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Separation and partial characterization of isolectins with different subunit compositions from *Datura stramonium* seeds

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The lectin from *Datura stramonium* seeds was separated into three individual isolectins by hydrophobic-interaction chromatography on phenyl-Sepharose. Two of these isolectins are homodimers made up of two A- or two B-subunits, whereas the third is a heterodimer composed of one A- and one B-subunit. Analysis of the homodimeric AA- and BB-isolectins revealed that the A- and B-subunits have similar but not identical M_r values (32 000 and 28 000, respectively), amino acid and carbohydrate compositions. The A-subunit has a higher affinity for *N*-acetyl-D-glucosamine oligomers than the B-subunit, whereas the latter is more specific for the carbohydrate determinants of some animal glycoproteins such as fetuin, asialofetuin and ovomucoid.

Lectin; Isolectin; Subunit composition; (*Datura stramonium*)

1. INTRODUCTION

Plant lectins represent a heterogenous group of (glyco)proteins that recognize and bind specific sugar residues [1,2]. Three groups of plant lectins have received extensive attention, namely the legume lectins, cereal lectins and Solanaceae lectins. Well characterized examples of the latter group are the lectins from potato tuber [3], tomato fruit [4] and Jimson weed (*Datura stramonium*) seeds [5,6]. These lectins share some particular characteristics. Firstly, they have an unusual amino acid composition, characterized by high contents of hydroxyproline, serine, glycine and cysteine; secondly, they are glycoproteins with a high carbohydrate content, most of which is arabinose; thirdly, they all exhibit specificity towards oligomers of *N*-acetyl-D-glucosamine. However, the Solanaceae lectins show differences with respect to their subunit structure. Whereas the agglutinins from potato and tomato are homodimeric proteins [3,4,7] the *D. stramonium* agglutinin (DSA) is considered to be a heterodimer [5,6,8,9]. The occurrence of two types of DSA subunits is based on the observation that upon SDS-PAGE, DSA migrates as two distinct bands with apparent M_r values of 45 000 (subunit A) and 40 000 (subunit B), respectively [5,8]. In this report, we describe the separation and characterization of three different isolectin forms of DSA that arise from AA, AB and BB combinations of the two non-identical A- and B-subunits.

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Abbreviations: DSA, *Datura stramonium* agglutinin; PBS, phosphate-buffered saline

2. MATERIALS AND METHODS

2.1. *Purification of DSA*

DSA was isolated from *D. stramonium* seeds as

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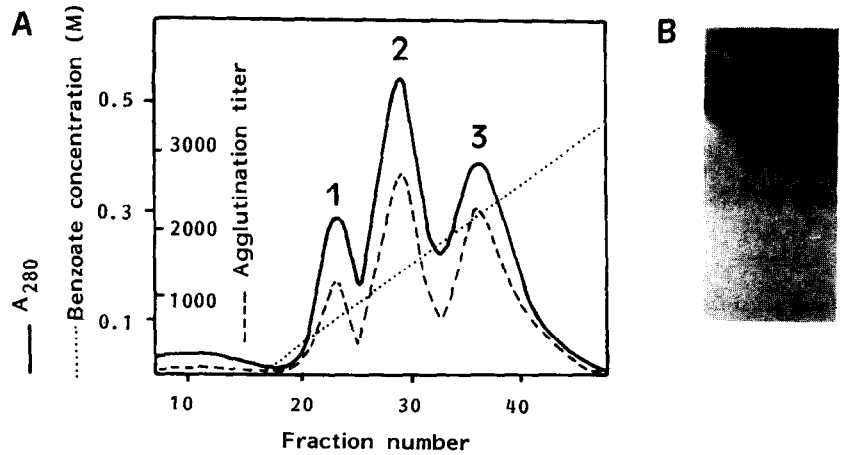

Fig.1. (A) Hydrophobic-interaction chromatography of DSA. A column of phenyl-Sepharose (2.7 × 20 cm) was equilibrated with PBS, loaded with 40 mg DSA (in 50 ml PBS) and washed with 100 ml of water, before application of a linear elution gradient (400 ml) from 0.0 to 0.5 M Na-benzoate. Fraction size was 10 ml. Agglutination assay was done immediately after chromatography and protein was monitored by measurement of A_{280} after extensive dialysis of the fractions against PBS. (B) SDS-PAGE of 25 μg samples from the peak fractions separated by hydrophobic-interaction chromatography. Numbers at the top of the lanes correspond to the peak numbers in (A).

described in [8]. Affinity-purified DSA was further separated from minor contaminating proteins [5,8] by ion-exchange chromatography on sulfopropyl-Sephadex (type C-50) (Pharmacia). DSA was applied to the column in 0.1 M Tris-acetate (pH 6) and eluted with a linear gradient from 0.1 to 0.4 M Tris-acetate (pH 6). DSA was eluted in a well resolved peak at a Tris concentration of 0.15 M.

