected to an online HPLC 120A system for identification of the phenylthiohydantoin derivatives, was used. The agglutination assay was done according to Cumsky and Zusman (1979). Serial two fold dilution of 25 µL aliquots of the isotonic lectin (200 µg/mL) were made in 25 µL of Tris buffer (50 mM Tris/HCl, 150 mM NaCl, 40 mM $CaCl_2$, pH 7.0) containing 12.5 µL of 2.5% erythrocytes in a microtiter V plate. The agglutination was achieved using the following carbohydrates at a concentration of 0.1 M in buffered saline: D(+) galactose, D(+) glucose, D(+) mannose, D(-) fructose, D(+) xilose, lactose, saccharose, N-acetyl-ß-D-galactosamine as well as with the alcohols mio-inositol and mannitol.

RESULTS

Affinity chromatography of 1 g of *E. fusca* extract on lactose-Agarose column is shown in Fig.1. The lectin was eluted with 0.2 M lactose peak (B). Agglutination of human RBC was observed with protein from tubes number 30 to 35. Fig.2 shows PAGE at pH 8.4 of *E. fusca* lectin. Gel number 1 shows the crude extract stained with Coomassie brilliant blue. Gels number 2 and 3 show the purified lectin with only one protein band and the crude extract with several protein bands, both gels stained with Schiff reagent. Fig.3 shows SDS-PAGE of *E. fusca* and *E. globocaliz* lectins; both lectins show single protein bands corresponding to molecular weight of 28 000 for the monomeric form of the proteins (lanes 1 to 6). The multimeric forms of the lectin are observed in lanes 7 to 12. The titer of the agglutination activity of *E. fusca* and *E. globocaliz* lectins is shown in Table 1, the inhibition of the agglutination activity in Table 2. The N-terminal amino acid sequence of the studied lectins compared with other *Erythrina* lectins is shown in Table 3.

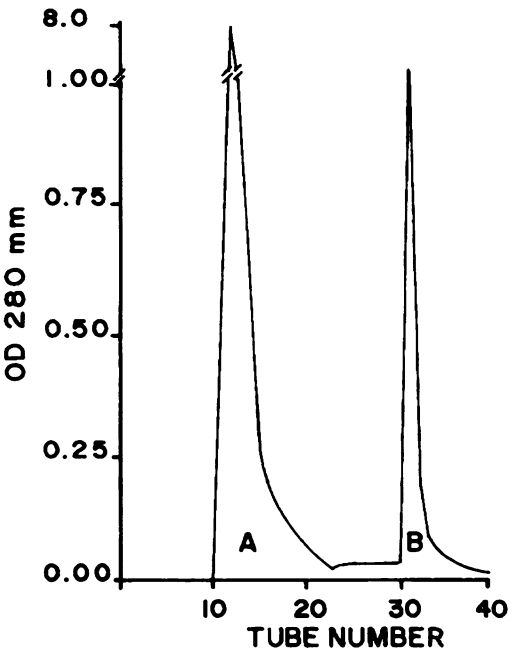

Fig.1. Affinity chromatography of 1 g of *E. fusca* extract on lactose-Agarose column. Protein from peak A was eluted with 0.01M Tris/HCl + 0.02M CaCl2, pH 7.2. Protein from peak B was obtained by elution with 0.2 M lactose + 0.15M NaCl. Agglutination of human RBC was observed with protein from tubes 30 to 35. Similar pattern of elution was obtained for the lectin from *E. globocaliz* and *E. costaricensis* (data not shown).

