Erythrina LECTINS - A REVIEW STRUCTURAL AND PHYSICOCHEMICAL PROPERTIES

by

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Resumen

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Este trabajo presenta una revisión de las principales propiedades fisícoquímicas y características estructurales de las lectinas aisladas de semillas del género *Erythrina*. Las lectinas muestran un alto grado de similitud y son una herramienta muy valiosa para estudiar interacciones proteína-carbohídrato.

Palabras clave: Leguminosae, *Erythrina*, lectinas.

Abstract

This article reviews the main physicochemical properties and structural features of the lectins isolated from seeds of the *Erythrina* genus. The lectins show a close similarity among them and are an invaluable tool to study protein-carbohydrate interactions.

Key word index: *Leguminoseae, Erythrina,* lectin, properties, structure.

Introduction

The *Erythrina* genus was systematically examined for the first time in the 40's due to the presence of curare - like alkaloids whose chemistry, biosynthesis and pharmacology have subsequently been studied (Dyke and Quesy, 1981). Botanical surveys have showed that ca 71 out of 106 *Erythrina* species are native of America where they grow as shrubs and trees in tropical and sub-tropical zones (Krukoff and Barneby, 1973; Krukoff, 1982).

The *Erythrina* genus has great potential economic interest as the plants frequently are N—fixers (Cardozo, pers. commun.); some species grow in arid zones and could be a source of proteins for humans or animals. In this regard the nutritional value of the proteins of the edible species *E. edulis* has been studied (Pérez et al, 1979). Research is currently done on the use of this species in animal nutrition (Acero, pers. commun.). Among the proteins being studied the trypsin inhibitors, which are common in Erythrina, as well as the chymotrypsin inhibitors, have received some attention (Hernández, de Sanabria and Pérez, unpublished results; Joubert and Sharon, 1985; Shieh et al, 1990).

The presence of lectins in the *Erythrina* genus was pointed out many years ago by several workers (Makela, 1957: Boyd et al, 1961; Martin and

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Bomchil, 1966; Lee et al, 1977) but no attempts were made to isolate them. In a preliminary study the lectin from *E. edulis* seeds was partially purified and some of its properties were described (Montes de Gómez and Pérez, 1974) but further purification was hindered by lack of a suitable support. Later, systematic studies were conducted on the *Erythrina* genus and currently the lectins from 18 species have been isolated and characterized to various degrees (Table 1). Since ca 70 lectins have been purified from leguminous plants, the *Erythrina* genus is therefore the most extensively studied in this regard and sufficient data is available to establish comparisons.

The following abbreviated names for the *Erythrina* lectins are used:

ECorL, lectin from *E. corallodendron;* ECL, *E. cristagalli;* ECafL, *E. caffra;* ECosL, *E. costaricensis;* EEL, *E. edulis;* EFL, *E. flabelliformis;* EHL, *E. humeana;* EIL, *E. indica;* ELatL, *E. latissima;* ELysL, *E. lysistemon;* EPL, *E. perrieri;* ERL, *E. rubrinervia;* ESubL, *E. suberosa;* ESL, *E. stricta;* EVL, *E. variegata;* EVesL, *E. vespertilio;* EZL, *E. zeryheri.*

RBC: red blood cells

Isolation

The *Erythrina* lectins have been purified exclusively from seeds and there is no information about attempts to detect/isolate them from other parts of the plant.

