Journal of Chromatography, 597 (1992) 197–200
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 830

Improved affinity chromatographic purification of D-mannose–N-acetyl-D-glucosamine-specific lectin from the bark of *Sophora japonica* eliminating the loss by sugar specific self-aggregation

Miyoko Ueno, Tomoko Arai, Kyoko Kojima, Haruko K. Ogawa, Isamu Matsumoto and Nobuko Seno*

Department of Chemistry, Faculty of Science, Ochanomizu University, Otsuka 2-1-1, Bunkyo-ku, Tokyo 112 (Japan)

ABSTRACT

A novel D-mannose–N-acetyl-D-glucosamine-specific lectin of *Sophora japonica* bark, B-SJA-II, which showed self-aggregation based on sugar specificity, was purified by affinity chromatography on maltamyl–Sepharose subsequent to chromatographic separation on lactamyl–Sepharose to remove a major D-galactose–N-acetyl-D-galactosamine specific lectin, B-SJA-I. However, the yield of this method was low as a result of the sugar-specific precipitation and binding to other glycoproteins. A modified method was developed to circumvent this problem. All the purification procedures, except for the final chromatographic separation, were carried out in the presence of the haptenic sugar and the sugar-specific adsorption of B-SJA-II onto the adsorbent was carried out in a dialysis bag by gradually removing the sugar. This method gave a yield eight times higher than the original method.

INTRODUCTION

Affinity chromatography is an effective purification method for proteins, especially the purification of lectins using their carbohydrate specificities [1,2]. It was reported previously that a D-mannose–N-acetyl-D-glucosamine-specific lectin (B-SJA-II) had been found in the bark of *Sophora japonica* and had been purified by two-step affinity chromatography. The crude bark extract was applied to lactamyl–Sepharose to remove a well known D-galactose–N-acetyl-D-galactosamine-specific lectin (B-SJA-I), and finally B-SJA-II was purified by affinity chromatography on maltamyl–Sepharose. In this method, however, the yield was low [3].

B-SJA-II is unique, not only because it is the first known *S. japonica* lectin specific for the mannosyl core of N-linked oligosaccharide chains, but also because of its property of self-aggregation, which is based on sugar specificity and its high carbohydrate content. The subunits recognize their own and the other N-linked oligosaccharide chains common in the plant glycoprotein and specifically aggregate sugar to form an insoluble complex in the absence of the specific sugar.

Sugar-specific binding and the precipitation of B-SJA-II interfered in the purification of B-SJA-II and resulted in the low yield. In this study, to reduce this interference and obtain a high yield, a modified affinity chromatographic procedure was developed.

EXPERIMENTAL

Materials

S. japonica bark was stripped from branches harvested in Tokyo. Sepharose 4B was purchased from Pharmacia (Uppsala, Sweden). Sodium cyanoborohydride ($NaCNBH_3$, special grade) was purchased from Nakarai Tesque (Kyoto, Japan). All other chemicals (special grade) were from Wako Pure

0021-9673/92/$05.00

Chemicals (Osaka, Japan). Lactamyl-Sepharose 4B (30 μmol of lactose per ml of gel) and maltamyl-Sepharose 4B (36 μmol of maltose per ml of gel) were prepared by the amination of epoxy-activated Sepharose and coupling by reductive amination to lactose and maltose, respectively, with slight modifications of previous methods [4–6]. Sepharose 4B was activated with epichlorohydrin in 2 *M* sodium hydroxide solution containing 0.2% (w/v) sodium borohydride at 40°C for 2 h with shaking. After washing with water, the epoxy-activated Sepharose was suspended in 1.5 volumes of concentrated ammonia solution, containing 0.2% (w/v) sodium borohydride, and was incubated at 40°C for 1.5 h with shaking. Coupling of amino-Sepharose to lactose and maltose was carried out in the presence of $NaCNBH_3$ at room temperature for 3–4 days with shaking. The remaining amino groups on the gel were then blocked by N-acetylation.

Methods

Purification of B-SJA-I (original method; Method 1). The bark was defatted by blending in acetone and was extracted with 20 volumes (v/w) of 15 m*M* Tris–HCl, pH 8.5, containing 0.15 *M* NaCl, 0.1 m*M* $CaCl_2$ and 0.05% NaN_3 (TBS) at 4°C overnight. The extract was centrifuged at 9000 *g* for 45 min at 4°C and filtered through cheesecloth. The residue was re-extracted with ten volumes (v/w) of TBS. The combined extracts were fractionated by the addition of ammonium sulphate to 70% saturation at 4°C. After centrifugation, the resulting precipitate was dissolved in distilled water and dialyzed against distilled water. The precipitate formed during the dialysis was removed by centrifugation at 10 000 *g* for 45 min at 4°C and the supernatant was lyophilized for use as crude bark *S. japonica* agglutinin (B-SJA). Crude B-SJA (0.5 g) was dissolved in 25 ml of TBS and applied onto a lactamyl–Sepharose 4B column (10 × 2.5 cm) to remove the D-galactose-specific lectin, B-SJA-I, which was eluted with 0.2 *M* lactose from the column. The passed over fractions, which had haemagglutinating activities inhibited by D-mannose and N-acetyl-D-glucosamine, were pooled and then applied to a maltamyl–Sepharose column (9.5 × 2.0 cm). After washing the column extensively with TBS, the B-SJA-II adsorbed on the column was eluted with 5 m*M* methyl-α-D-mannoside.