2.2. *Analytical methods*

Agglutination assays were done with trypsin-treated human blood-group A erythrocytes as described [10]. Protein was determined by the method of Lowry et al. [11], using bovine serum albumin as a standard. SDS-PAGE was done on 12.5–25% (w/v) polyacrylamide gels, using a discontinuous system according to Laemmli [12]. Gels were stained with Coomassie brilliant blue. Amino acid and carbohydrate analyses were carried out as in [13].

2.3. *Carboxyamidation of proteins*

Protein samples were dissolved in 6 M guanidinium-Cl/0.4 M Tris-HCl (pH 8.7)/5 mM dithiotreitol and heated during 2 h at 45°C. Iodacetamide was added to a final concentration of 20 mM and samples were kept in ice for 30 min. The reaction was quenched by the addition of dithiotreitol to a final concentration of 20 mM, followed by a heating for 30 min at 50°C.

2.4. *Preparation of asialofetuin and* N*-acetyl-D-glucosamine oligomers*

Sialic acid was removed from fetuin (Sigma, St. Louis, MO) by treatment at 80°C for 1 h in 0.1 N sulfuric acid, followed by extensive dialysis against PBS. *N*-Acetyl-D-glucosamine oligomers were prepared as described [14].

3. RESULTS

3.1. *Isolation of the DSA isolectins*

Fig.1A shows the elution behavior of DSA chromatographed on phenyl-Sepharose (Pharmacia) and eluted with a linear gradient from 0 to 0.5 M sodium benzoate. Under these chromatographic conditions the lectin is resolved into three peaks. SDS-PAGE analysis of the peak fractions (fig.1B) revealed that the first peak consisted exclusively of subunit A, the last peak of subunit B, whereas the middle peak contained both subunits in equal amounts. All fractions exhibited hemagglutinating activity, indicating that the subunit association is unaffected by the presence of chaotropic ions. Ideed, since DSA has only one carbohydrate binding site per subunit [15], the lec-

