Studies on plant gums. Isolation and characterisation of the major polysaccharide from Neem (Azadirachta indica) gum

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Abstract. Azadirachta indica (neem) exudate gum was treated with pronase for 48 h followed by chromatography on TEAE-cellulose and the major polysaccharide was isolated. The polysaccharide covalently associated with remnant protein, was homogeneous as indicated by rechromatography on TEAE-cellulose, paper electrophoresis, gel chromatography under dissociating conditions on Bio-Gel P-100 and P-300. The monosaccharide units, galactose, arabinose, glucuronic acid, fucose and glucosamine were present in a molar ratio of 86 : 70 ; 30 : 10 : 1. Thirteen amino acids constituted the protein portion. The linkage between the polysaccharide and the protein was a glucosaminyl asparginyl bond. Limited hydrolysis showed that fucose and arabinose were at the non-reducing ends of the polysaccharide and galactose and glucuronic acid were in the central core.

Keywords. Azadirachta indica (neem) gum; polysaccharide composition; glucosaminyl asparginyl bond.

1. Introduction

Neem (Azadirachta indica) exudate gum which belongs to the family of 'galactan' gums is known to contain D-galactose, L-arabinose, D-glucuronic acid and L-fucose as the monosaccharide units (Mukherjee and Srivatsava 1955). Usha Lakshmi and Pattabiraman (1967) identified an additional sugar, D-glucosamine and also showed the presence of appreciable amounts of protein in the neem gum. Fractionation studies revealed the presence of carbohydrate-rich and protein-rich components in this gum (Satya Narayan and Pattabiraman 1973). In this paper, the isolation of the major polysaccharide fraction after pronase treatment of the neem gum and its characterisation are reported. Evidence is provided to show that the remnant protein in the fraction isolated, is linked covalently to the polysaccharide.

2. Materials and methods

2.1. Materials

Neem gum samples used in these studies were hand-picked during the summer months of March to June and were stored at room temperature until use. The powdered gum was dissolved by stirring in water at a concentration of 6 % (wt/vol). The cloudy
solution was centrifuged at 12000 g for 20 min. The supernatant solution was treated with charcoal to remove colouring matter and filtered. The clear pale yellow solution was dialysed against 50 vol of water for 16 h with one change after 8 h. The solution was stored at -5°C until use.

TEAE-cellulose, CM-cellulose, Bio-Gel P-300, P-100 and P-2, Dowex-50 (X4, 200–400 mesh H+) and Dowex-1 (X8, 100-200 mesh, Cl⁻) were obtained from Bio-Rad Laboratories, Richmond, CA, USA. Pronase was purchased from Sigma Chemical Company, St. Louis, MO, USA. Other reagents were of analytical reagent grade.

2.2. Methods

2.2.1. General methods: Total carbohydrate was estimated by the method of Dubois et al. (1956) using galactose as the standard. Protein and Folin-positive materials were estimated by the method of Lowry et al. (1951) using bovine serum albumin as the standard. L-Arabinose was determined by the phloroglucinol method (Dische and Borenfreund 1957). Glucuronic acid was estimated by the method of Bitter and Muir (1957). Glucosamine was assayed in the hydrolysates by the method of Levvy and McAllan (1959). Reducing sugar was determined by the method of Nelson (1944).

2.2.2. Pronase digestion: Three ml of the gum solution (106 mg carbohydrate and 54 mg protein) was mixed with 3 ml of 0.1 M sodium phosphate buffer, pH 7.0, heated for 10 min at 98°C and cooled. Trace amounts of calcium present in the gum were precipitated at this stage. To the clear supernatant obtained after centrifugation at 12000 g for 20 min, 1 mg of pronase (in 0.1 ml water) was added. After 48 h incubation at 28–30°C, the solution was heated at 98°C for 10 min and centrifuged.

2.2.3. Isolation of the major polysaccharide: The clear supernatant of the pronase digest was applied to a column of TEAE-cellulose (1.8 x 30 cm, bed vol 70 ml) equilibrated with 0.1 M sodium phosphate buffer, pH 7.0, washed with 140 ml of the equilibration buffer, and eluted with 140 ml of 0.1 M phosphate buffer, pH 5.0, containing 1 M NaCl at a flow rate of 40 ml per h. Ten ml fractions were collected and assayed for carbohydrates and proteins. Fractions 20 and 21 (figure 1) were pooled and dialysed against water. Several such dialysed fractions were pooled and concentrated by lyophilisation. This fraction designated as the major polysaccharide, contained 31.3 mg carbohydrate and 1.75 mg protein.

2.2.4. Rechromatography of the major polysaccharide on TEAE-cellulose: The major carbohydrate fraction was dissolved in 1 ml, was applied to a column of TEAE-cellulose (1.8 x 16 cm, bed volume 38 ml) equilibrated with 0.1 M phosphate buffer, pH 5.0. A gradient of 150 ml 0.1 M phosphate buffer, pH 5.0 in the mixing chamber and 150 ml of 0.1 M phosphate buffer, pH 5.0, containing 1 M NaCl in the outer chamber was applied at a flow rate of 25 ml per h. Ten ml fractions were collected and assayed for carbohydrate and protein.