Table 1
Ocurrence of lectins in *Erythrina* genus

Species	Yield mg/100 g flour)	Reference
*E. indica**	120	Horejsi et al, 1980
*E. corallodendron**	n.d.	Gilboa-Garber and Mizrahi, 1980
E. corallodendron	125–166	Lis et al, 1985
E. latissima	166–200	Lis et al, 1985
E. caffra	133–166	Lis et al, 1985
E. flabelliformis	n.d.	Lis et al, 1985
E. humeana	n.d.	Lis et al, 1985
E. perrieri	n.d.	Lis et al, 1985
E. striata	n.d.	Lis et al, 1985
E. zeyheri	n.d.	Lis et al, 1985
E. indica	40	Bhattacharyya et al, 1986
E. lithosperma	40	Bhattacharyya et al 1986
E. arborescens	40	Bhattacharyya et al, 1986
E. suberosa	2	Bhattacharyya et al, 1986
*E. variegata**	540	Datta and Basu, 1981
E. variegata		Fukuda et al, 1990
E. cristagalli	166	Iglesias et al, 1982
*E. edulis**	n.d.	Montes de Gómez and Pérez, 1974
E. edulis	121	Perez, 1984
E. vespertilio	220	Kortt, 1986
E. rubrinervia	152	Peña et al, 1988
E. costaricensis	103	Perez, (unpublished results)
E. costaricensis	n.d.	Nanne and Aragón, 1991
E. bogotensis, E. glauca		Makela, 1957
E. poeppigiana, E. sumatrana,		Boyd et al, 1961
E. berteroana		Boyd et al, 1961
E. falcata		Martin and Bomchil, 1966
E. guineensis		Lee et al, 1977

* Partially characterized

The purification scheme generally used involves extraction of defatted flour with 1% NaCl or PBS, fractionation with 50–60% saturated ammonium sulphate and affinity chromatography. As the lectins are inhibited by galactose, several types of supports have been used such as O–galactosyl polyacrylamide gels (Horejsi et al, 1980; Pérez, 1984; Peña et al, 1988), acid–treated Sepharose 4B (Gilboa-Garber and Mizrahi, 1981; Yamasaki et al, 1992), acid–treated Sepharose 6B (Bhattacharyya et al, 1981; Datta and Basu, 1981) and galactose or lactose coupled to Sepharose 6B with divinyl sulphone (Iglesias et al, 1982; Lis et at, 1985; Kortt, 1986).

The reported yields of the lectins vary between 2–540 mg/100 g flour but for most species the range is 100 to 160 mg/100 g flour. The achieved degrees of purification are between 19.5 to 114.8 as calculated from the specific titers obtained with the affinity–purified proteins (Gilboa-Garber and Mizrahi, 1981: Datta and Basu, 1981; Iglesias et al, 1982; Pérez 1984; Peña et al, 1988).

Physicochemical properties

Molecular properties

The *Erythrina* lectins have several common structural features as shown in Table 2:

- All are dimeric proteins with molecular weights in the range of 56000 to 68000, with identical subunits of Mr between 26000–32000. In some instances, one subunit appears to be slightly heavier than the other; this difference may arise during the post-transcriptional processing of the lectins that is likely to occur in the seeds. Experimental evidence supporting this hypothesis has recently been obtained by *Yamasaki* et al (1992) with the isolation of three isolectins from *E. variegata* seeds. The alternative explanation implicating proteolytic cleavage occuring during extraction or purification steps, is not likely to occur in view of the results obtained with ERL (Peña et al, 1998).
- All are glycoproteins with 2.8–11.2% of neutral sugars which, in the studied lectins, consist of fucose, xylose, mannose and glucosamine (N–acetylglucosamine) (Table 3). The presence of xylose indicates that the oligosaccharide core is different from that of animal N–glycoproteins. It may be similar to the structure present in bromelain and suggested for the carbohydrate unit of the lectins of *Sophora japonica*, *Vicia graminea* and *Wistaria floribunda* (Lis et al, 1985; Goldstein and Poretz, 1986) The structure of the oligosaccharide has been elucidated by Ashford et al (1991). It consists of the heptasaccharide Manα6 (Manα3) (Xylβ2) Manβ 4Gl cNAcβ4 (L–Fucα3) G1cNAcβ N–linked to AsN 17 of each subunit (Adar et al, 1989) which is part of the characteristic sequon NXT (see Table 8). Chemical modification of the carbohydra-

