Amino Sugars in the Wall of *Pseudomonas fluorescens*

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SUMMARY

3-Amino-3,6-dideoxy-D-galactose has been identified as a component of the wall of *Pseudomonas fluorescens* (NCTC10038). This amino sugar was present in the lipopolysaccharide fraction of the wall: other amino compounds present were glucosamine, galactosamine, quinovosamine, alanine and ethanolamine. The distribution of amino sugars within different regions of the lipopolysaccharide was studied briefly.

INTRODUCTION

In addition to muramic acid, glucosamine and galactosamine (Wilkinson, 1970), amino sugars present in the wall of *Pseudomonas fluorescens* include quinovosamine (2-amino-2,6-dideoxyglucose) and an unidentified compound, Unknown V (Wilkinson & Carby, 1971). Unknown V has been identified as 3-amino-3,6-dideoxy-D-galactose and has been shown to occur in the lipopolysaccharide fraction of the cell wall.

METHODS

Pseudomonas fluorescens (NCTC10038) was grown and its walls were prepared as described previously (Wilkinson, 1970). Methods for the isolation and characterization of amino sugars were those used in previous studies (Wilkinson & Carby, 1971). Lipopolysaccharide was extracted from defatted walls (Fensom & Gray, 1969) by a single treatment for 15 min at 72° with 45 % (w/v) phenol, and was purified by the method of Key, Gray & Wilkinson (1970). The lipopolysaccharide was hydrolysed to lipid and polysaccharide fractions with 1 % acetic acid for 1 h at 100° (Müller-Seitz, Jann & Jann, 1968). The crude polysaccharide was fractionated by chromatography on Sephadex G-50 (Schmidt, Jann & Jann, 1969), and the eluate was analysed for phosphorus (Bartlett, 1959) and for carbohydrate (Dubois *et al.* 1956).

Infrared spectra were recorded by using a Unicam SP 200 spectrophotometer, with samples dispersed in discs of KCl. Optical rotation was measured with a Bendix NPL automatic polarimeter, Model 143A.

RESULTS

Unknown V was present in the basic fraction obtained by high-voltage paper electrophoresis of wall hydrolysates (Wilkinson & Carby, 1971), and was separated from basic amino acids and other amino sugars (quinovosamine, glucosamine and galactosamine) by preparative paper chromatography using solvent system A: ethyl acetate + pyridine + water + acetic acid (5 + 5 + 3 + 1, by vol.). Using solvent system A, unknown V had $R_{\text{glucosamine}}$ 1.19 and on high voltage electrophoresis at pH 5.3 it had $R_{\text{glucosamine}}$ 1.09. The compound reduced alkaline AgNO₃ strongly and reacted with ninhydrin on heating to give a bright yellow colour which changed to pink-purple on standing. A slight reaction, regarded as

Fig. 1. Infrared spectra of the *N*-acetyl derivative of Unknown V from *Pseudomonas fluorescens* (A) and of 3-acetamido-3,6-dideoxygalactose (B). The samples were dispersed in KCl discs.

negligible, was obtained in a test for 2-amino-2-deoxyhexoses (Rondle & Morgan, 1955), but a test for 3-amino-3-deoxyhexoses (Ashwell, Brown & Volk, 1965) was strongly positive. Acetaldehyde was formed on oxidation of the compound with periodate. Thus, Unknown V had the properties of a 3-amino-3,6-dideoxyhexose.