Purification of B-SJA-II (modified method; Method 2). Extraction of the bark and ammonium sulphate fractionation of the extract were carried out in the same way as Method 1. After dialysis against distilled water, the whole dialysate, including the precipitate, was lyophilized. The crude B-SJA (0.5 g) thus obtained was dissolved in 25 ml of TBS containing 0.1 *M* methyl-α-D-mannoside and applied onto a lactamyl–Sepharose column (5.0 × 2.0 cm). The passed over fractions were combined and mixed with a half volume (v/v) of maltamyl–Sepharose. After dialysis against distilled water for 2 days at 4°C, the content of the dialysis bag was transferred into a column and the maltamyl–Sepharose gel (5.0 × 2.0 cm) was washed extensively with TBS. The B-SJA-II adsorbed on the gel was eluted with 5 m*M* methyl-α-D-mannoside in the same manner as Method 1. The procedure for Method 2 is shown in Fig. 1.

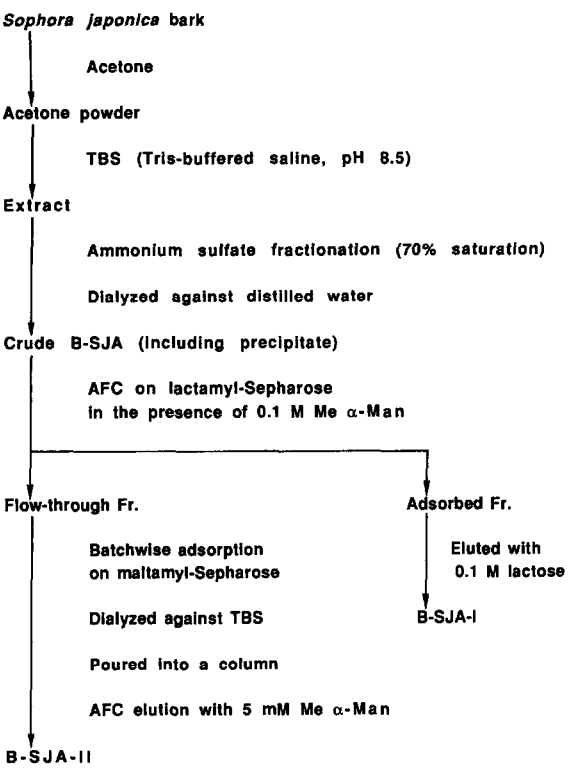

Fig. 1. Purification of B-SJA-II by Method 2. AFC = affinity chromatography; Me α-Man = methyl-α-D-mannoside; Fr = fraction.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis (PAGE) in the presence of 0.1% sodium dodecyl sulphate (SDS) was performed according to the method of Laemmli [7] using 13% acrylamide separation gels.

RESULTS

Purification of B-SJA-II (Method 1)

The yield of crude B-SJA from 50 g of acetone-defatted powder of *S. japonica* bark was 1.5 g. The galactose-specific lectin B-SJA-I was adsorbed onto lactamyl–Sepharose and eluted with 0.2 *M* lactose. The passed over fractions, showing haemagglutinating activities, were pooled and applied to a maltamyl–Sepharose column and the adsorbed B-SJA-II was eluted with 5 m*M* methyl-α-D-mannoside. The yields of B-SJA-II and B-SJA-I from 50 g of the acetone powder were 24 and 177 mg, respectively (Table I).

Purification of B-SJA-II (Method 2)

The yield of crude B-SJA from 50 g of the acetone-defatted powder increased to 3.1 g. To prevent the formation of an insoluble aggregate during purification, the subsequent affinity chromatography was carried out in the presence of methyl-α-D-mannoside. The elution patterns of lactamyl– and maltamyl–Sepharose chromatography were similar to those of Method 1 (data not shown). The addition of 0.1 *M* methyl-α-D-mannoside to the crude B-SJA solution did not interfer with the lactamyl-Sepharose chromatography and enhanced the separation of B-SJA-II from B-SJA-I. The dialysis of the mixture of the passed over fractions and maltamyl–Sepharose gave an effective binding of B-SJA-II onto maltamyl–Sepharose. Using these modifications, the yield of B-SJA-II and B-SJA-I from 50 g of the acetone-defatted powder increased to 194 and 276 mg, respectively (Table I).

TABLE I

YIELDS OF B-SJA-II AND B-SJA-I

The results are expressed as dry weights (g and mg) after lyophilization and yields (%) from 50 g acetone powder of *S. japonica* bark.

