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Purification and partial characterization of two lectins from the cactus *Machaerocereus eruca*

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Two lectins (MEA_I and MEA_{II}) were isolated from the cactus *Machaerocereus eruca* by affinity chromatography on mucin-Sepharose and partially characterized with respect to their biochemical and carbohydrate binding properties. Both are oligomeric glycoproteins consisting of 35 kDa monomers. Amino acid analysis indicates that both lectins have similar composition with high amounts of glycine, glutamic acid and serine. MEA_I and MEA_{II} contain ~ 36 and 24% (w/w) of carbohydrates, respectively. They agglutinate erythrocytes from several animal species. Binding specificity was directed to galactose-containing oligosaccharides and glycopeptides. The *M. eruca* lectins are the first lectins to be isolated from a species belonging to the plant family of Cactaceae.

Cactus lectin; Affinity chromatography; Erythrocyte agglutination; (*Machaerocereus eruca*)

1. INTRODUCTION

Lectins are specific-binding proteins or glycoproteins widely distributed in plants, animals and micro-organisms (for recent review, see [1]). Although plant lectins have been mostly found in seeds, increasing interest has been drawn to lectins occurring in various types of vegetative tissues of several plant species: roots [2], leaves [3] and fruits [4]. In the present paper, we describe the isolation and the partial characterization of two lectins from the stems of 'chirinola' (*Machaerocereus eruca*), a representative of the plant family Cactaceae.

2. MATERIALS AND METHODS

2.1. *Materials*

The cactus *Machaerocereus eruca* was collected in Baja California Sur (Mexico). Sepharose 4B and protein markers for polyacrylamide gel electrophoresis were purchased from Pharmacia Fine Chemicals (Bois d'Arcy, France). Ultrogel ACA-202 was from IBF (Villeneuve-La-Garenne, France) and Bio-Gel P-2 from Bio-Rad (Richmond, CA, USA). Bovine serum albumin, pig stomach mucin grade II, fetuin grade III and all sugars were from Sigma Fine Chemicals (St. Louis, MO, USA). Human sero- and lactotransferrins, human milk and serum IgA were gifts from Professor G. Spik and Dr A. Pierce. Sheep submaxillary gland mucin and 2,3- and 2,6-sialyllactoses were gifts from Drs G. Strecker and J.-C. Michalski. Hen ovomucoid was prepared by Professor B. Fournet. β-Galactosidase from jack bean meal was kindly provided by Professor S. Bouquelet. Human α_1 acid glycoprotein (orosomucoid) was isolated according to [5]. All other reagents were of analytical grade and obtained from different commercial sources.

2.2. *Preparation of mucin and fetuin glycans and glycosylpeptides*

O-Glycosidically linked glycans were liberated from glycoproteins by alkaline reductive treatment and purified by gel-filtration on ACA-202 Ultrogel and Bio-Gel P-2 columns as described earlier [6]. N-Glycosylpeptides from fetuin released during the alkaline reductive treatment were enzymatically desialylated and degalactosylated according to [6].

2.3. *Extraction and purification of the lectins*

The juice of 1 kg of cactus stem was obtained with a food processor and centrifuged at 3000 × *g*, for 15 min at 4°C. To supernatant solid ammonium sulphate was added to a final con-

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All sugars are of the D configuration unless otherwise stated

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centration of 66%. The precipitate was dialysed against 0.02 M phosphate/0.09% NaCl, pH 7.4 (PBS) and then applied to a column (1 × 17 cm) of pig stomach mucin immobilized on Sepharose 4B equilibrated with PBS. Unbound proteins were eluted with PBS until A_{280} falls below 0.01. A first lectin fraction (MEA_I) was desorbed with distilled water and a second fraction (MEA_{II}) was eluted with 0.2 M glycine/HCl, pH 2.5.

2.4. *Hemagglutination assays*

Agglutination of human A erythrocytes (2×10^6 cells/ml) and inhibition of the agglutination by glycoproteins and sugars were performed in micro-titer U-plates (Titertek, Linbro Scientific Co., Hamden, USA) by a 2-fold serial dilution procedure [7]. Inhibition was expressed as the minimum concentration (in μM) of either glycoproteins, glycopeptides or glycans required to inhibit 4 hemagglutinating units of the lectin.

2.5. *Analytical methods*

2.5.1. Sugars

Total neutral sugars were determined by applying a phenol-sulphuric method [8]. Carbohydrate composition of MEA_I and MEA_{II} was determined by GLC of the trifluoroacetyl alditols [9].