DISCUSSION

It is inferred that the lectins from *E. fusca* and *E. globocaliz* are obtained as single band proteins, with a carbohydrate moiety. They are multimeric proteins and the molecular weight of the monomeric denatured protein was shown to be around 28 000. A similar staining behaviour and molecular weight was also reported for the lectin of *E. costaricensis* (Nanne-Echandi and Aragón-Ortiz 1991). The lectins did not differentiate between the human blood types studied. This activity was shown to be

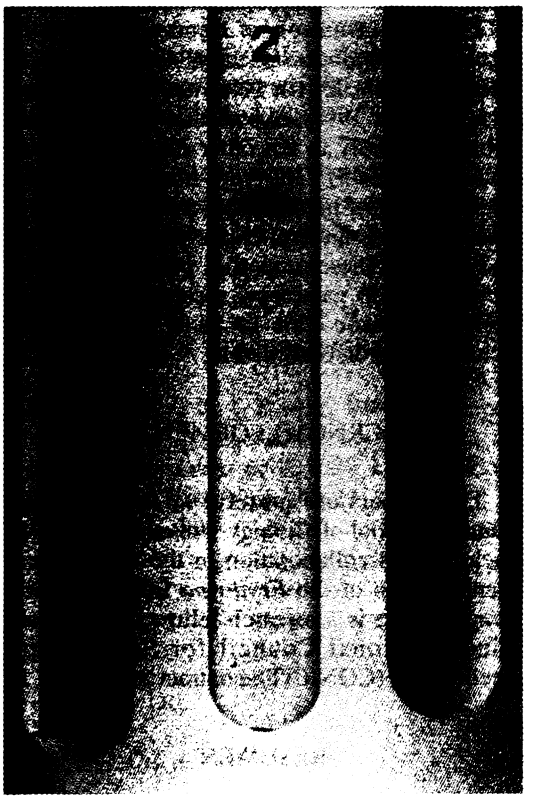

Fig. 2. Polyacrylamide gel electrophoresis of *E. fusca* lectin. Gel 1 shows the crude extract stained with Coomassie brilliant blue. Gels 2 and 3 show the purified lectin and the crude extract both stained with Schiff reagent.

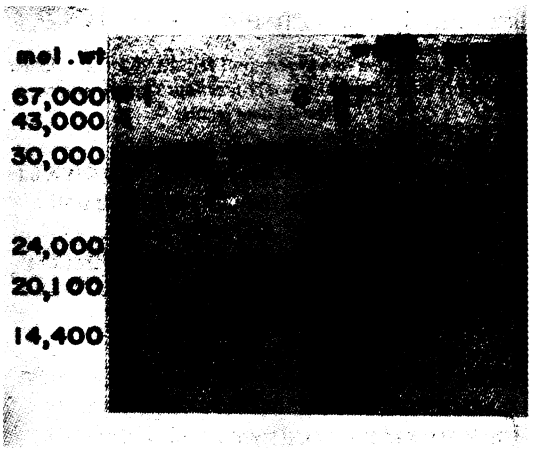

Fig. 3. **SDS-PAGE of the lectins from *E. fusca* and *E. globocaliz*..** (A) left lane : mol. wt standards (10 µg each of the following proteins were applied: B. albumin, ovalbumin, carbonic anhydrase, PMSF trypsinogen and soybean trypsin inhibitor). Lanes 1 to 3 EFusL: Lanes 4 to 6 EGL in both cases; 10, 15 and 20 µg of lectin were applied respectively. (B) Left lane: mol. wt standards (same as decribed above). Lanes 7 to 12 corresponds to EFusL and EGL in absence of SDS ; 10, 15 and 20 µg of the lectins were applied respectively in each case.

Table 1

Titer of the agglutinating activity of the lectins from E. fusca *and* E.globocaliz, *over the human blood groups ABO $Rh_0(D)$ positive and negative*

	Blood group			
Lectin titer	A	B	AB	O
1:2	+	+	+	+
1:4	+	+	+	+
1:8	+	+	+	+
1:16	-	-	-	-

Table 2

Inhibitory action of carbohydrates over the hemagglutinating activity of lectins from E. fusca *and* E. globocaliz *toward human blood groups ABO, Rh_0 (D) positive and negative*

