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Note

N-Acetyl-β-D-galactosaminidase activity of *E. coli* phage 44 and the sequencing of *E. coli* K44 capsular polysaccharide by mass spectrometry

GUY G. S. DUTTON, ZAMAS LAM, AND ANDREW V. S. LIM
Department of Chemistry, University of British Columbia, Vancouver, B.C., V6T 1Y6 (Canada)
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Few bacteriophages have been described for *E. coli* and those that have[1–3] act on strains whose capsular polysaccharides (CPS) are devoid of amino sugars—except in the case of *E. coli* K1 whose capsule consists of neuraminic acid[4]. It was, therefore, of interest to examine the action of the bacteriophage for which *E. coli* K44 acts as the host strain and whose CPS has been shown[5] to contain both 2-acetamido-2-deoxy-D-galactose and 2-acetamido-2-deoxy-D-glucose. This note describes the phage depolymerization of *E. coli* K44 and, more importantly, demonstrates how a polysaccharide may be sequenced by desorption chemical ionization mass spectrometry (d.c.i.-m.s.) of the derived phage oligosaccharide.

The hydrolyzate resulting from the phage depolymerization of the CPS of *E. coli* K44 was subjected to dialysis, decationized, and fractionated by preparative paper chromatography to yield **P1**. The n.m.r. spectra (^{1}H and ^{13}C) of **P1** were substantially identical with those of the original CPS, although they showed additional peaks, presumably from the phage preparation. It is of interest that the use of unpurified phages[6] has given these spurious peaks with other *E. coli*, *e.g.*, K26, K31, and K34 (ref. 7), although the same experimental procedure applied to *Klebsiella* CPS gives spectra devoid of additional signals[8].

Methylation of **P1** and its alditol (**P1R**) confirmed the results of standard analyses of the CPS, and demonstrated that the nonreducing terminus of **P1** was glucuronic acid and the reducing end was 2-acetamido-2-deoxy-D-galactose (*N*-acetyl-D-galactosamine). The phage *E. coli* φ44 therefore exhibits *N*-acetyl-β-D-galactosaminidase activity, and is believed to be the first example of this type. The ^{1}H-n.m.r. spectrum of **P1** did not exhibit fractional anomeric signals indicative of a mutarotational equilibrium, but suggested that the reducing amino sugar exists predominantly as the β anomer.

Oligosaccharide **P1** is comprised of uronic acid and deoxy and amino sugar residues, each of which has a different formula weight. It is thus an excellent model compound for investigating whether mass spectrometry may be used to sequence *E. coli* K44 polysaccharide. The technique cannot differentiate between the two

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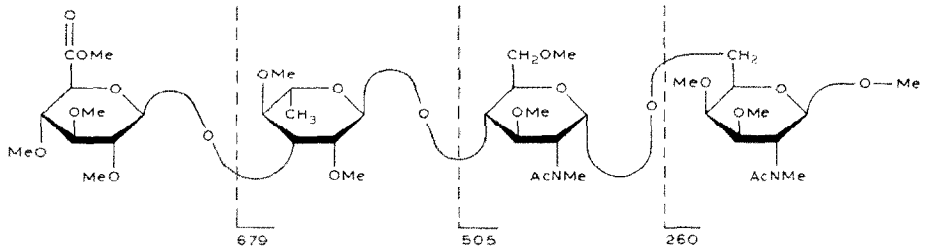

Fig. 1. Structure of methylated oligosaccharide **P1** showing its fragmentation on d.c.i.-m.s.

amino sugars, but, once the reducing-end residue of **P1** is identified, the remaining residues can in principle be sequenced by the characteristic mass-fragments of the oligosaccharide. No conclusive evidence could be obtained from native **P1**, whose f.a.b.-m.s. spectrum (positive ion mode) lacked a peak for the molecular ion and showed extensive fragmentation and a noisy background. In contrast, a d.c.i.-m.s. spectrum of the permethylated derivative of **P1** gave major fragments having *m/z* (relative intensity) 679 (33), 505 (100), and 260 (65), with minor peaks at 434 (48) and 189 (45). This fragmentation establishes the sequence of the monosaccharide residues in **P1** as shown in Fig. 1, and is consistent with the structure of the CPS already deduced by classical methods. The peaks at *m/z* 434 and 189 arise by loss of an *N*-methylacetamido group from *m/z* 505 and 260, respectively.

Previous papers from our laboratory have demonstrated the advantages of phage-derived oligosaccharides for determining the location of *O*-acetyl substituents[9] and 1-carboxyethylidene groups[10], and for sequencing by n.m.r.[8]. To these examples may now be added the ability to sequence a polysaccharide of M.W. *ca.* 10^6 by application of suitable mass spectrometric techniques[11] to a phage-produced oligosaccharide.