Table 2
Molecular properties of *Erythrina* lectins

	$ERL_{(1)}$	$EIL_{(2)}$	$ECL_{(3)}$	$EEL_{(4)}$	$ECorL_{(5)}$	$EVesL_{(6)}$	$ECosL_{(7)}$	$EVL_{(8)}$
Mr protein (kDa)	62	68.2	56.8	56	60.2	59	58	52.5 48.5
Mr subunits (kDa)	29.5	30 33	26 28	27	28	32	29.5	36 33
Bands in SDS–PAGE	1	2	2	1	1	1	1	2
Bands in alkaline PAGE	2	1	1	2	n.d.	3	n.d.	n.d.
%Neutral sugars	10	$11.2_{(9)}$	2.8	7.8	5.5	9.7	6.5	n.d.
Ca atoms/mol	16	$2.9–3.5_{(9)}$	1.9	6	n.d.	n.d.	n.d.	n.d.
Mn atoms/mol	1	$1.8–2.3_{(9)}$	1	traces	n.d.	n.d.	n.d.	n.d.
pI	5.19 5.02 5.12	4.83 5.09 5.44	n.d.	5.40 5.50	n.d.	4.8 5.3	5.7 5.9 6.13 6.50	n.d.

(1) Peña et al, 1988
(2) Bhattacharyya et al, 1981
(3) Iglesias et al, 1982
(4) Pérez, 1984
(5) Lis et al, 1985
(6) Kortt, 1986
(7) Nanne and Aragón, 1991
(8) Yamasaki et al, 1992
(9) Bhattacharyya et al, 1986

Table 3.
Carbohydrates of *Erythrina* lectins

Lectin	Ratio of monosaccharide / subunit				Reference
	Fucose	Xylose	Mannose	Glucosamine	
ECorL	1.0	1.0	3.3	2.5	Lis et al, 1985
ELatL	1.0	1.2	3.0	0.8	Lis et al, 1985
ECafL	1.0	1.1	3.8	1.1	Lis et al, 1985
ECL	1.0	1.0	3.5	1.9	Iglesias et al, 1982
EVesL*	0.5	2.5	7.5	2.0	Kortt, 1986
EIL+	3.0	3.7	8.8	2.9	Bhattacharyya et al, 1986

* 25 mols Galactose/mol protein are also present
\+ 3.3 mols Arabinose/mol protein and 1.7 mols Galactose/mol protein are also present

te unit does not alter the erythroagglutinating properties of EEL showing that it is not essential for the lectin activity (Montes de Gómez and Pérez, 1974).

— The lectins examined have varying amounts of Ca+2 and Mn+2. "Demetallization" by usual procedures, using 0.1 M EDTA and 1.0 M AcOH, does not decrease the metal content of the proteins, suggesting that metal ions are tightly bound to the lectins (Iglesias et al, 1982; Bhattacharyya et al, 1986; Peña et al 1988).

— The lectins are microheterogeneous as observed by isoelectric focusing and this is probably due to partial deamidation of AsN and GIN residues. At least in one instance the existence of isolectins has been explained by varying degrees of glycosilation which presumably originate differences in affinity for acid–treated Sepharose 4B (Yamasaki et al, 1992) .

Interactions with animal cells

Human erythrocytes of groups A, B, O are agglutinated with similar specific titers by most

Table 4.
Agglutination of animal erythrocytes by *Erythrina* lectins

	Rabbit		Cow		Dog		Sheep		Ref.
	NT	T	NT	T	NT	T	NT	T	
ERL	6–12	n.d	–	–	–	6–12	–		*
ECorL	–	n.d.	n.d.	n.d.	–	62.5&	125&	125&	%
ECorL	5–20	0.25–1.0	n.d.	n.d.	n.d.	n.d.	–	–	#
EPL	250–500	12.5–25	n.d.	n.d.	n.d.	n.d.	–	–	#
EIL	3.9	0.24	250	62.5	–	4.9	n.d.	n.d.	**
ECL	10	0.25–0.50	n.d.	n.d.	n.d.	n.d.	–	–	##
EVesL	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	%%
EVL	–	n.d.	–	n.d.	–	n.d.	–	n.d.	@
EEL EL	+	+	+	+	–	+	–	n.d.	@@
ECosL	+	n.d.	n.d.	n.d.	–	n.d.	–	n.d.	%%%

Data taken or calculated from references. Values are expressed as minimal agglutination concentrations (ug/ml).

&: Papain–treated erythrocytes.
NT: Non– trypsinized erythrocytes
T: Trypsinized erythrocytes

* Peña et al, 1988
% Gilboa–Garber and Mizrahi, 1981
%%% Nanne and Aragón, 1991
# Lis et al, 1985
** Bhattacharyya et al, 1981
Iglesias et al, 1982
%% Kortt, 1986
@ Datta and Basu, 1981
@@ Perez, 1984; Montes de Gómez and Pérez, 1974.