2.5.2. Proteins

Proteins were determined by the method of Lowry [10] with bovine serum albumin as standard.

2.5.3. Molecular masses

SDS-polyacrylamide gel electrophoresis was performed on 5–25% (w/v) acrylamide gradient gels and the molecular masses of the two lectins were determined by comparison of their relative electrophoretic mobilities with those of proteins with known molecular mass [11].

2.5.4. Amino acid analysis

Proteins were hydrolyzed in vacuo at 110°C, in 6 N HCl for 72 h. One drop of phenol was added to prevent degradation of tyrosine residues [12]. Analyses were carried out on a Beckman 119 C L amino acid analyser.

2.5.5. Isoelectric focusing

Isoelectric focusing was performed in cylindrical polyacrylamide gels (0.5 × 10 cm) containing 2% (w/v) of pH 3.5 to 10 Ampholines (LKB, Sweden) and 1% Tween X-100 (Sigma, St. Louis, MO, USA) as described in [13]. Gels were run at 600 V for 14 h at 4°C. After removal of detergent and Ampholines by incubation in 50% methanol-water for 12 h at room temperature, proteins were stained with Coomassie blue. The pH gradient established after isoelectric focusing was measured in blank gels by slicing the gels into 0.4 cm section, eluting the Ampholines with 0.5 ml of degassed water, for 12 h and measuring the pH of the solution. The gradient was linear over the pH range 4 to 8.5.

2.6. *Antibody production*

Production of rabbit antiserum to purified MEA_I lectin has been described previously [14]. Antigen-antiserum double diffusion tests were carried out on 1.5% Agarose (IBF, Villeneuve-La-Garenne, France) plates in PBS according to [15].

3. RESULTS

3.1. *Purification of* M. eruca *lectins*

Table 1 summarizes the purification procedure and yields of the *M. eruca* lectins, and fig.1 describes the last step, fractionation on immobilized mucin.

3.2. *Molecular properties of* M. eruca *lectins*

On SDS-polyacrylamide gel electrophoresis in 2% (v/v) β-mercaptoethanol, both lectins migrate as single bands of M_r 35000 (fig.2). Gel filtration on Sephadex G-200 or Sepharose 4B columns under non-denaturing conditions gave for both lectins apparent molecular masses of over 200000 (not shown) suggesting that in the native form they are oligomers. Analysis of the purified lectins by isoelectric focusing revealed three major molecular species for MEA_I with pI values of 3.6, 4.1 and

Table 1

Purification of *Machaerocereus eruca* lectins (from 1 kg of stem)

Fractions	Protein (mg)	Protein recovery (%)	Hemagglutinating activity		Specific activity
			Total units	Recovery (%)	
Crude extract	1680	100	415000	100	247
$(NH_4)_2SO_4$ precipitation (0–66% saturation)	1008	60	415000	100	412
Mucin-Sepharose affinity chromatography					
Non-retained fraction	860	51	0	0	0
Fraction F-I (MEA_I)	50.4	3	124500	30	2470
Fraction F-II (MEA_{II})	10	0.6	166000	40	9326

