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INTERACTION OF PNEUMOCOCCAL S-14 POLYSACCHARIDE WITH LECTINS FROM *Ricinus communis*, *Triticum vulgaris*, AND *Bandeiraea simplicifolia*

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ABSTRACT

Two purified lectins, namely, wheat-germ agglutinin (from *Triticum vulgaris*) and the hemagglutinin from *Ricinus communis* seeds, readily form a precipitate with pneumococcal S-14 polysaccharide, whereas the *Bandeiraea simplicifolia* lectin (BS I) does not. Exhaustive periodate oxidation and borohydride reduction of S 14 modifies terminal β-D-galactopyranosyl residues, as well as chain D-glucopyranosyl residues, and abolishes reactivity with both the *R. communis* lectin and wheat-germ agglutinin. Controlled periodate oxidation followed by Smith degradation cleaves only terminal β-D-galactopyranosyl residues, giving a linear polymer, the structure of which was determined by methylation analysis. This derived polymer, containing (1→6)-linked 2-acetamido-2-deoxy-β-D-glucosyl residues, readily precipitated wheat-germ agglutinin, but not the *R. communis* lectin.

INTRODUCTION

Carbohydrate-binding proteins (lectins) are proving to be useful reagents for probing structural features of polysaccharides (including *Pneumococcus* S-12 capsular polysaccharide)[1–3] and glycoproteins[4–6]. These plant and animal agglutinins, especially in their immobilized form, are also finding application in the isolation of carbohydrate-containing macromolecules, including cell-surface glycoproteins[7–12].

We now report on the use of several plant lectins for investigating *Pneumococcus* type-14 capsular polysaccharide, (S 14), the structure of which is reported in the preceding communication[13]. The interaction of S 14 with crude extracts of *R. communis* and *T. vulgaris* has been reported previously[14–17].

RESULTS AND DISCUSSION

S 14, obtained from the same source as reported in the previous paper[13], gave precipitin-like curves with both wheat-germ agglutinin and the lectin from *R.*

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communis (RCA_I) (Fig. 1), but not with the α-D-galactopyranosyl-binding lectin from *B. simplicifolia* seeds. The *R. communis* lectin cannot distinguish α- and β-D-galactopyranosyl residues[18-20], whereas the *B. simplicifolia* lectin (BS I) interacts only with α-D-galactopyranosyl end-units (and to a lesser extent with 2-acetamido-2-deoxy-α-D-galactopyranosyl residues, which are not present in S 14). These results confirm the presence of β-D-galactopyranosyl end-groups in S 14 (*cf.* Ref. 22).

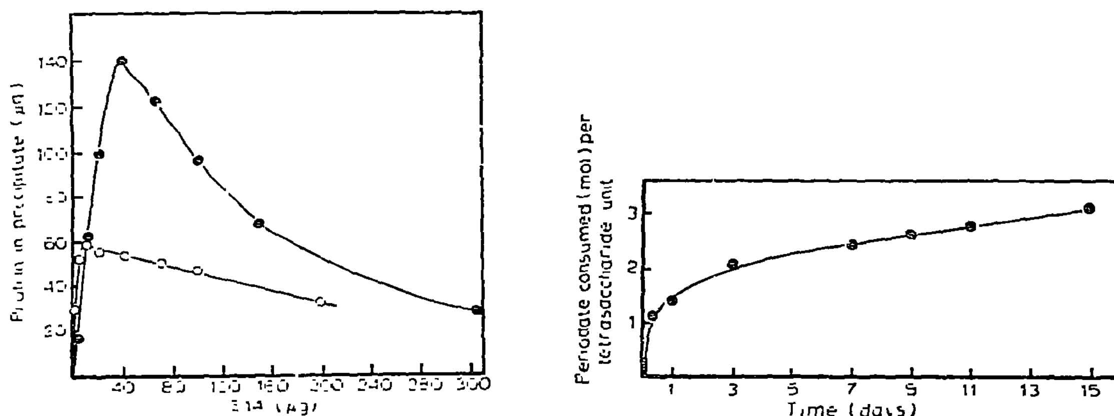


Fig. 1. Precipitation of S 14 by wheat-germ agglutinin and *R. communis* lectin: (●——●), wheat-germ agglutinin (150 µg of protein per tube); (○——○), *R. communis* lectin (65 µg of protein per tube); in a total volume of 250 µl.