Erythrina lectins with a slight preference for 0 cells; EIL and ESubL showed significantly higher activity towards the 0 group (Bhattacharyya et al, 1981; Datta and Basu, 1981). Sudakevitz et al (1991) have shown that EIL and ECorL exhibit H/HI blood group preference and were not inhibited by the saliva from ABO (H) secretors. Trypsinization of cells usually increases the susceptibility to agglutination by 4—67 fold. The minimal concentrations of lectins necessary for agglutination are in the range 3 to 15 μg/ml.

Table 4 shows the agglutination of animal erythrocytes by *Erythrina* lectins. In all cases, excepting EVarL, rabbit erythrocytes were agglutinated whereas dog and sheep RBCs were not. Horse erythrocytes, whenever tested, have showed a consistent lack of agglutinability (Data and Basu, 1981; Pérez, 1984). Agglutination of guinea pig RBCs has been reported with *E. guineensis* and *E. edulis* (Lee et al, 1977; Montes de Gómez and Pérez, 1974); EIL even at 20 mg/ml did not agglutinate guinea pig erythrocytes (Bhattacharyya et al, 1981).

The *Erythrina* lectins differ significantly in their mitogenic ability as shown by Table 5. ECorL and ECL exhibited specificity towards human T lymphocytes; the former requiring treatment of lymphocytes with neuraminidase. Lis et al (1985) have reported mitogenic activity for most of the *Erythrina* lectins studied by them but no data about the doses required for stimulation is available.

Table 5
Mitogenic activity of *Erythrina* lectins

Species	Dose for maximum stimulus (μg/ml)	Reference
E. corallodendron	50	Gilboa-Garber and Mizrahi, 1980.
E. cristagalli	100	Iglesias et al, 1982
E. humeana	non-mitogenic	lis et al., 1985
E. zeyheri	non-mitogenic	Lis et al, 1985
E. edulis	non–mitogenic	Peña et al, 1988

Information on interactions with other cell types is scarce. Mouse thymocytes or splenocytes were not stimulated by *Erythrina* lectins (Lis et al, 1985). ECL specifically recognized feline monocytes, discriminating them from feline lymphocytes; in contrast ECorL behaves similarly towards the two cell classes (Whitehurst et al, 1990).

Carbohydrate binding

All the Erythrina lectins are inhibited by the galactosyl moiety with varying strength as indicated in Table 6. Common mono—, di—or trisaccharides devoid of galactose (not included in the Table), showed no inhibitory ability even at 100 mM.

The most potent inhibitors of agglutination are N—acetyllactosamine and biantennary or

Table 6
Carbohydrate inhibition of agglutination by *Erythrina* lectins

Carbohydrate	Relative inhibitory activity					
	ERL*	EEL**	ECL+	EIL#	EVesL&	ECorL%
D–galactose	1.0	1.0	1.0	1.0	1.0	1.0
N–Acetylgalactosamine	2.5	5.1	2.0	2.0	5.0	1.9
Methyl–α–D–galactoside	2.2	4.4	2.8	2.0	2.0	1.5
Methyl–β–D–galactoside	2.2	2.2	1.0	4.0	1.0	1.0
p–Nitrophenyl–α–D–galactoside	n.d	n.d	3.0	2.0	10.0	1.6
p–Nitrophenyl–β–D–galactoside	8.3	8.4	6.7	8.0	20.5	3.2
D–Galactosamine	–	0.3	0.7	0.1	0.3	n.d.
D–Lactose	3.7	14.9	6.7	4.0	8.0	2.7
N–Acetyllactosamine	n.d.	n.d.	33.7	n.d.	n.d.	19.0
D–Melibiose	3.6	1.8	n.d.	2.0	2.0	n.d.
D–Raffinose	–	0.5	1.9	1.0	1.0	n.d.