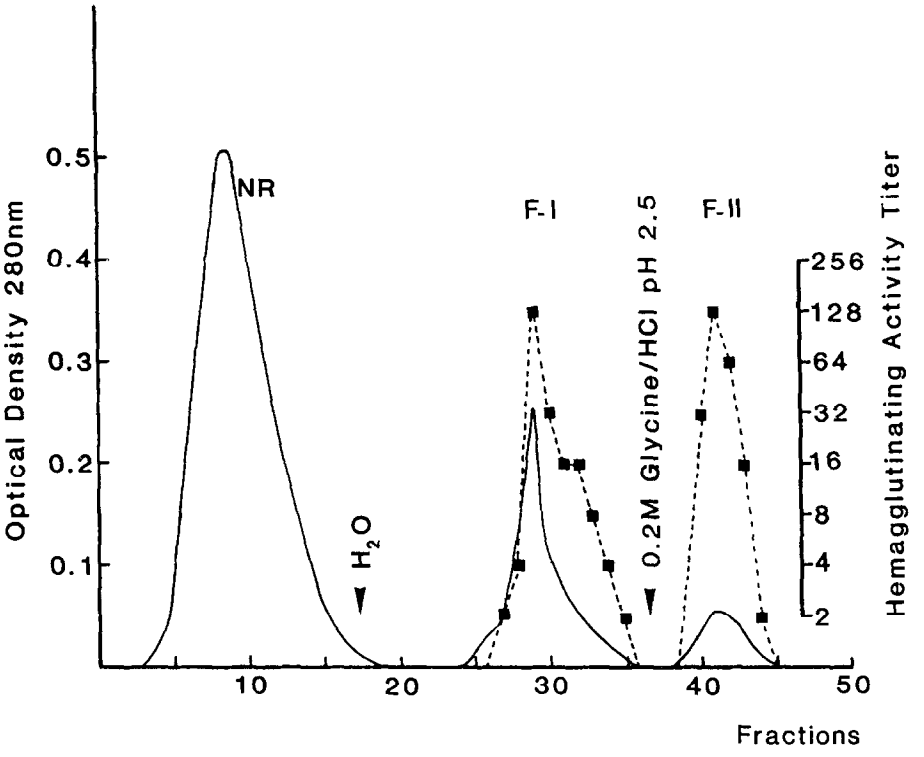

Fig.1. Affinity chromatography of a *Machaerocereus eruca* extract (114 mg of protein) on mucin-Sepharose 4B column. Fractions (2.2 ml) were collected at a flow rate of 9 ml/h. NR, non-retained fraction, F-I, fraction eluted with distilled water; F-II, fraction desorbed with 0.2 M glycine/HCl, pH 2.5, buffer. Ionic strength and pH of each fraction were adjusted by adding concentrated phosphate buffer, pH 7.4. (——) Absorbance at 280 nm; (■—■) hemagglutinating activity with human A erythrocytes.

4.6, and two molecular components for MEA_{II} with p*I* values of 3.5 and 4.1 (fig.3).

Both lectins contain high amounts of glycine, glutamic acid and serine and are devoid of cysteine, asparagine and methionine (table 2). The neutral sugar content of MEA_I was 36% and of MEA_{II} 24%. The monosaccharide composition of the purified lectins is reported in table 3.

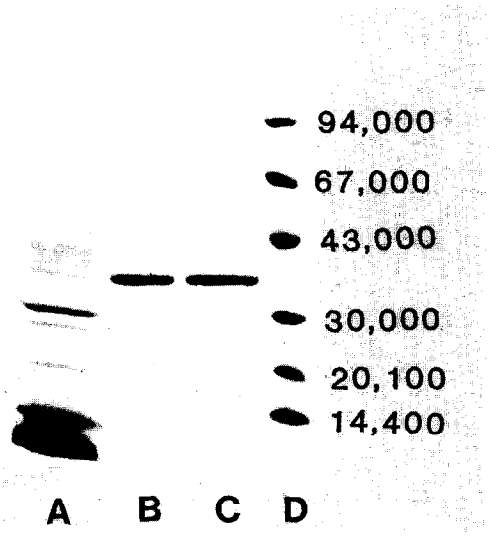

Fig.2. SDS-gel electrophoresis of *Machaerocereus eruca* lectins. Crude extract (line A), purified lectins I (line B) and II (line C). M_r standards: phosphorylase *b*, 94000; albumin, 67000; ovalbumin, 43000; carbonic anhydrase, 30000; soybean trypsin inhibitor, 20100 and lysozyme, 14400.

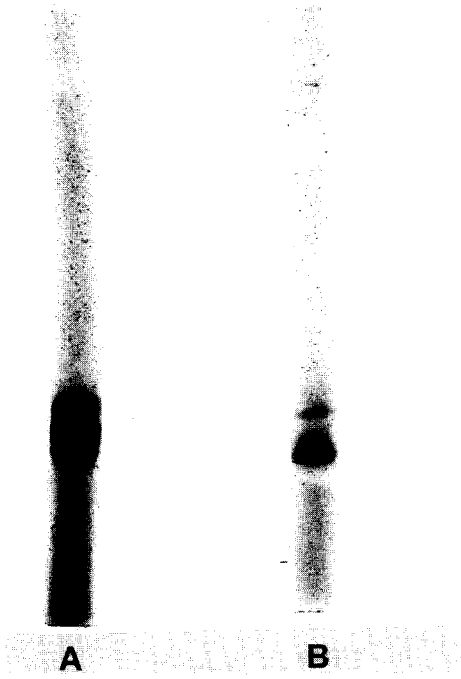

Fig.3. Isoelectric focusing in polyacrylamide gel with a pH 3.5–10 Ampholine gradient of MEA_I (line A) and MEA_{II} (line B).