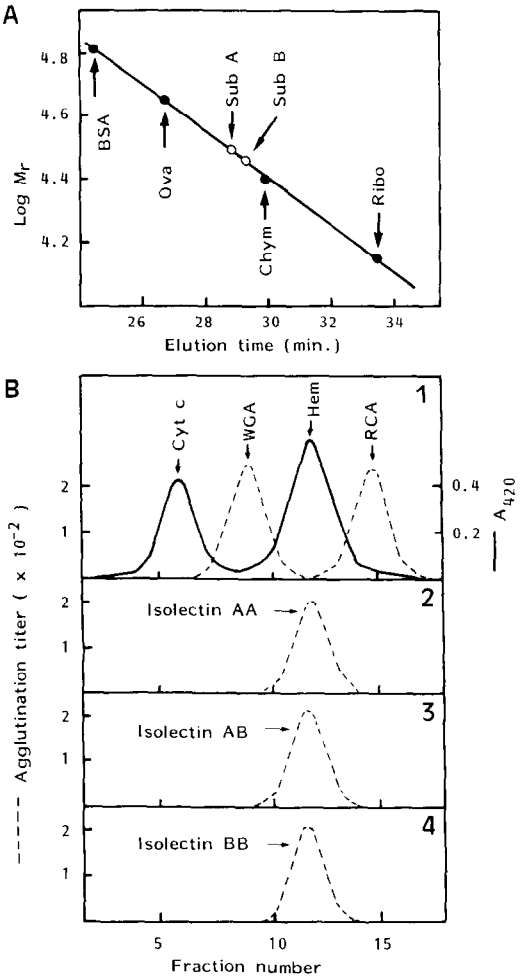

Fig.2. (A) M_r determination of carboxyamidated subunit A (Sub A) and subunit B (Sub B) by HP-gel filtration on a FPLC system (Pharmacia) equipped with a Superose-12 column (HR 10/30) (Pharmacia) and an in-line UV-monitor (280 nm). The column was calibrated with 20 μg amounts of carboxyamidated derivatives of bovine serum albumin (BSA, M_r 65 000), ovalbumin (Ova, M_r 45 000), chymotrypsinogen (Chym, M_r 25 000) and ribonuclease (Ribo, M_r 13 500). Running buffer was 6 M guanidinium-Cl/100 mM Tris-HCl (pH 8.7)/5 mM dithiotreitol, and elution rate was 20 ml/h. (B) Sucrose density gradient centrifugation of native DSA isolectins. Protein samples containing 20 μg amounts of each tested protein were centrifuged in a 12–38% sucrose gradient (in PBS) at 2°C for 24 h in a Beckman SW 50.1 rotor. Gradients were fractionated in 0.2 ml portions and assayed for agglutination activity and A_{420} (to trace the position of cytochrome *c* and haemoglobin). Cytochrome *c* (Cyt c, M_r 12 300), wheat germ agglutinin (WGA, M_r 36 000), hemoglobin (Hem, M_r 65 000) and *Ricinus communis* agglutinin (RCA, M_r 120 000) were used as M_r markers. M_r markers (panel 1), isolectin AA (panel 2), isolectin AB (panel 3) and isolectin BB (panel 4) were run in parallel gradients.

tin should be devoid of agglutinating activity in its monomeric state. It must hence be concluded that the three peak fractions represent dimeric isolectins with different subunit compositions: a homomeric AA-isolectin (peak 1), a heteromeric AB-isolectin (peak 2) and homomeric BB-isolectin (peak 3). The sequence of elution in fig.1 can then be explained by assuming a lower affinity of subunit A relative to subunit B for the phenyl-Sepharose matrix. After extensive dialysis against PBS, the pooled peak fractions were rechromatographed on phenyl-Sepharose, whereby each peak fraction retained its typical elution position (not shown). This is indicative for the stability of the isolated isolectins.

3.2. *Molecular structure of the DSA subunits*

The separation of different DSA isolectins, and especially the availability of the homomeric AA and BB forms, enabled us to compare the A- and B-type subunits of DSA with respect to their biochemical properties. According to Desai et al. [6], dimeric DSA molecules can be dissociated into monomers after reduction of the disulfide bonds. We have prepared A- and B-subunits by reduction and carboxyamidation of the AA- and BB-isolectins, respectively. The molecular mass of these subunits was determined by high-performance gel filtration on a Superose-12 column (Pharmacia, Uppsala, Sweden), using 6 M guanidinium-Cl/100 mM Tris-Cl (pH 8.7)/5 mM dithiotreitol as a running buffer (fig.2A). Under these conditions, M_r values of 32 000 and 28 000 were determined for subunit A and B, respectively. The M_r of native DSA isolectins was also estimated under non-denaturing conditions, by sucrose density gradient centrifugation. As shown in fig.2B, the DSA isolectins sedimented in symmetric peaks either coincident (isolectin AA) or slightly slower (isolectins AB and BB) than hemoglobin (M_r 65 000). These experiments confirm thus that the isolectins isolated by hydrophobic interaction chromatography each consist of two subunits.

The amino acid and carbohydrate composition of subunits A and B (determined by analyses of samples of isolectins AA and BB, respectively) is given in table. 1.

3.3. *Carbohydrate binding properties of the DSA isolectins*

The isolated DSA isolectins were found to have slightly different specific agglutinating activities: minimal concentrations required for agglutination of human trypsin-treated erythrocytes were 75, 100 and 150 ng/ml for isolectins AA, AB and BB, respectively.