Fig. 2. Periodate consumption of S 14 in 0.02M sodium metaperiodate at 4° in the dark.

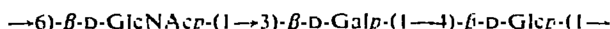
Formation of a specific precipitate between S 14 and wheat-germ agglutinin is noteworthy, because this is the first time that a 2-acetamido-2-deoxy-β-D-glucopyranosyl residue substituted at *both* the O-4 and O-6 positions has been shown to interact with the purified lectin. Wheat-germ agglutinin has a combining site complementary to a tri-*N*-acetylchitotriosyl unit[23-25]. The lectin also reacts with terminal, non-reducing 2-acetamido-2-deoxy-β-D-glucopyranosyl groups and with non-contiguous, internal, (1→4)-linked 2-acetamido-2-deoxy-β-D-glucopyranosyl residues, as they occur, for example, in keratan[26].

S 14 reduced three molar equivalents of periodate per tetrasaccharide unit (Fig. 2) over a period of 15 days; this is consistent with the oxidation of the terminal, as well as the (1→4)-linked, β-D-glucopyranosyl units. Not surprisingly, the derived S-14 polyalcohol, obtained by reduction of the polyaldehyde with sodium borohydride, no longer reacted with the *R. communis* lectin.

On the other hand, the lack of reactivity of S-14 polyalcohol with wheat-germ agglutinin was somewhat surprising; perhaps the lectin is sterically hindered by the hydroxyl groups of the oxidized-reduced residue attached to O-4 of (1→6)-linked 2-acetamido-2-deoxy-β-D-glucosyl residues.

In another experiment, the periodate oxidation of S 14 was terminated after

1 day, at which time ~ 1.4 molar equivalents of periodate per tetrasaccharide unit had been consumed. The product was reduced with sodium borohydride and subjected to a Smith degradation. The resulting polymer, isolated by dialysis followed by freeze-drying, was methylated by the Hakomori technique[27]. G.l.c.–m.s.[28,29] analysis of the resulting *O*-methyl sugars, as their alditol acetates, gave 2,4,6-tri-*O*-methyl-D-galactose and 2,3,6-tri-*O*-methyl-D-glucose in the ratio of 1:0.91. A peak corresponding to 2-acetamido-2-deoxy-3,4-di-*O*-methyl-D-glucose was also obtained, but no 2,3,4,6-tetra-*O*-methyl-D-galactose was found. The results clearly indicate that preferential oxidation and cleavage of the single-unit, β-D-galactopyranosyl stubs in S 14 had occurred, and indicate that the repeating unit of the Smith-degraded S 14 has structure **1**.



1

The observation that this polymer (**1**) forms a precipitate with wheat-germ agglutinin (Fig. 3) marks the first time that (1→6)-linked 2-acetamido-2-deoxy-D-glucosyl residues have been shown to interact with wheat-germ agglutinin. It has been noted previously that methyl 2-acetamido-2-deoxy-6-*O*-methyl-α-D-glucopyranoside inhibited agglutination of rabbit erythrocytes by wheat-germ agglutinin[23]. In accordance with the result of the methylation analysis, **1** did not form a precipitate with *R. communis* lectin (Fig. 3).