It is of interest to note in a recent review[12] of d.c.i.-m.s. of carbohydrates the statement that "effecive application of m.s. to large biopolymers must, in the final analysis, be dependent on methods of partial degradation". The article further points out the lack of specific cleavage procedures available in the carbohydrate field; in contrast to the situation in protein chemistry. The use of viral enzymes for bacterial polysaccharides serves to redress this balance.

EXPERIMENTAL

General methods. — These are as described in the accompanying paper[5].

Isolation and propagation[6] of bacteriophage ϕ44. — The phage was isolated from Grahamstown (South Africa) sewage and was propagated on *E. coli* K44 using Mueller–Hinton broth. Cultures were grown successively in tubes, then in flasks, until 10^{13} p.f.u. were obtained. The phage suspension was concentrated to 400 mL and dialyzed (M.W. cutoff 3,500) against 1 L of volatile buffer [NH_4OAc and $(NH_4)_2CO_3$, pH 7]. The retentate was concentrated to 400 mL and added to *E. coli* K44 CPS (380 mg) dissolved in the same volatile buffer (50 mL). Depolymerization

was effected at 37° in the presence of chloroform and after 2 d, by which time there was a marked drop in viscosity, the reaction mixture was dialyzed against distilled water (3 × 1 L). Concentration of the dialyzate, passage through Amberlite IR 120(H^+) and lyophilization yielded a pale brown product (120 mg).

Paper chromatographic examination (2:1:1 1-butanol–acetic acid–water) showed three fast-moving components and one presumptive oligosaccharide having $R_{lactose}$ 0.33. Preparative paper chromatography in the same solvent yielded **P1** (90 mg).

Methylation of **P1**. — A sample of **P1** (15 mg) was methylated by the method of Hakomori and the product was extracted with dichloromethane and purified on a column of Sephadex LH-20. A portion was hydrolyzed with 2M hydrochloric acid for 7 h on a steam bath and the sugars were analyzed as alditol acetates.

Another sample of **P1** (70 mg) was reduced with aqueous sodium borohydride for 4 h at room temperature to give **P1R**, a sample (15 mg) of which was similarly methylated. This product was reduced with lithium aluminum hydride, hydrolyzed using 2M hydrochloric acid for 16 h at 95°, and analyzed as alditol acetates.

Mass spectrometry. — Native **P1** was subjected to f.a.b.-m.s. on an AEI MS9 instrument fitted with a saddle-field gun operating at 1 mA and 7 to 8 kV. Xenon was used as the bombarding gas and glycerol as the matrix.

D.c.i.-m.s. was performed on permethylated **P1** using a Nermag R10-10C quadrupole mass spectrometer with methane as reagent gas. The spectrum was recorded in the positive ion mode.

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REFERENCES

1 S. STIRM, *J. Virol.*, 2 (1968) 1107–1114.
2 S. STIRM AND E. FREUND-MOLBERT, *J. Virol.*, 8 (1971) 330–342.
3 C. WHITFIELD AND M. LAM, *FEMS Microbiol. Lett.*, 37 (1986) 351–355.
4 S. TOMLINSON AND P. W. TAYLOR, *J. Virol.*, 55 (1985) 374–378.
5 G. G. S. DUTTON, D. N. KARUNARATNE, AND A. V. S. LIM, *Carbohydr. Res.*, 183 (1988) 111–122.
6 G. G. S. DUTTON, J. L. DI FABIO, D. M. LEEK, E. H. MERRIFIELD, J. R. NUNN, AND A. M. STEPHEN, *Carbohydr. Res.*, 97 (1981) 127–138.
7 L. BEYNON, G. G. S. DUTTON, AND A. KUMA-MINTAH, unpublished results.
8 G. G. S. DUTTON, H. PAROLIS, J.-P. JOSELEAU, AND M.-F. MARAIS, *Carbohydr. Res.*, 149 (1986) 411–423.
9 G. G. S. DUTTON AND D. N. KARUNARATNE, *Carbohydr. Res.*, 138 (1985) 277–291.
10 J. L. DI FABIO, G. G. S. DUTTON, AND H. PAROLIS, *Carbohydr. Res.*, 133 (1984) 125–133.
11 G. G. S. DUTTON, G. EIGENDORF, Z. LAM, AND A. V. S. LIM, *Biomed. Environ. Mass Spectrom.*, 15 (1988) 459–460.
12 V. N. REINHOLD, *Methods Enzymol.*, 138 (1987) 59–84.