Localization of *O*-Acetyl Groups of Bacterial Alginate

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INTRODUCTION

Acetyl groups have been widely recognized as substituents of bacterial exopolysaccharides. Such polymers are repeating structures containing two to six monosaccharides (Sutherland, 1967). *O*-Acetyl groups occur regularly as part of the repeating units as revealed by enzymic hydrolysis of the polysaccharides and characterization of the products. In one exceptional example, *O*-acetyl groups were located on alternate repeating units of *Klebsiella aerogenes* type 54 polysaccharide, i.e. although the repeating units were tetrasaccharides, the *O*-acetyl groups occurred regularly on each octasaccharide (Sutherland & Wilkinson, 1968).

Azotobacter vinelandii and certain strains of *Pseudomonas aeruginosa* are known to produce exopolysaccharides which resemble the algal polysaccharide, alginic acid, in being copolymers of guluronic acid and mannuronic acid (Gorin & Spencer, 1966; Linker & Jones, 1966). One known difference between algal and bacterial alginates is the presence of *O*-acetyl groups in the latter polysaccharide. The lack of any obvious repeating structure in alginic acid, in contrast to the polysaccharides described above, suggested the acyl groups might be specifically associated with certain regions of the macromolecule. Studies with alginate lyases have now provided evidence for the location of the *O*-acetyl groups in bacterial alginate and have indicated a possible role for these acyl groups.

METHODS

Enzyme preparation. Polyguluronate lyase (EC. 4.2.2.3) was recovered and purified from an unidentified marine pseudomonad grown in a defined medium containing sodium alginate as sole carbon source (Davidson, Sutherland & Lawson, 1976). Polymannuronate lyase (EC. 4.2.2.3) was recovered from bacteriophage-infected cultures of *A. vinelandii* (Davidson, Lawson & Sutherland, 1977).

Alginates. Algal alginate was obtained from BDH. *Azotobacter* alginate was prepared using the procedure described by Pindar & Bucke (1975). *Pseudomonas aeruginosa* alginate was prepared from a strain of the bacterium isolated from a patient suffering from cystic fibrosis and was kindly provided by Dr L. Evans, University of Utah, Salt Lake City, U.S.A. *Pseudomonas aeruginosa* was grown in nitrogen-deficient medium (Sutherland & Wilkinson, 1965) and the polysaccharide was purified using the method of Dudman & Wilkinson (1956).

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Bacterial alginates were deacetylated by treatment with 2 M-NH₄OH at 60 °C for 60 min. After dialysis against distilled water, non-dialysable material was lyophilized.

Analytical procedure. *O*-Acetyl groups were determined by the method of Hestrin (1949). Carbohydrate was estimated using phenol/sulphuric acid (Dubois *et al.*, 1956).

Gel filtration. Products of alginate lyase action were fractionated in a column (1.6 × 30 cm) of Sephadex G-50 using pyridinium acetate buffer (4 ml pyridine plus 10 ml acetic acid diluted to 1 l with distilled water) as eluant. Fractions (1 ml) were collected and analysed for carbohydrate and *O*-acetyl groups. High molecular weight material recovered from Sephadex G-50 was further fractionated in columns of Sephadex G-100 and Bio-Gel P30 (dimensions 1.5 × 27 cm and 1.2 × 34 cm respectively).

Enzymic hydrolysis of alginates. Alginates (50 mg) were dissolved in 5 ml 10 mM-Mops/NaOH buffer, pH 7.5 [Mops, 3-(*N*-morpholino)propanesulphonic acid], and sufficient alginate lyase added to give maximum hydrolysis in 48 h at 30 °C. Microbial growth was inhibited by the addition of toluene. Fractions recovered from Sephadex columns were hydrolysed by heating with 2 M-HCl for 2 h at 100 °C. The hydrolysates were evaporated to dryness, HCl was removed by repeated evaporation of water, and they were then examined by paper chromatography and high-voltage electrophoresis. Comparative paper chromatography was carried out by descending irrigation of Whatman no. 1 paper using butan-1-ol/acetic acid/water (50:12:25, by vol.) as solvent. Paper electrophoresis was performed on Whatman no. 1 paper in pyridinium acetate buffer pH 5.3 using a current of 80 to 100 mA at 3.5 kV for 1.5 h on Locarte (London) equipment with a 75 × 20 cm cooled plate area. Sugars were detected with alkaline silver nitrate (Trevelyan, Procter & Harrison, 1950). Mannuronic and guluronic acid contents were determined as previously described (Davidson *et al.*, 1976).