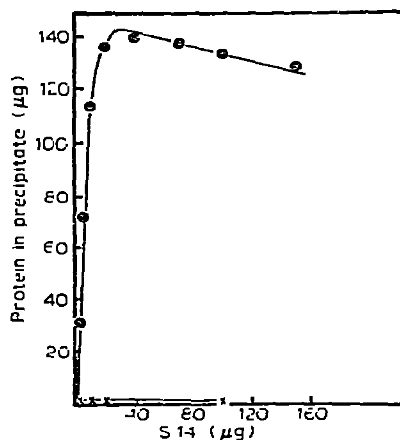


Fig. 3. Precipitation of Smith-degraded S 14 by wheat-germ agglutinin and *R. communis* lectin ●——●, wheat-germ agglutinin (150 µg of protein per tube); ×——×, *R. communis* lectin (65 µg of protein per tube); in a total volume of 250 µl.

This study represents a further example of how pure lectins of known specificity may be used as structural probes and as tools for assessing the results of chemical modification and degradation studies.

EXPERIMENTAL

Materials. — Wheat-germ agglutinin was obtained from Calbiochem, San Diego, Cal., U.S.A. *Ricinus communis* lectin RCA_I was a gift from Dr. M. E. Etzler of the University of California, Davis, Cal.; *Bandeiraea simplicifolia* lectin (BS I) was prepared by the procedure of Hayes and Goldstein[21].

The capsular polysaccharide of *Pneumococcus* type 14 (lot 116R) was obtained from E. R. Squibb & Sons, New Brunswick, N.J., U.S.A.

Precipitin reaction. — Quantitative precipitin reactions were performed by a microprecipitin technique[30]. Increasing amounts of S 14 or modified S 14 dissolved in PBS-Ca [phosphate-buffered saline (0.01M phosphate, pH 7.0; 0.15M NaCl) with 0.1mM $CaCl_2$ (for *R. communis* lectin and *B. simplicifolia* lectin)] or PBS [phosphate-buffered saline (0.01M phosphate, pH 7.0; 0.15M NaCl) (for wheat-germ agglutinin)] were added to duplicate tubes containing lectin and PBS-Ca or PBS in a final volume of 250 μl. After incubation at room temperature for 48 h, the precipitates were collected by centrifugation and washed with PBS-Ca or PBS as described. Protein in the precipitates was determined with the Folin-Ciocalteau phenol reagent by the method of Lowry *et al.*[31]; crystalline, bovine serum albumin was used as standard.

Periodate oxidation. — Periodate consumption was determined by the method of Fleury and Lange[32]. S 14 (10 mg) was dissolved in 0.02M sodium metaperiodate (20 ml). The solution and a control lacking S 14 were incubated in the dark at 4°. After complete oxidation (15 days; periodate consumption 3.1 mol./tetrasaccharide unit), excess of periodate was precipitated with barium carbonate. The polyaldehyde was reduced with sodium borohydride (30 mg) for 20 h at room temperature. Acidification (acetic acid) to pH 6.3 destroyed residual borohydride. The reaction mixture containing the S-14 polyalcohol was dialyzed against water and lyophilized (yield, 7.6 mg).

Preparation of Smith-degraded S 14. — S 14 (4 mg) was treated with 0.02M sodium metaperiodate (8 ml) in the dark at 4° for 24 h. After neutralization ($BaCO_3$), the oxidized S 14 was reduced with sodium borohydride (15 mg) in the usual way. The solution was acidified to pH 1.0 with 0.5M sulfuric acid and kept at 25° for 24 h[33]. The solution containing Smith-degraded S 14 was neutralized ($BaCO_3$), dialyzed against water, and lyophilized (yield, 2.0 mg).

Methylation analysis of Smith-degraded S 14. — The polysaccharide (~1 mg) was methylated according to Hakomori[27], and the product dialysed free of reagents. The recovered material was hydrolysed with 90% aqueous formic acid followed by 0.13M aqueous sulfuric acid. After neutralization ($BaCO_3$), the sugars were transformed into alditol acetates by reduction with sodium borohydride followed by acetylation with acetic anhydride–pyridine[28]. G.l.c.-m.s. analysis was performed on columns of 3% of OV-225 at 170° (for neutral sugar derivatives) and 3% of OV-17 at 190° (for acetamido sugar derivatives). The compounds were identified from their T values[28] and mass spectra[13,28,29].

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