A SIMPLE METHOD FOR THE PREPARATION OF AN AFFINITY ABSORBENT FOR SOYBEAN AGGLUTININ USING GALACTOSAMINE AND CH-SEPHAROSE

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1. Introduction

Soybean agglutinin (SBA) is a lectin. Lectins are cell-agglutinating, carbohydrate-binding proteins with a number of interesting properties, which are increasingly being used for the investigation of glycoproteins, particularly those of cell surfaces (see reviews [1,2]). SBA has been purified [3,4] and its properties have been investigated by Sharon et al. [4,5]; its agglutination reaction is specifically inhibited by *N*-acetyl-D-galactosamine and, to a lesser extent, by D-galactose [6]. Gordon et al. [7,8] made an effective affinity system for the direct purification of SBA from an extract of soybean meal by linking *N*-ε-aminocaproyl-β-D-galactopyranosylamine to Sepharose. Although the purification system was straightforward and easy to use, the preparation of the affinity absorbent involves four steps, is fairly time-consuming and requires some of the facilities of an organic chemistry laboratory. We have found that an alternative affinity absorbent can easily be prepared by coupling galactosamine to CH-Sepharose (by a carbodiimide reaction in aqueous solution) with the production of a caproylamido linkage to C-2 of galactosamine, rather than a linkage to C-1 of galactose in the system of Gordon et al. [7,8]. The system that we have developed has a high affinity for SBA and the absorbed lectin can be eluted with D-galactose. From 12.5 g of soybean meal 40 mg of purified SBA were obtained. An affinity system for binding wheat-germ agglutinin can be prepared in a similar manner from CH-Sepharose and glucosamine. We suggest that these affinity systems are effective because the lectins are specific for *N*-acyl hexosamines rather than simply for *N*-acetyl hexosamines.

2. Materials and methods

CH-Sepharose 4B was a product of Pharmacia (Uppsala, Sweden), D-galactose and D-galactosamine-HCl were from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K., 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl was from E. Merck, Darmstadt, W. Germany and soybean meal (Batch 1397250) was obtained from BDH Chemicals, Poole, Dorset, UK. All other reagents were commercial preparations of BDH AnalaR grade or the highest purity available.

Protein was determined photometrically, and agglutinating activity was measured using trypsin-treated rabbit red blood cells as described previously [9]. Phosphate-buffered saline contained 7.20 g NaCl, 1.48 g Na_2HPO_4, 0.43 g KH_2PO_4 per litre; the final pH was 7.2.

2.1. *Preparation of the Sepharose-N-caproylgalactosamine conjugate*

The following coupling procedure was basically that recommended by Pharmacia. CH-Sepharose 4B (1.5 g) was swollen in 0.5 M NaCl and washed three times with the same solution by centrifugation, the NaCl was then removed by washing with water. The gel was suspended in water to make a slurry of 25 ml and stirred at room temperature. Galactosamine HCl (100 mg) was added and the pH was adjusted to 5.0 with 0.1 M NaOH. Solid 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (50 mg) was added over a period of 5 min, and after 10 min the pH had risen to 5.3 where it then remained constant. The reaction mixture was stirred for an hour and then left at room temperature for 20 hr without stirring. The gel was then

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washed successively with 1 M NaCl, 1 M NaCl containing 0.1 M Tris–HCl buffer (pH 8.6), 1 M NaCl containing 0.05 M-HCOONa buffer (pH 3.0), 1 M NaCl containing 0.1 M Tris–HCl buffer (pH 8.6), water and phosphate-buffered saline. The gel (6 ml) was then ready for use; if not immediately required it was stored in 2 M NaCl.

2.2. *Purification of SBA*

Soybean meal (12.5 g) was defatted by stirring with 10 vol light petroleum (b.p. 40°–60°C), filtered, and the remaining solvent was removed by drying in a fume-cupboard at room temperature. The defatted meal (10 g) was stirred with phosphate-buffered saline (200 ml) overnight at 4°C, centrifuged at 9000 *g* for 15 min and the supernatant was retained. This supernatant was applied at room temperature to a column (7 cm × 1 cm) of the Sepharose-*N*-caproylgalactosamine. Of the protein which was applied, 99% passed through the column but the lectin was retained. The column was then washed with phosphate-buffered saline until the absorbancy of the effluent was below 0.02 at 280 nm. Fractions (5 ml) were then collected and SBA was eluted with 0.1 M galactose in phosphate-buffered saline at 4°C. Those fractions which contained active material were pooled, dialysed extensively against water and freeze-dried.

3. Results and discussion

As was reported by Gordon et al. [7,8] the SBA which was eluted from the affinity column consisted of one major lectin and a number of minor isolectins which could be separated on DEAE-cellulose. The freeze-dried material was homogenous on disc-gel electrophoresis in SDS (pH 7.2 in 10% gel, staining with Coomassie Blue). Its amino acid and carbohydrate compositions were in agreement with previous reports [4,5]. When saturated 6 ml of gel were found to be capable of retaining about 80 mg of SBA.

The *N*-caproylgalactosamine affinity system for SBA which we report here is much easier to prepare than is the *N*-caproylgalactosylamine system of Gordon et al. [7,8], as the former synthesis requires an overnight incubation in aqueous solution and the latter a rather lengthy 4-stage chemical preparation. The gel produced by the method reported here has a very high capacity for SBA (about 12 mg/ml gel) and like all such systems can be used repeatedly. The procedure for the actual purification of SBA is essentially similar to that of Gordon et al. [7,8] excepting that, with this batch of soybean meal, it was possible to omit the ammonium sulphate fractionation and obtain a hundredfold purification on the column in a single step.

Lis et al. [6] have shown that derivatives of *N*-acetylgalactosamine are tenfold better inhibitors of SBA than are derivatives of galactose. The product of coupling CH-Sepharose to galactosamine is Sepharose-*N*-caproylgalactosamine. This is an analogue of *N*-acetylgalactosamine and is likely to have a greater affinity for SBA than is the derivative of galactose(caproylgalactosylamine) produced by Gordon et al. [7,8]. The specificity of SBA is therefore directed towards *N-acyl*-galactosamines in general rather than *N-acetyl*galactosamine in particular.

The general specificity for *N*-acyl, rather than particularly for *N*-acetyl hexosamines may be a general feature of lectins, since wheat-germ agglutinin has already been shown to be as effectively inhibited by *N*-propionylglucosamine as by *N*-acetylglucosamine [9]. This was emphasised by the finding that we could couple glucosamine to CH-Sepharose, (in a similar manner to the coupling of galactosamine) to produce an affinity system that extracted wheat-germ agglutinin from a crude extract of wheat-germ. We also found that wheat-germ agglutinin could be eluted from the column with acetic acid in the same manner as from the Sepharose-2-acetamido-*N*-(ε-aminocaproyl) β-glucosylamine column of Lotan et al. [10].

It is probable that other *N*-acetylgalactosamine-binding lectins (see list in [1,2]) such as those from *Dolichos biflorus, Sophora japonica* and *Phaseolus lunatus* could also be purified using this *N*-caproylgalactosamine affinity system.

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