Table 2

Amino acid composition of *Machaerocereus eruca* lectins[a]

	MEA_I	MEA_{II}
Aspartic acid	15.4	18.3
Threonine	17.6	13.2
Serine	42.8	56.5
Glutamic acid	43	38.4
Proline	8.7	11.5
Glycine	82.8	107.1
Alanine	40.8	33.7
Valine	16.3	11.2
Isoleucine	7.5	6.2
Leucine	12.9	9.3
Tyrosine	4.3	3.3
Phenylalanine	4.7	3.3
Histidine	5.8	6.1
Lysine	9.0	5.9
Arginine	5.0	3
Half-cystine	0	0
Methionine	0	0
Total	316.6	327.0

[a] Results are in residues of amino acids per subunit of 35 kDa

Table 3

Carbohydrate composition of *Machaerocereus eruca* lectins[a]

	MEA_I	MEA_{II}
L-Rhamnose	34 ± 3	35 ± 3
Galactose	31 ± 3	34 ± 3
Mannose	5 ± 1	1 ± 0.5
Glucose	87 ± 7	109 ± 8
L-Arabinose	1 ± 0.5	0
Xylose	44 ± 5	17 ± 2
N-Acetylgalactosamine	0	0
N-Acetylglucosamine	0	0
L-Fucose	0	0
Sialic acid	0	0

[a] Expressed in moles per subunit of 35 kDa

3.3. *Immunological characterization*

Antiserum produced in rabbit immunized with MEA_I gave a single and continuous immunoprecipitation line in the presence of total juice extract and with the MEA_{II} when examined in double-diffusion agar plates (fig.4).

3.4. *Hemagglutinating activity and sugar specificity of* M. eruca *lectins*

Crude extracts from *M. eruca* exhibited high specificity for donkey erythrocytes. Erythrocytes of several other animal species were also agglutinated, but to a lesser extent (table 4). No

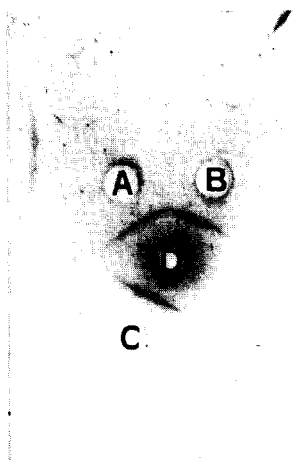

Fig.4. Double immunodiffusion of *Machaerocereus eruca* lectins on agar plates. (A) MEA_I; (B) MEA_{II}; (C) crude extract. Central well: antiserum against MEA_I.

Table 4

Hemagglutinating activity of *Machaerocereus eruca* extracts

Erythrocytes	Titer[a]
Human A	32
Human B	16
Human O	16
Human AB	16
Donkey	2048
Rabbit	256
Sheep	8
Rat	4
Mouse	16
Hamster	64
Guinea pig	128
Chicken	4
Cow	0

[a] Titer represents the highest dilution factor with agglutinating activity induced by 2.4 mg of crude protein extract

specificity for human red blood cells belonging to different blood groups was observed. As shown in table 5, galactose, L-fucose and *N*-acetylgalactosamine inhibited agglutination, although only at relatively high concentrations. Glycosylproteins such as mucin, fetuin or IgA from human serum and milk, were inhibited 100-fold more than *N*-glycosylproteins (orosomucoid, ovomucoid, human sero- and lactotransferrins) (table 6). Glycans and glycopeptides released from the above *O*-glycosylproteins by reductive alkaline treatment

Table 5

Inhibition of agglutinating activity of *Machaerocereus eruca* crude extracts and of MEA_I and MEA_{II} by 0.1 M monosaccharide solution[a]

Sugar	Titer		
	Crude extract	MEA_I	MEA_{II}
Galactose	8	4	8
N-Acetylgalactosamine	16	16	16
Glucose	32	32	32
N-Acetylglucosamine	32	32	32
L-Fucose	16	16	16
Mannose	32	32	32
Mannosamine	32	ND	ND

[a] Crude extract, MEA_I and MEA_{II} concentrations were adjusted to 32 hemagglutinating units in the presence of human erythrocytes A. ND, not determined

Table 6

Minimal concentration (μM) of glycoproteins necessary to inhibit 4 hemagglutinating units of *Machaerocereus* lectins