	Improved method (Method 2)	Previous method (Method 1)
Crude B-SJA	3.1 g (6.2%)	1.5 g (3.0%)
B-SJA-II	194 mg (0.4%)	24 mg (0.05%)
B-SJA-I	276 mg (0.6%)	177 mg (0.4%)

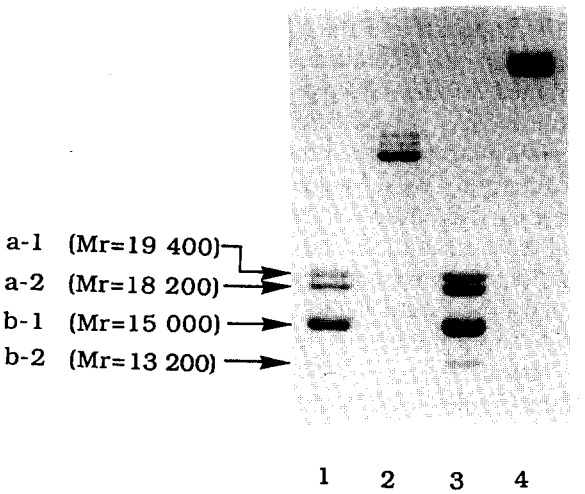

Fig. 2. SDS-PAGE of purified B-SJA-II and B-SJA-I on 13% polyacrylamide gel (pH 8.8). B-SJA-II purified by Method 2 showed four bands corresponding to a-1 [relative molecular mass (M_r = 19 400)], a-2 (M_r = 18 200), b-1 (M_r = 15 000) and b-2 (M_r = 13 200) purified by Method 1 [3] in the presence (lane 1) and absence (lane 3) of 2-mercaptoethanol. B-SJA-I showed M_r = 31 000–34 000 subunits in the presence of 2-mercaptoethanol (lane 2) [8] and broad bands (M_r = 50 000–60 000) in the absence of 2-mercaptoethanol (lane 4).

B-SJA-II purified by Method 2 gave bands on SDS-PAGE identical to the four subunits a-1, a-2, b-1 and b-2 purified by Method 1 (Fig. 2). The chemical compositions and haemagglutinating activity of B-SJA-II purified by Method 2 were also identical with those reported previously [3].

DISCUSSION

In this study, every precipitate containing aggregates of B-SJA-II formed during the purification steps was considered. To circumvent the loss of B-SJA-II by precipitation, two steps of modified affinity chromatography were performed: (1) the whole dialysate, including the precipitate, was used as crude B-SJA so that the B-SJA-II insoluble complex was not lost; (2) separation of B-SJA-II from B-SJA-I by affinity chromatography on lactamyl–Sepharose was performed in the presence of methyl-

α-D-mannoside and the addition of a specific sugar enhanced the solubilization of B-SJA-II and contributed to the effective separation of B-SJA-II from B-SJA-I; (3) batchwise incubation with maltamyl–Sepharose in the presence of methyl-α-D-mannoside and the subsequent removal of the sugar were carried out for effective adsorption. The gradual removal of the specific sugar prevented the formation of a precipitate and B-SJA-II was specifically adsorbed onto maltamyl–Sepharose and eluted with 5 m*M* methyl-α-D-mannoside.

The increase in the yield of B-SJA-II was significant (Table I). The yield of B-SJA-II in Method 1 was 24 mg and that of Method 2 was 177 mg. The yield of crude B-SJA increased two-fold using the modified method. The yield of B-SJA-I was also improved; 194 mg from 50 g of acetone powder in Method 1 to 276 mg in Method 2.

This modified method is useful for detecting lectins which bind to endogenous inhibitors. Using this method, trace amounts of D-mannose–N-acetyl-D-glucosamine-specific lectin was found in *S. japonica* leaves, but not in its seeds (data not shown). The high content of B-SJA-II in the *S. japonica* extract was shown in this study. The high content of B-SJA-II and the distribution of similar lectins in other tissue suggests that B-SJA-II has some biological role or mechanism depending on the type of aggregation present.

ACKNOWLEDGEMENT

This study was supported in part by a Grant-in-Aid (03858047) for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1 C. R. Lowe and P. D. G. Dean, *Affinity Chromatography*, Wiley, London, 1974.
2 I. J. Goldstein and C. E. Hayes, *Adv. Carbohydr. Chem. Biochem.*, 35 (1978) 127.
3 M. Ueno, H. Ogawa, I. Matsumoto and N. Seno, *J. Biol. Chem.*, 266 (1991) 3146.
4 I. Matsumoto, Y. Mizuno and N. Seno, *J. Biochem. (Tokyo)*, 85 (1979) 1091.
5 I. Matsumoto, N. Seno, A. M. Golovtchenko-Matsumoto and T. Osawa, *J. Biochem. (Tokyo)*, 87 (1980) 535.
6 I. Matsumoto, H. Kitagaki, Y. Akai, Y. Ito and N. Seno, *Anal. Biochem.*, 116 (1981) 103.
7 U. K. Laemmli, *Nature (London)*, 227 (1970) 680.
8 C. N. Hankins, J. I. Kindinger and L. M. Shannon, *Plant Physiol.*, 86 (1988) 67.