* Data taken from Peña et al, 1988
** Data calculated from Pérez, 1984
\+ Data calculated from Iglesias et al, 1982
\# Data calculated from Bhattacharyya et al., 1981
& Data calculated from Kortt, 1986
% Data taken from Lis et al, 1985

triantennary oligosaccharides containing two or three terminal N—acetyllac tosamines (Lis et al, 1985). The strong inhibition of all *Erythrina* lectins with p—Nitrophenyl—D—galactoside suggests that a hydrophobic binding site is close to the carbohydrate binding site. This has been confirmed by structural studies on ECorL (Shaanan et al, 1991). The carbohydrate specificity of ECL has been studied in detail by quantitative precipitin reaction, hapten—inhibition assays and spectrofluorometry. It was shown that the lectin has an extended binding site that accommodates N—Acetylllactosamine with a significant thermodynamic contribution of the 2—acetamido group of N—acetylglucosamine (Kaladas et al, 1982; de Boeck et al, 1984).

The requirement of free C' —3, C' —4 and C' —6 hydroxyls in the galactosyl residue is a general feature of the carbohydrates that inhibit the *Erythrina* lectins as can be deduced from Table 6. It is noteworthy to point out that there are some differences between the lectins in regard to their inhibition by galactosamine and raffinose.

Structural properties

Primary structure

The amino acid composition of the *Erythrina* lectins (Table 7) shows the characteristic small content of methionine and the absence, with the exception of ERL, of cysteine observed with most lectins. As a whole, the number of residues/mol is similar between the examined lectins, the acidic and hydroxy amino acids being predominant.

Table 7
Amino acid composition of the *Erythrina* lectins

Amino acid	EVesL*	ECL**	EIL#	ERL%	ECorL+
Lys	22	20	18	22	19
His	12	8	10	6	9
Arg	13	11	11	7	11
Asp	65	62	63	65	60
Thr	43	43	44	28	41
Ser	48	47	51	71	47
Glu	52	55	61	64	58
Pro	39	39	34	23	39
Gly	40	39	38	47	41
Ala	43	40	40	22	40
Cys	0	0	0	8	0
Val	44	42	42	20	33
Met	6	6	6	6	4
Ile	31	30	29	17	29
Leu	38	37	37	18	36
Tyr	20	20	22	10	18
Phe	28	28	29	11	28
Trp	9	11	13	16	n.d.

Results are expressed as residue/mol

* Taken from Kortt, 1986
** Taken from Iglesias et al, 1982
\# Taken from Horejsi et al, 1980
% Taken from Peña et al, 1988
\+ Taken from Lis et al, 1985

Table 8 shows the N—terminal sequence of some studied lectins where ECorL is representative of nine Erythrina lectins (not included in Table 8) analyzed by Lis et al (1985); these lectins have identical N—terminal sequences down to the ninth residue. A high degree of homology is evident among the lectins shown in Table 8; Glu at position 2 and 12, Ser at position 7 and Phe at positions 6, 8 and 11 are invariant. Most of these residues are also highly conserved in the sequences of lectins from various tribes; Phe at positions 6 and 11 is the most striking example.

In some lectins Val is replaced by Ala as N—terminal and interestingly the sequence Asn—Leu—Thr of ECorL, where the glycosylation site has been assigned (Adar et al, 1989), is replaced by Ser—Leu—Thr in EVesL.

Among the *Erythrina* lectins only ECorL has been completely sequenced (Adar et al, 1989) and it exhibits an extensive homology with lectins from the tribes *Vicieae*, *Diocleae* and *Lotoideae* (Sharon and Lis, 1990; Pérez et al, 1991). It is remarkable that from the nine amino acids involved in metal binding in ConA seven are conserved not only in ECorL but in the rest of lectins as well. This suggests that the metal binding site should be the same in all lectins. In contrast, only two out of the five amino acids of ConA involved in carbohydrate binding, are conserved in the other lectins.

The sequence of *E. corallodendron* lectin cDNA was determined by Arango et al (1990). These authors compared the deduced amino acid sequence to the chemically—determined sequence of the lectin and found a very high homology between them except at seven positions corresponding to variable regions of the legume lectins. The deduced sequence shows at the N—terminus a putative signal leader peptide and at the C—terminus a short peptide (12 amino acids) which suggests a post-transcriptional modification of the protein. In this respect the processing of the *Erythrina* lectins would be very similar to that observed for pea lectin (Higgins et al, 1983).

