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Note

Abnormal affinity of *Staphylococcus aureus* N-acetyl glucosamine ribitol teichoic acid for wheat-germ agglutinin

ANTOINETTE NDULUE*
Laboratoire d'Immunochimie Microbienne, Centre de Recherches Epidémiologiques des Staphylocoques et Streptocoques, Faculté de Médecine Alexis Carrel, Rue Guillaume Paradin, F-69372 Lyon Cedex 2 (France)
JEAN-PIERRE FLANDROIS
Laboratoire de Bactériologie et Virologie, Faculté de Médecine Lyon-Sud, Hopital Jules Courmont/Ste Eugenie, F-69310 Pierre Benite (France)
and
DANIELLE MARMET
Laboratoire d'Immunochimie Microbienne, Centre de Recherches Epidémiologiques des Staphylocoques et Streptocoques, Faculté de Médecine Alexis Carrel, Rue Guillaume Paradin, F-69372 Lyon Cedex 2 (France)
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The increasing use of *Staphylococcus aureus* ribitol teichoic acid (RTA), which possesses a β-N-acetylglucosamine residue, in serological diagnosis especially in bacteremia and osteoarticular infections[1–7] prompted us to purify this antigen. In the course of this purification, the wheat-germ agglutinin immobilized on Ultrogel (WGA-Ultrogel) which has affinity for the N-acetylglucosamine residue[8–16] was employed to separate RTA from other contaminating antigens.

This study is concerned with the abnormal affinity of the β-N-acetylglucosamine ribitol teichoic acid of *Staphylococcus aureus* for the WGA-Ultrogel which is dependent on the ionic forces and the nature of the contaminants.

MATERIALS AND METHODS

Strains and culture

Staphylococcus aureus strains 830 and Wood 46 were used. These were cultured in a 20-l fermentor (Chemapec Sarl, Bron, France) at 37°C for 24 h with aeration (0.44 l l^{-1} min^{-1}). The medium consisted of brain–heart infusion (450 g), yeast extract (100 g) and glucose (750 g), all in 15 l of distilled water.

Extraction and purification of the RTA of Staphylococcus aureus

Two methods were used. First, Armstrong's method[17] in which 100 g (wet weight) of bacteria were suspended in 3 l of 10% trichloroacetic acid solution for 24 h at 4°C and then centrifuged at 8300 *g* for 30 min. A 6-l volume of acetone was added to the supernatant at −15°C and after 24 h the mixture was centrifuged. The supernatant was kept apart. The precipitate was washed three times with distilled water and centrifuged. The four supernatants were pooled, dialysed against distilled water and freeze-dried.

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Secondly, Haukenes' method[18] was used in which 100 g of bacteria were suspended in 1 l of 1/15 *M* phosphate buffer, pH 6.5, at 37°C with continuous stirring for 24 h. The suspension was centrifuged and the extraction repeated twice with half the original volume of buffer. The three supernatants were pooled and 1 *N* HCl was added to pH 4.2 at 4°C. After 24 h the mixture was centrifuged, four volumes of absolute alcohol were added to the supernatant at pH 5.2 which was allowed to stand overnight at −15°C. The precipitate was dissolved in water, dialysed against distilled water and freeze-dried.

The freeze-dried samples were dissolved and applied on a column (40 × 2.8 cm) of DEAE-cellulose (DE-52; Whatman, Maidstone, Great Britain). Elution was performed with a gradient of 0.5 *M* KCl in 0.02 *M* phosphate buffer, pH 7.4, at a flow-rate of 30 ml/h. The positive fractions, detected by double diffusion in agar, were desalted on a column (40 × 2.8 cm) of Bio-Gel P-2 (Bio-Rad Labs., Richmond, CA, U.S.A.).

The final product from strain Wood 46 is pure ribitol-I-phosphate polymer substituted by N-acetylglucosamine in the β-position, as shown by nuclear magnetic resonance. That from strain 830 consists of RTA and a_5 agglutinogen, a type of antigen of *Staphylococcus aureus*[19].

Affinity chromatography of RTA

A 10-mg amount of RTA was applied on a column (10 × 1.8 cm) of WGA-Ultrogel (Industrie Biologique Francaise, Clichy, France) at a flow-rate of 15 ml/h. Two different washing buffers were used: 0.05 *M* Tris–HCl, pH 7.8, containing 0.15 *M* NaCl; and 0.01 *M* Tris–HCl, pH 7.8, containing 0.01 *M* NaCl. The elution was performed with 0.05 *N* HCl. Ultraviolet (UV) detection of the polysaccharides was carried out at 206 nm in an LKB detector (Uvicord 2138; LKB, Bromma, Sweden).