RESULTS AND DISCUSSION

Algal and bacterial alginates containing 48 % and 45 % mannuronic acid respectively were degraded with a specific polyguluronate lyase from a marine pseudomonad (Davidson *et al.*, 1976) and the products were fractionated in Sephadex G-50; differences were noted in the elution profiles. Algal alginate yielded almost entirely low molecular weight products (Fig. 1*a*), whereas *A. vinelandii* alginate yielded material near the void volume as well as smaller fragments (Fig. 1*b*). The high molecular weight fragment was applied to Bio-Gel P30 and Sephadex G-100 and behaved as a single peak on both filtration gels. Analysis for *O*-acetyl groups indicated that all the detectable acetate from the original polymer was associated with the residual high molecular weight material. On total acid hydrolysis of this larger fragment, mannuronic acid was the only identifiable monosaccharide. Repetition of the experiments with alginates of either bacterial or algal origin having different mannuronic acid contents, gave essentially similar profiles but different relative proportions of high molecular weight and oligosaccharide material.

The elution profile of the enzyme digest was not affected by prior deacetylation (Fig. 1*c*) of the polysaccharide or extended incubation with or without more enzyme. In addition, similar results were obtained when *A. vinelandii* alginates of differing mannuronic acid: guluronic acid ratios were used as substrates. *Pseudomonas aeruginosa* polysaccharide treated with the polyguluronate lyase gave rise to elution profiles similar to those obtained using *Azotobacter* alginate as substrate.

Bacterial alginates treated with a polymannuronate lyase obtained from *A. vinelandii* phage (Davidson *et al.*, 1977) gave almost entirely low molecular weight fragments, thus

Fig. 1. Elution profiles of products of alginate hydrolysis. Samples of (a) algal alginate, (b) *Azotobacter* alginate and (c) deacetylated *Azotobacter* alginate were dissolved in 5 ml 10 mM-Mops/NaOH buffer (pH 7.5) and sufficient polyguluronate lyase was added to give maximum hydrolysis in 48 h at 30 °C. The products were fractionated in a column (1.6 × 30 cm) of Sephadex G-50 using pyridinium acetate as buffer eluant. — (E_{490}), Carbohydrate; --- (E_{540}), acetate.

confirming the polymannuronic acid nature of the high molecular weight material described above. Many of the oligosaccharides were acetylated.

In algae, biosynthesis of alginic acid is thought to occur via polymerization of the uronic acids derived from GDPmannuronic acid and GDPguluronic acid (Lin & Hassid, 1965*a*, *b*). In contrast the biosynthesis of the bacterial polymer involves the formation of a homopolymer, polymannuronic acid, alginic acid being formed by the epimerization of some of the D-mannuronic acid residues to L-guluronic acid (Pindar & Bucke, 1975). The difference in susceptibility to enzymic hydrolysis shown by algal and bacterial alginates may reflect the alternative biosynthetic mechanisms and the consequent different structures. The high molecular weight material present after polyguluronate lyase treatment of *A. vinelandii* (or *P. aeruginosa*) polysaccharide may correspond to the original product of biosynthesis by these species.

Since *O*-acetyl groups do not occur regularly in the polymer, but are associated with one

portion of it, the acyl groups may protect certain of the mannuronic acid residues from epimerization during the biosynthesis of bacterial alginate.

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