Tertiary structure

Table 9 shows the crystalline parameters of ECorL and ERL. The crystals were obtained by vapour diffusion in hanging drops equilibrated against solvents that differ in their composition (Saper et al, 1987; Rojas and Pérez, 1990; Shaanan et al, 1991). In the absence of carbohydrate the two lectins show the same behaviour as they crystallize in the hexagonal space group $P6_1$, ($P6_5$);

Table 8
N–terminal sequence of *Erythrina* lectins

	Sequence	Reference
	1 10 20	
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 L P. . . .	*
	###	
EVesL	V E T I S F S F S E F E A G N D S L T L Q G A S L P	**
EIL	V E V L(F) F (A) F	***
EPL	V E T I S F S F S K F E A G	****
EFL	A E T I S F S F S E F E P G N	****
ECosL	A E T M T F	*****
EVL	V E T I S F S F S E F E A G N D X L T L Q G A A L I.	******

###: Glycosilation site
* : Adar et al, 1989
** : Kortt, 1986
*** : Bhattacharyya et al, 1986
**** : Lis et al, 1985
***** : Perez (unpublished results), 1991
****** : Yamasaki et al, 1992

the unit cells have the same dimensions and in both cases there is one dimer of Mr 60000 in the assymetric unit. The crystals diffract to at least 2.5Å and are stable; therefore they are suitable for high–resolution X–ray analysis. If ECorL is crystallized as the lectin–lactose complex (Shaanan et al, 1991) the crystals are monoclinic C2 and contain one monomer in the assymetric unit. The three–dimensional structure of ECorL, solved at 2 Å resolution with an R value of .190, is very similar to those described for ConA, pea, fava bean and *Griffonia simplicifolia* lectins (Sharon and Lis, 1990). The main structural element is β–sheet and the folding of the polypeptide chains in the carbohydrate binding site is very similar in all lectins; the galactosyl moiety is estabilized at this site by means of hydrogen bonds and hydrophobic interactions. A distinctive feature is the well–ordered N–linked heptasaccharide chain that constrains the protein to acquire a quaternary structure different to that observed with mannose/glucose–binding lectins. Comparison of the tertiary structures of lectins illustrate the fact that carbohydrate–binding sites are flexible enough to orientate the specific ligands (sugars) with respect to the amino acid residues involved in the binding.

Recently Rojas et al (1992, unpublished results) have solved the tertiary structure of *E. rubrinervia* lectin at 3Å resolution with an R value 0.21. The protein has a conformation very similar to that determined for *E. corallodendron* lectin; refinement of the model awaits the determination of its primary structure.

Concluding remarks

The considerable amount of available information about *Erythrina* lectins has allowed to establish the general features of this class of proteins and comparison with ConA, the most extensively studied lectin, has revealed unsuspected common characteristics that may well be valid for lectins from other tribes.

Table 9
Crystalline parameters of *Erythrina* lectins

	*E.corallodendron**	*E. rubrinervia***	*E. corallodendron****
Space group	$P6_1$ ($P6_5$)	$P6_1$ ($P6_5$)	C 2
Unit cell dimensions			
a (Å)	136.3	135.1	84.40
b (Å)	136.3	135.1	73.05
c (Å)	83.2	83.0	71.40
β			113.42°
Crystal dimensions (mm)	1.0 x 0.4 x 0.4	0.6 x 0.3 x 0.2	
Subunits per assymetric cell	2	2	1

* Saper et al, 1987
** Rojas and Pérez, 1990
*** Shaanan et al, 1991

In spite of the close homology existing between the *Erythrina* lectins, they show several differences in their ability to interact with animal cells. This may result from fine, yet undetected, variations in tertiary structure. More information on this field will probably help to clarify the point.

Studies on the biosynthesis of these proteins are virtually nonexistent and it is foreseeable that research will be done in this field. For example, to establish the glycosylation pattern, if and how post-transcriptional events lead to the mature protein and how the lectin is channeled to protein bodies will help to propose a general biosynthetic pattern for lectins.

Last but not least, to establish if "in vivo" these proteins are associated with other seed proteins and how the complex works, may throw new light on the physiological function (s) of lectins.

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