Infrared (IR) spectroscopy

This analysis was performed with a potassium bromide (KBr) disk. A 1-mg amount of the sample was ground in 250 mg of KBr and compressed at a pressure of 10 tons in a press (30-ton press C-30; Research and Industrial Instruments Co., London, Great Britain) for 20 min *in vacuo* to obtain a clear disk which was then placed in the IR spectrophotometer (Model 237E; Perkin-Elmer, Beaconsfield, Great Britain).

Sugar analysis by gas chromatography

The sugars were analysed as their trimethylsilyl alditol derivatives. A 0.5-mg amount of the sample was hydrolysed in 2 *N* HCl for 3 h at 100°C, then evaporated. A reducing mixture (150 μl) of sodium borohydride (1 mg) and 1 *M* ammonium hydroxide (300 μl) was added at 20°C. After 2 h, 4 ml of acetic acid were added and the solvent evaporated. A 2-ml volume of methanol–conc. HCl (1000:1, v/v) was added and the solvent origin evaporated. Trimethylsilylimidazole (TSIM) (300 μl) and 300 μl of pyridine were added and the mixture left to stand for 20 min at 70°C. A 150-μl volume of N-heptafluorobutyrylimidazole was then added and the mixture again left for 20 min at 70°C. The derivatized product was then extracted with 250 μl of hexane and 150 μl of water. A 1.5-μl volume of the hexane layer was injected into a SE-30 wall-coated open tubular glass capillary column (50 m × 0.35 mm I.D.) (Chrompack, Middelburg,

The Netherlands) in a Packard 427 gas chromatograph equipped with a flame ionization detector (Packard Instruments, Delft, The Netherlands). The oven temperature was programmed at 2°C/min from 140°C to 270°C. The temperature of the injector was 200°C and that of the detector was 320°C. Quantification was done by using xylose as internal standard.

Detection of positive fractions

The rabbit immune sera used for the detection of the antigens were prepared by intravenous injections of formalin-killed bacteria[20].

The antigens were detected by double diffusion on immuno slides (Gelman, Ann Arbor, MI, U.S.A.) with 1.2% noble agar dissolved in 0.1 *M* Tris–HCl buffer, pH 7.4, and by counter-current immunoelectrophoresis on glass plates (10 × 10 cm) with 1% agarose dissolved in barbital buffer, pH 8.6 (Behringwerke Immunoelectrophoresis Chamber; Behringwerke, Lahn, G.F.R.).

RESULTS

Only a small amount of the pure preparation from Wood 46 was bound to WGA-Ultrogel when 0.05 *M* Tris–HCl buffer, pH 7.8, containing NaCl was used to wash the column before and after application of the sample (Fig. 1). Approximately the same amount was bound, using the same buffer, when the strain 830 preparation was applied, but this was heavily contaminated by agglutinogen a_5 (see Fig. 2). When the ionic strength was reduced, *i.e.*, in 0.01 *M* Tris–HCl containing 0.01 *M* NaCl, most of the antigen was bound to the WGA column (see Fig. 3).

IR spectra of both the free and bound RTA had the same characteristics with respect to the N-acetylation of the glucosamine amino group, as shown in Figs. 4 and 5. Gas–liquid chromatography (GLC) shows that the unbound RTA fraction is composed of N-acetylated ribitol and glucosamine, a_5 antigen is also composed of N-acetylated ribitol and glucosamine.

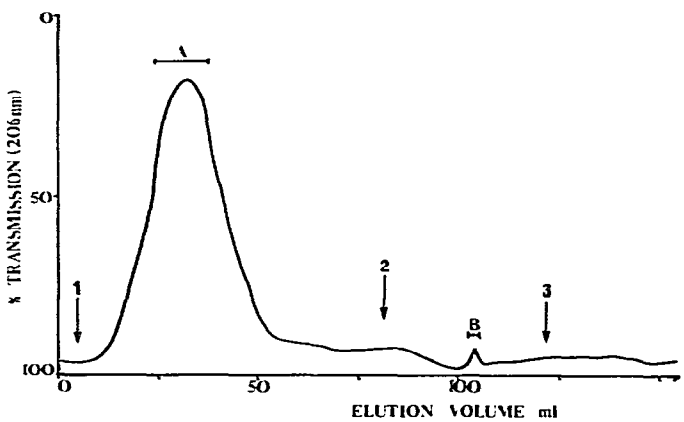

Fig. 1. Affinity chromatography of pure RTA on WGA column. The buffer: 1 and 3 were 0.05 *M* Tris–HCl, pH 7.8, containing 0.15 *M* NaCl. Elution was effected with 0.05 *N* HCl (2). Fractions in regions A and B contained RTA; the B fractions were detectable only in counter-current immunoelectrophoresis.

