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The paper chromatographic separation of naturally acetylated, formylated and pyruvylated oligosaccharides

The discovery of hydrolytic enzymes releasing pyruvylated, O-acetylated and O-formylated fragments from bacterial extracellular polysaccharides[1-3] led to a requirement for suitable systems for their chromatographic separation. Of particular importance was the separation of such fragments from the corresponding non-acylated material which may either be present naturally or as an artifact of preparation. The relatively large yields of oligosaccharides obtained by enzymic hydrolysis made paper chromatography a method of choice for preparative separation as well as for comparative studies.

Experimental

A number of naturally O-acetylated, O-formylated and pyruvylated oligosaccharides were available[1,2]. The complete carbohydrate structure of each was known, but the position of the acyl groups was only known for the pyruvylated fragments. The compounds tested and their known structures are shown in Table I.

TABLE I

FRAGMENTS ISOLATED FROM BACTERIAL EXOPOLYSACCHARIDES

Fraction	*Carbohydrate structure*	*Substituents*	[a]M_{Glca}	*Source*	*Reference*
F 1	Gal → Glc → GlcA → Fuc	—	0.46	*Escherichia coli* K27	1
F 2	Gal → Glc → GlcA → Fuc	Acetyl	0.41	*E. coli* K27	1
F 3	Glc → Glc → GlcA → Fuc	—	0.47	*Klebsiella aerogenes* Type 54	4
F 4	Glc → Glc → GlcA → Fuc	Formyl	0.47	*K.aerogenes* Type 54	2, 3
F 5	Glc → Glc → GlcA → Fuc	Formyl + acetyl	0.45	*K.aerogenes* Type 54	2, 3
F 6	Glc → GlcA → Fuc	Formyl	0.53	*K.aerogenes* Type 54	5
F 7	Glc → GlcA → Fuc	Formyl + acetyl	0.51	*K.aerogenes* Type 54	5
F 8	Gal → GlcA → Gal	—	0.54	*E.coli* K12	6
F 9	Gal → GlcA → Gal	Pyruvyl	0.87	*E.coli* K12	6
F 10	Gal	Pyruvyl	0.95	*E.coli* K12	6
F 11	Gal	Pyruvyl	0.95	*Salmonella typhimurium*	7

[a]Electrophoretic mobility relative to glucuronic acid in pyridinium acetate (pH 5.3).

The corresponding non-acylated fragments were obtained from the polysaccharides by partial acid hydrolysis, as were some of the pyruvylated fractions[4-6]. As the products of enzymic hydrolysis and also some of those obtained by acid hydrolysis contained varying amounts of salts these were removed by paper electrophoresis in pyridinium acetate buffer (pH 5.3). As well as oligosaccharides, two isomeric forms of pyruvylated galactose were tested, as such pyruvylated fragments are commonly found in bacterial exopolysaccharides. The first of these, the 4,6-carboxyethylidene derivative was obtained from the polysaccharide of *Escherichia coli* K12 by partial acid hydrolysis of the periodate-oxidised polymer[6], while the 3,4-carboxyethylidene galactose was prepared in the same way from the polysaccharide of a *Salmonella typhimurium* strain[7].

The solvents tested comprised a large number of those commonly employed for the paper chromatographic separation of sugars and oligosaccharides. Several of those tested were basic solvents, while others were acidic or neutral. From preliminary experiments it was clear that most of the solvents tested, failed to give satisfactory separation of the pairs of oligosaccharides which differed only in the acyl groups present. Four solvents were eventually selected for further study: (A) butan-1-ol–pyridine–water (6:4:3); (B) ethyl acetate–pyridine–acetic acid–water (5:5:1:3); (C) acetic acid–formic acid–ethyl acetate–water (3:1:18:4); and (D) butanol–acetic acid–water (4:1:5). All were run as descending systems at 20° using Whatman No. 1 paper. Irrigation times were normally 24 h for solvents A, B and C, and 96 h for solvent D. The carboxyethylidene sugars were run for 10 h, in solvent C. The oligosaccharides were applied as approximately 10 nmoles amounts and were detected after irrigation, with alkaline silver nitrate reagent.

TABLE II

THE PAPER CHROMATOGRAPHIC MOBILITIES OF ACYLATED AND OTHER OLIGOSACCHARIDES
All values are given as R_{Glc}.

Fraction	*Mobilities in solvent*			
	A	*B*	*C*	*D*
F 1	0.03	0.34[a]	0.05	0.02
F 2	0.05	0.49	0.13	0.09
F 3	0.04	0.35[a]	0.08	0.01
F 4	0.05	0.49	0.08	0.01
F 5	0.07	0.63	0.18	0.03
F 6	0.13	0.55	0.18	0.26
F 7	0.18	0.68	0.21	0.29
F 8	0.05	0.13	0.08	0.19
F 9	0.34	0.73	0.17	0.28
F 10	1.47	1.35	1.95	1.02
F 11	1.09	1.00	1.67	0.63
Gal	0.89	0.93	0.93	0.91

[a] These oligosaccharides streaked and accurate values could not be obtained.

The results for the four solvent systems tested are shown in Table II. It is clear that neither of the two acid solvents C and D gave satisfactory separation of the oligosaccharides. Solvent C did however give good differentiation of the two carboxyethylidene derivatives of galactose from each other and from the free sugar, despite its poor resolution of the pyruvylated trisaccharide (F9) from the corresponding non-pyruvylated fragment. The neutral solvent A gave good separation of these two trisaccharides. For oligosaccharides which were acetylated or formylated, the solvent system of FISCHER AND DÖRFEL[8] gave good separation. This was not unexpected as it was originally used for the separation of amino sugars and the corresponding N-acetylated compounds. It was also observed that in this solvent system the acylated oligosaccharides gave discrete spots whereas glucuronic acid and aldobiouronic acids streaked. It thus appears that this solvent is the best of those tested for the separation of naturally acylated oligosaccharides of the type indicated in Table I.

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