	Blood group							
Inhibitor (0.1M)	$Rh_0(D)+$				$Rh_0(D)-$			
	A	B	AB	O	A	B	AB	O
Fucose	+	+	+	+	+	+	+	+
Galactose	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
N-acetyl-mannosamine	+	+	+	+	+	+	+	+
N-acetyl-galactosamine	-	-	-	-	-	-	-	-
Xylose	+	+	+	+	+	+	+	+
Inositol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-
Saccharose	+	+	+	+	+	+	+	+

Table 3

Comparison of the amino terminal sequences of the studied lectins with several known N-terminal sequences from other legume lectins

EFusL	V E T I S F N F S E F E P G N N D L T L Q G A A L I
EGL	A E T M S F S F S Q F Q P G N N D L T L Q G V
ECosL	A E T M S F S F
ECorL	V E T I S F S F S E F E P G N D N L T L Q G D S * P
ECL	V E T I S F S F S E F E P G N
ECafL	V E T I S F S F S E F E P G N
EFL	A E T I S F S F S E F E P G
EHL	V E T I S F S F S E F E P G N
ELysL	V E T I S F S F S E
EPL	V E T I S F S F S K F E A G
EZL	V E T I S F S F S E F E P G N
PNA	A E T V S F N F N S F S E G N
SBA	A E T V S F S W N K F V P K E

* Leucine or isoleucine. Data from Lis *et al.* (1985) and Adar *et al.* (1989).

The following abbreviated names for the *Erythrina* lectins are used: EFusL, *E. fusca;* EGL, *E. globocaliz;* ECosL, *E. costaricensis;* ECorL, *E. corallodendron;* ECL, *E. cristagalli;* ECafL, *E. caffra;* EFL, *E. flabelliformis;* EHL, *E. humeana;* ELysL, *E. lysistemon;* EPL, *E. perrieri;* EZL, *E. zeyheri.* For other legume lectins: PNA, peanut agglutinin; SBA, soybean agglutinin.

inhibited by galactose, N-acetyl-galactosamine and lactose.

A comparison of the N-terminal amino acid sequence of the lectins from *E. fusca*, *E. globocaliz* and *E. costaricensis* with the N-terminal sequences of other 10 lectins (Lis *et al.* 1985) as well as with the amino acid sequence of *E. corallodendron* (Adar *et al.* 1989), a high percentage of homology was observed, providing further evidence for the conservation of the lectin gene in legume plants.

The lectins from *E. fusca*, *E. globocaliz* and from *E. costaricensis* (Nanne-Echandi and Aragón-Ortiz 1991), exhibit many of the physicochemical properties already adscribed to other lectins characterized from the same family (Lis *et al.* 1985, Nanne-Echandi and Aragón-Ortiz 1991). All are glycoproteins with a neutral sugar content between 3-10%. After PAGE, they all give a positive reaction for carbohydrates with the Schiff reagent (Fig.1). The proteins are multimeric with a monomeric molecular weight around 28 000.

The agglutination of human RBC by *E. fusca* and *E. globocaliz* lectins as well as by *E. costaricensis* lectin (Nanne-Echandi and Aragón-Ortiz 1991), shows that these proteins behave similarly to ten other *Erythrina* lectins (Lis *et al.* 1985). They are unspecific in the agglutination of human RBC and the activity is inhibited by galactose, N-acetyl-galactosamine and lactose. These properties point out that the lectins bind to galactosyl receptors of the red cell membrane (Lis *et al.* 1985, Nanne-Echandi-Aragón Ortiz 1991).

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RESUMEN

Tres lectinas obtenidas de extractos crudos, preparados de semillas de la *Erythrina fusca, E. globocaliz* y *E. costaricensis* fueron aisladas utilizando cromatografía de afinidad. Las lectinas son glicoproteínas y en su forma monomérica presentan un peso molecular de 28 000. Las lectinas aglutinan eritrocitos humanos, independientemente del grupo sanguíneo. La actividad aglutinante es inhibida por N-acetil-galactosamina, galactosa y lactosa. La secuencia N-terminal de cada lectina fue determinada mediante la degradación automatizada de Edman. Al compararlas con las secuencias de diez lectinas de leguminosas, se encontró una extensa homología.

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