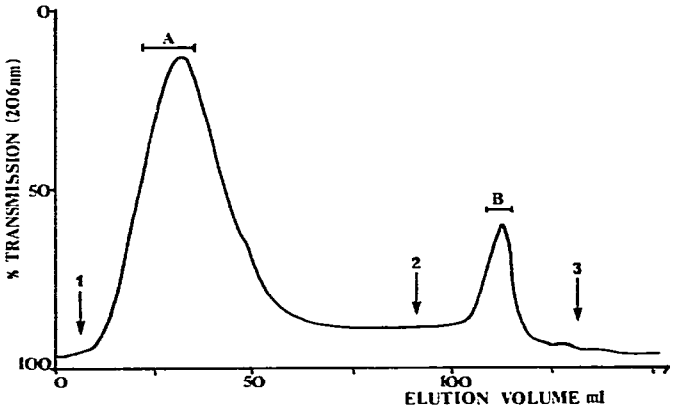

Fig. 2. Affinity chromatography on WGA column of the partially purified RTA extract from strain 830 of *Staphylococcus aureus*. The washing and elution buffers were as in Fig. 1. The fractions in region A contained RTA and a_5 agglutinogen which was not bound to the gel; region B consisted mainly of a_5 (*ca.* 2 mg) and RTA (*ca.* 0.125 mg).

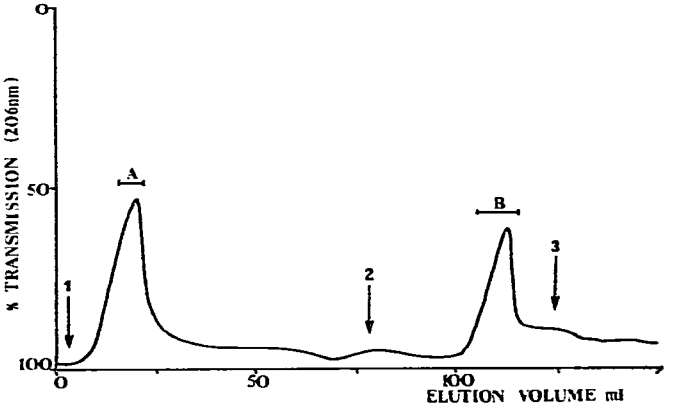

Fig. 3. Affinity chromatography of pure RTA on WGA column. Buffers 1 and 3 were 0.01 *M* Tris–HCl, pH 7.8, containing 0.01 *M* NaCl. Elution was effected with 0.05 *N* HCl (2). The fractions A and B contained pure RTA; the B fractions were obtained by double diffusion in agar.

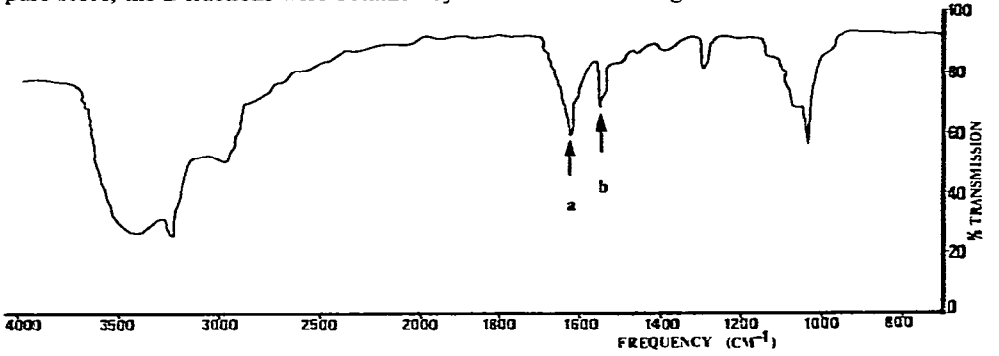

Fig. 4. IR spectrum of a compressed disk of RTA (1 mg) and KBr (250 mg), from the RTA fixed on WGA-Ultrogel, recorded on a Perkin-Elmer IR spectrophotometer. Peaks: a = 1630–1650 cm^{-1} band corresponding to the ν (C=O) vibrations of the N-acetyl group; b = 1550 cm^{-1} band corresponding to the δ (NH) vibration of the N-acetyl group.