On paper chromatography using solvent systems A and B – *n*-butanol + acetic acid + water (5 + 1 + 2, by vol.) – Unknown V had the same mobility as an authentic sample of 3-amino-3,6-dideoxygalactose and was clearly differentiated from 3-amino-3,6-dideoxyglucose. Unknown V and 3-amino-3,6-dideoxygalactose behaved identically in the following experiments: (a) high-voltage paper electrophoresis at pH 5.3; (b) ion-exchange chromatography using a Technicon AutoAnalyzer; (c) paper chromatography of the *N*-acetyl derivatives using three solvent systems; (d) high-voltage paper electrophoresis in molybdate buffer (pH 5.0) of the amino sugar alcohols (Mayer & Westphal, 1968). Although 3-amino-3,6-dideoxygalactose and 3-amino-3,6-dideoxyglucose were virtually indistinguishable ($R_{\text{glucosamine}}$ values 1.28 and 1.29, respectively, for successive chromatograms) using the AutoAnalyzer with a standard 21 h elution system, resolution of the two amino sugars is possible with a pyridine-acetic acid buffer system, pH 3.85 (Brendel, Roszel, Wheat & Davidson, 1967; Lüderitz *et al.* 1967). Insufficient *N*-acetyl derivative of Unknown V was available for crystallization, but the infrared spectrum of a dried sample closely resembled that of crystalline 3-acetamido-3,6-dideoxygalactose (Fig. 1). The specific rotation determined with an aqueous solution of the *N*-acetyl derivative (concentration estimated using 3-acetamido-3,6-dideoxygalactose as standard) was $+110 \pm 4^\circ$: $[\alpha]_D^{25}$ for 3-acetamido-3,6-dideoxy-D-galactose is $+114^\circ$ (Ashwell & Volk, 1965). Thus Unknown V may be identified

Fig. 2. Fractionation of partly degraded polysaccharide on Sephadex G-50. The material (10.5 mg) was applied to a column (50 cm length $\times$ 1.2 cm diam.) and eluted with pyridine-acetic acid buffer (pH 5.4) at a flow rate of 10 ml/h. Fractions (1 ml) were screened for contents of phosphorus (---) and carbohydrate (—) with glucose as standard.

as 3-amino-3,6-dideoxy-D-galactose. The yield of compound from *Pseudomonas fluorescens* was about 10 μ moles/100 mg walls.

Analysis of the lipopolysaccharide fraction of the wall showed that glucosamine, galactosamine, quinovosamine and 3-amino-3,6-dideoxygalactose were all components, with the last-named being the major amino sugar. Other ninhydrin-positive components were alanine, ethanolamine and probably phosphates of both ethanolamine and glucosamine. The lipid A released on acid hydrolysis of lipopolysaccharide had only glucosamine and glucosamine phosphate as amino components: the remaining amino compounds and part of the glucosamine were present in the polysaccharide fraction. The partly degraded polysaccharide was separated on Sephadex G-50 into the three fractions indicated in Fig. 2. Fraction I contained most of the 3-amino-3,6-dideoxygalactose and only trace amounts of other amino sugars and alanine. Fraction II contained almost all the alanine, glucosamine, galactosamine and quinovosamine of the total polysaccharide, together with a small amount of 3-amino-3,6-dideoxygalactose and most of the ethanolamine and ethanolamine phosphate. Fraction III contained only small or trace amounts of amino compounds.

DISCUSSION

The presence of a 2-amino-2,6-dideoxyhexose (quinovosamine) and a 3-amino-3,6-dideoxyhexose in the lipopolysaccharide fraction from *Pseudomonas fluorescens* (and possibly *P. synxantha*, Wilkinson & Carby, 1971) is unusual. Although amino sugars of both types occur in lipopolysaccharides from members of the Enterobacteriaceae, apparently they do not occur together in the same organism (Lüderitz, Jann & Wheat, 1968). 3-Amino-3,6-dideoxygalactose has been found in strains of *Salmonella*, *Arizona*, *Escherichia* and *Citro-*

bacter (Lüderitz *et al.* 1968; Keleti, Lüderitz, Mlynarčík & Sedlák, 1971), as well as in *Xanthomonas campestris*, a member of Pseudomonadaceae (Ashwell & Volk, 1965). As strain NCTC10038 has been proposed (Rhodes, 1959) as the neotype strain for *P. fluorescens* biotype A (Stanier, Palleroni & Doudoroff, 1966), it will be of interest to examine the lipopolysaccharides of other strains for the presence of unusual amino sugars. If the results obtained on gel filtration of the partly degraded polysaccharide (Fig. 2) can be interpreted in the same way as those of other similar experiments (e.g. Müller-Seitz *et al.* 1968; Fensom & Meadow, 1970), 3-amino-3,6-dideoxygalactose may be a component of the side-chain region of the polysaccharide and the other amino sugars and alanine are possibly components of the core region. Further studies will be necessary to establish this point.