The carbohydrate binding specificity of the DSA isolectins was determined by comparing the inhibitory effect on hemagglutination of some oligosaccharides and glycopolypeptides that are known from previous work [5,15] to bind to the lectin. As shown in table 2, β-(1-4) oligomers of *N*-acetyl-D-glucosamine were much more inhibitory for the AA- than for the BB-isolectins. Indeed, the *N*-acetyl-D-glucosamine dimer is 16-fold and the trimer and tetramer are 32-fold more potent inhibitors of AA than of BB. The effect of the oligomers on the AB-isolectin is intermediate, since their activity on this isolectin is about 4-fold lower than that on AA-isolectin. The inhibition pattern of the AB-isolectin by the distinct *N*-acetyl-D-glucosamine oligomers correlates well with previously reported data on the specificity of a total DSA preparation [15]. In contrast to the *N*-acetyl-D-glucosamine oligomers, the aminal glycoproteins (fetuin, asialofetuin and ovomucoid) have a greater affinity for isolectin BB than for isolectin AA and AB. Differences are most prominent with ovomucoid, whose inhibitory effect on BB is 5-fold and 10-fold as high as on AB and AA, respectively.

Table 1

Amino acid and carbohydrate composition of the A- and B-subunits of DSA

Amino acid/carbohydrate	Subunit A	Subunit B
Hyp	25	18
Asp	13	13
Thr	12	13
Ser	27	23
Glu	18	18
Pro	13	15
Gly	23	27
Ala	8	9
1/2 Cys	26	31
Val	2	3
Met	0	0
Ile	0	1
Leu	2	3
Tyr	4	4
Phe	1	1
His	0	0
Lys	2	2
Try	4	5
Arg	6	8
Ara	95	49
Gal	7	4

Results are expressed in residues per molecule assuming a M_r of 32000 and 28000 for the A- and B-subunits, respectively. Values are to the nearest whole residue

Table 2

Carbohydrate-binding specificity of DSA isolectins

	Isolectin		
	AA	AB	BB
GlcNAc	>200	>200	>200
$(GlcNAc)_2$	0.6	2.5	10
$(GlcNAc)_3$	0.15	0.6	5
$(GlcNAc)_4$	0.075	0.3	2.5
Fetuin	0.12	0.06	0.03
Asialofetuin	0.03	0.015	0.0075
Ovomucoid	0.03	0.015	0.003

Results are expressed as the minimal concentration (in mM for the carbohydrates and mg/ml for the glycopolypeptides) required for 50% inhibition of agglutination

4. DISCUSSION

The molecular structure of DSA has been discussed in several publications [5,6,8,9], in which the lectin has been described as a heterodimeric glycoprotein, composed of two non-identical subunits. However, the results presented here indicate that DSA consists of a mixture of three different isolectins separable by hydrophobic-interaction chromatography. From SDS-PAGE analysis and from molecular mass determination of the native and reduced isolectins it could be concluded that the three distinct isolectins have AA, AB and BB subunit compositions respectively. On the basis of our determinations of M_r values by HP-gel filtra-

tion of the A- and B-subunits (M_r 32000 and 28000, respectively), we propose M_r values of 64000, 60000 and 56000 for isolectins AA, AB and BB, respectively. The earlier reported M_r values (M_r 85000 for the native lectin and M_r 45000 and 40000 for subunits A and B, respectively [5,8]) are believed to be less reliable, as they were determined by SDS-PAGE, a method which is inappropriate for the determination of the M_r values carbohydrate-rich glycoproteins.

Biochemical and physicochemical analysis of the AA- and BB-isolectins revealed that the A- and B-subunits have similar but not identical properties. Although the overall amino acid composition of both subunits is very similar, some differences are obvious, for instance the higher hydroxyproline content of subunit A (25 residues/mol) relative to that of subunit B (18 residues/mol). Moreover, subunit A contains about twice as much covalently linked arabinose and galactose as subunit B. Subunits A and B share in common a carbohydrate binding specificity for β-(1-4) linked oligomers of *N*-acetyl-D-glucosamine and for glycoproteins such as fetuin. However, quantitative differences in the inhibitory potency of these compounds on hemagglutination by AA-, AB- and BB-isolectins are apparent. From our data we can conclude that subunit A has the highest affinity for oligomers of *N*-acetyl-D-glucosamine, whereas subunit B exhibits a higher specificity towards the carbohydrate determinants of fetuin, asialofetuin and ovomucoid.

The isolectin composition of *Datura* lectin described in this report shows some striking similarities to that of the lectins from *Phaseolus vulgaris* (PHA) and from *Griffonia simplicifolia* (GSL I) [16,17]. These lectins too are composed of two types of subunits that can be arranged into 5 types of tetrameric isolectins (A_4, A_3B, A_2B_2, AB_3 and B_4). In both cases the distinct subunits have a similar but not identical structure and exhibit different carbohydrate binding properties.

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