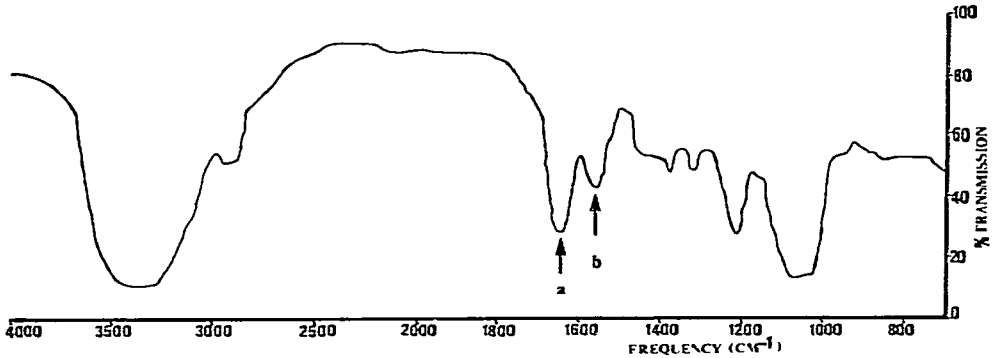

Fig. 5. IR spectrum of the RTA which did not bind to the WGA-Ultrogel. The conditions and peaks are as in Fig. 4.

DISCUSSION

The use of affinity chromatography on immobilized lectins to purify polysaccharides has been reported[9,21–23]. Thus Concanavalin A, which is chemically bound on Sepharose gel, is used to purify polysaccharides and glycoproteins having α-D-mannoside, α-D-glucoside or internal O-α-D-mannoside groups[23–25].

Surprisingly the WGA column was quickly saturated when RTA was applied. Only a small quantity of sample was bound. However, a greater quantity of the contaminating a_5 (see Fig. 2) was bound to the WGA-Ultrogel, showing that the gel still contains wheat-germ agglutinin. The bound a_5 was shown to have the same characteristics as RTA by IR spectroscopy (see Fig. 6) and GLC analysis. More RTA was bound to the WGA-Ultrogel when the molarity and salt content of the buffer were reduced.

The affinity of β-N-acetylglucosamine for WGA immobilized on gel is in agreement with the findings of Goldstein *et al.*[9]. Although we observed that ionic forces play an important role in the affinity of RTA for WGA, we cannot explain why not all of the RTA was bound when the column was saturated.

The binding of a_5 to the WGA column at high ionic strength is also unexpected because a_5 has a teichoic-like structure containing ribitol and β-N-acetylglucosamine and exhibits a very characteristic immunological activity, but no immunological cross

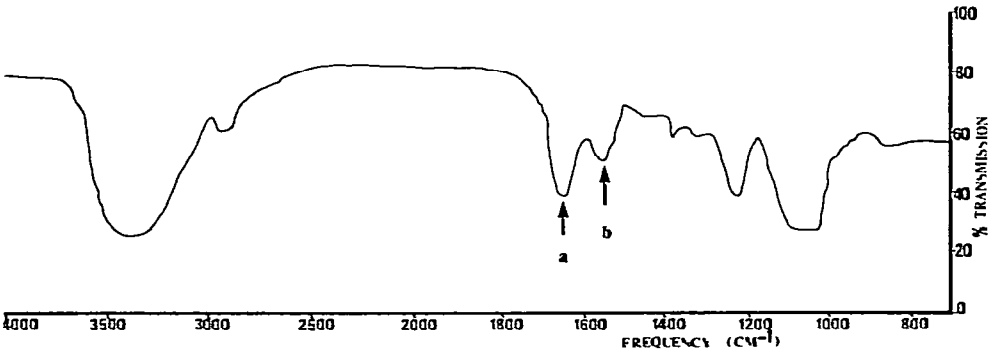

Fig. 6. IR spectrum of a_5 agglutinogen bound to WGA-Ultrogel. The conditions and peaks are as in Fig. 4.

reactivity either with β-N-acetylglucosamine RTA or with α-N-acetylglucosamine RTA. This shows that although the compositions of both antigens are identical, their structural formulae must be quite different.

CONCLUSION

The WGA-Ultrogel was employed in the purification of RTA with some success. Its use in the purification of a_5 gives a reasonably good yield. This work points out the danger in drawing structural analysis conclusions from lectin affinity.

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