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REFERENCES

- ASHWELL, G., BROWN, N. C. & VOLK, W. A. (1965). A colorimetric procedure for the determination of *N*-acetylated-3-amino hexoses. *Archives of Biochemistry and Biophysics* **112**, 648–652.
- ASHWELL, G. & VOLK, W. A. (1965). Isolation and identification of *N*-acetyl-3-amino-3,6-dideoxy-D-galactose, a cell wall constituent of *Xanthomonas campestris*. *Journal of Biological Chemistry* **240**, 4549–4555.
- BARTLETT, G. R. (1959). Phosphorus assay in column chromatography. *Journal of Biological Chemistry* **234**, 466–468.
- BRENDEL, K., ROSZEL, N. O., WHEAT, R. W. & DAVIDSON, E. A. (1967). Ion-exchange separation and automated assay of some hexosamines. *Analytical Biochemistry* **18**, 147–160.
- DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. & SMITH, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**, 350–356.
- FENSOM, A. H. & GRAY, G. W. (1969). The chemical composition of the lipopolysaccharide of *Pseudomonas aeruginosa*. *Biochemical Journal* **114**, 185–196.
- FENSOM, A. H. & MEADOW, P. M. (1970). Evidence for two regions in the polysaccharide moiety of the lipopolysaccharide of *Pseudomonas aeruginosa* 8602. *FEBS Letters* **9**, 81–84.
- KELETI, J., LÜDERITZ, O., MLYNARČÍK, D. & SEDLÁK, J. (1971). Immunochemical studies on *Citrobacter* O antigens (lipopolysaccharides). *European Journal of Biochemistry* **20**, 237–244.
- KEY, B. A., GRAY, G. W. & WILKINSON, S. G. (1970). Purification and chemical composition of the lipopolysaccharide of *Pseudomonas alcaligenes*. *Biochemical Journal* **120**, 559–566.
- LÜDERITZ, O., JANN, K. & WHEAT, R. (1968). Somatic and capsular antigens of Gram-negative bacteria. In *Comprehensive Biochemistry*, vol. 26A. *Extracellular and Supporting Structures*, pp. 105–228. Edited by M. Florkin & E. H. Stotz. Amsterdam: Elsevier Publishing Co.
- LÜDERITZ, O., RUSCHMANN, E., WESTPHAL, O., RAFF, R. & WHEAT, R. (1967). Occurrence of 3-amino-3,6-dideoxyhexoses in *Salmonella* and related bacteria. *Journal of Bacteriology* **93**, 1681–1687.
- MAYER, H. & WESTPHAL, O. (1968). Elektrophoretische Trennungen von Hexosamin- und Hexuronsäurederivaten als Molybdatkomplexe. *Journal of Chromatography* **33**, 514–525.
- MÜLLER-SEITZ, E., JANN, B. & JANN, K. (1968). Degradation studies on the lipopolysaccharide from *E. coli* 071:K?:H12. Separation and investigation of *O*-specific and core polysaccharides. *FEBS Letters* **1**, 311–314.
- RHODES, M. E. (1959). The characterization of *Pseudomonas fluorescens*. *Journal of General Microbiology* **21**, 221–263.
- RONDLE, C. J. M. & MORGAN, W. T. J. (1955). The determination of glucosamine and galactosamine. *Biochemical Journal* **61**, 586–589.
- SCHMIDT, G., JANN, B. & JANN, K. (1969). Immunochemistry of R lipopolysaccharides of *Escherichia coli*. Different core regions in the lipopolysaccharides of O group 8. *European Journal of Biochemistry* **10**, 501–510.

- STANIER, R. Y., PALLERONI, N. J. & DOUDOROFF, M. (1966). The aerobic pseudomonads: a taxonomic study. *Journal of General Microbiology* **43**, 159–271.
- WILKINSON, S. G. (1970). Cell walls of *Pseudomonas* species sensitive to ethylenediaminetetraacetic acid. *Journal of Bacteriology* **104**, 1035–1044.
- WILKINSON, S. G. & CARBY, K. A. (1971). Amino sugars in the cell walls of *Pseudomonas* species. *Journal of General Microbiology* **66**, 221–227.