Glycoproteins	Source	Lectin	
		MEA_I	MEA_{II}
Fetuin	calf serum	0.01	0.005
sIgA	human milk	0.2	0.1
Mucin	pig stomach	0.04	0.02
Mucin	sheep	0.05	0.03
IgA	human serum	0.6	0.1
Lactotransferrin	human milk	12.0	1.5
Serotransferrin	human serum	25.0	3.0
Orosomucoid	human serum	210.0	105.0

are also inhibitory (table 7). A glycan from pig stomach mucin containing galactose, L-fucose, *N*-acetylgalactosamine, *N*-acetylgalactosaminitol and *N*-acetylglucosamine in the molar ratio of 2:1:1:1:3, respectively, was the most powerful inhibitor. Enzymatic cleavage of galactose residues from the asialo-*N*-glycosylpeptides of fetuin abolished their inhibitory effect on the agglutination reaction (not shown).

Table 7

Minimal concentration (μM) of glycans and glycopeptides necessary to inhibit 4 hemagglutinating units of *Machaerocereus eruca* lectins

Substrates	Lectins	
	MEA_I	MEA_{II}
1. Galβ1-4Glc (lactose)	NI	NI
2. NeuAcα2-3(6)Galβ1-4Glc(sialyllactose)	NI	NI
3. Galβ1-3GalNAc-ol	12.5	75
4. NeuAcα2-3Galβ1-3GalNAc-ol	10	15
5. $Gal_2GlcNAc_3Fuc_1GalNAc_1GalNAc$-ol	0.6	0.3
6. $Gal_6GlcNAc_4GalNAc_3Fuc_5GalNAc$-ol	1.8	0.6
7. $Gal_7GlcNAc_4GalNAc_3Fuc_5GalNAc$-ol	2	1
8. $NeuAc_2Gal_4GlcNAc_5Man_3GalNAc_{0.8}Asn$	58	29
9. $Gal_4GlcNAc_5Man_3Asn$	12	15
10. $GlcNAc_5Man_3Asn$	NI	NI
11. $NeuAc_2Gal_1GlcNAc_5Man_3Asn$	NI	NI

NI, not inhibitory at 100 μM. The expressed values are relative to 1 galactosaminitol residue for glycans released by reductive alkaline treatment and to 3 mannose residues for *N*-glycosylpeptides. Sugars 1,2: from human milk; 3,4: from fetuin; 5–7: from pig stomach mucin; 8–10: *N*-glycosylpeptides from fetuin; 11: from hen ovomucoid

4. DISCUSSION

The presence of lectins in the plant family of Cactaceae has been previously suggested [16,17] and preliminary studies with *M. eruca* have identified more than one lectin in the extracts [17]. As shown in this work, two lectins from *M. eruca* can be isolated by affinity chromatography on a pig stomach mucin-Sepharose column. Polyacrylamide gel electrophoresis in the presence of SDS shows that both lectins consist of subunits with an apparent molecular mass of 35 kDa. Purified lectins are glycoproteins containing 36% (MEA_I) and 24% (MEA_{II}) of total carbohydrate, respectively. They do not contain sialic acid, but are rich in glucose, galactose, L-rhamnose and xylose; in addition, mannose is present as well as some L-arabinose in MEA_I.

Both lectins gave a single immunoprecipitation line when tested against anti-MEA_I antiserum showing that the two lectins are immunologically indistinguishable.

Isoelectric focusing of the *M. eruca* lectins reveals three main molecular species with p*I* values of 3.6, 4.1 and 4.6 for MEA_I and two molecular components of p*I* 3.5 and 4.1 for MEA_{II}.

Hemagglutination induced by the crude *M. eruca* lectins is not species-specific although a high affinity for donkey erythrocytes has been shown. Sugar specificity of both purified lectins seems directed toward complex glycan structures containing galactose residues, but the highest affinity presented toward *O*-glycosylproteins and their derived glycans suggests that *M. eruca* lectins can be considered as an additional example of lectin specific for *O*-glycosidically linked glycans, like *Vicia graminea* [18], *Agaricus bisporus* [19] and *Dolichos biflorus* [20].

These lectins are the first ones to be isolated in a purified form from a species belonging to the Cactaceae family. Moreover, the bands observed in isoelectric focusing could represent closely related allelomorphic forms of the same lectin as occurred with *Pisum sativum* [21] or *Lens culinaris* [22] lectins. However, the possibility that both lectins are different forms of the same protein that differ in their extent of glycosylation cannot be discounted.

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