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STRUCTURE AND SEROLOGICAL PROPERTIES OF THE CAPSULAR K11 ANTIGEN OF *Escherichia coli* O13:K11:H11

MARIA-LUISA RODRIGUEZ, BARBARA JANN, AND KLAUS JANN*
Max-Planck-Institut für Immunbiologie, Stübeweg 51, D-7800 Freiburg-Zähringen (F.R.G.)
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ABSTRACT

The capsular K11 antigen of *Escherichia coli* contains glucose, fructose, and phosphate in the molar ratios 2:1:1, and a backbone of -4)-β-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl phosphate-(1→ to which β-D-fructofuranose is linked at position 3 of the β-D-glucopyranosyl residue. The fructose, which is the immunodominant sugar of the K11 antigen, is released from the polysaccharide under mild acidic conditions (70°, pH 5.0).

INTRODUCTION

The capsular polysaccharides of *E. coli* can be classified into two groups[1–3] on the basis of molecular weight, mobility in electrophoresis, nature of their acidic components, and the chromosomal location of their biosynthesis gene-cluster.

Some group II capsular polysaccharides contain phosphate groups and resemble the teichoic acids of Gram-positive bacteria. The K18, K22, and K100 antigens are poly(ribosyl-ribitol phosphates)[4] that are related structurally to the capsular antigen[5] of *Haemophilus influenzae* type b. The structures of the K18 and K22 antigens differ only in the partial *O*-acetylation of the K18 antigen. The K51 antigen is a poly(2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate)[6], the K2 antigen a poly(α-D-galactofuranosyl-glycerol phosphate)[7], and the K52 antigen a poly(α-D-galactopyranosyl phosphate) substituted at position 2 by β-D-fructofuranose[8]. Polysaccharides of group II are generally not expressed at growth temperatures below 20°. There are only a few exceptions known, such as K3, K10, K11, and K54 which belong to group II but are also expressed at low temperature[9].

We now report on the K11 antigen, which was isolated from a uropathogenic strain of *E. coli* and consists of glucose, fructose, and phosphate. Fructose is not a common constituent of bacterial heteropolysaccharides and hitherto has been found only in the capsular K4 antigen[10] and K52 antigen[8] of *E. coli* and the capsular polysaccharide of *H. influenzae* type e[11]. Each of these antigens contains terminal β-D-fructofuranosyl units which are important for their serological properties.

*Author for correspondence.

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RESULTS AND DISCUSSION

Isolation and characterization. — The capsular K11 antigen, isolated[12-13] from *E. coli* strain Su4321-41 (O13:K11:H11) grown in liquid culture in a yield of 291 mg/L, consisted of glucose, fructose, and phosphate in the molar ratios 2:1:1. That the glucose and fructose were D was indicated by their reaction in the combined D-glucose/D-fructose enzyme test (Boehringer, Mannheim). The ^{31}P-n.m.r. spectrum of the K11 antigen indicated the presence of phosphodiester linkages (−1.64 p.p.m.).

Heating in water at pH 5.0 (2 h, 100°) degraded the K11 antigen to phosphorylated glucosylglucose and fructose, and, with 0.1M HCl (1 h, 100°), glucose 1-phosphate, glucose, and fructose were obtained. Of the fructose, ~90% was liberated at pH 5 (4 h, 70°), but the polymer chain remained intact. The composition of the defructosylated polymer (K11-dF) is shown in Table I.

Treatment of the K11 antigen with 0.5M NaOH (4 h, 4°) yielded a phosphorylated trisaccharide with a free reducing end. Its incubation with alkaline phosphatase yielded a trisaccharide (glucose–fructose, 2:1) which was purified by column chromatography. Similarly, treatment of the defructosylated K11 antigen with alkali resulted in a phosphorylated glucosylglucose with a free reducing end. This was also dephosphorylated with alkaline phosphatase and purified by column chromatography. These results show that one glucose residue carried a phosphate group.

The native and defructosylated K11 polymer, in phosphate-buffered saline (pH 7.2), each consumed 2 mol of periodate per repeating unit (two glucose, one fructose, and one phosphate). The periodate-oxidised products were reduced with sodium borohydride and hydrolysed. As shown in Table I, periodate oxidation destroyed the fructose residue and one glucose residue in the native polymer and both glucose residues in the defructosylated K11 antigen. Periodate oxidation of the trisaccharide showed that the glucose residue at the reducing end (corre-

TABLE I

CHEMICAL COMPOSITION DATA

Preparation[a]	*Glucose*		*Fructose*		*Phosphate*	
	[%]	*mol/repeating unit*	[%]	*mol/repeating unit*	[%]	*mol/repeating unit*
K11-PS	55	2.0	25	1.0	13	1.0
K11-dF	75	2.0	5	0.1	18	1.0
K11-TS	54	2.0	27	1.0	—	—
$K11_{Ox}$	28	1.0	<1	0	13	1.0
$K11\text{-}dF_{Ox}$	8	0.3	<1	0	18	1.0

[a]K11 antigen before (K11-PS) and after periodate oxidation ($K11_{Ox}$), the defructosylated antigen before (K11-dF) and after periodate oxidation ($K11\text{-}dF_{Ox}$), and the K11 trisaccharide (K11-TS).

sponding to D-glucose 1-phosphate in the polymer) and the fructose residue were destroyed. This result indicated that the subterminal glucose residue was protected from periodate oxidation by 3-*O*-fructosylation.

Methylation. — The K11 antigen was methylated with methyl sulfoxide–potassium hydride–methyl iodide[14–16] and then hydrolysed. The products were reduced with sodium borodeuteride and acetylated. The partially methylated alditol acetates were identified by g.l.c.–m.s. as shown in Table II. The data indicated the presence of terminal fructofuranose (2,5-di-*O*-acetyl-1,3,4,6-tetra-*O*-methyl derivatives of glucitol and mannitol), 4-substituted glucose (1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol), and 3,4-disubstituted glucose (1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylglucitol).

^{13}C-N.m.r. spectroscopy. — The K11 antigen gave 18 ^{13}C signals, three of which (δ 104.4, 103.5, and 95.9) were in the region for anomeric carbons. In an APT experiment[17,18], the signal at δ 104.4 was negative and was assigned to C-2 of the β-D-fructofuranose residue, whereas the signals at δ 103.5 and 95.9 were positive and were assigned to C-1 of the D-glucopyranose residues. The region δ 61–64 contained four signals, all negative in the APT experiment[17,18], due to CH_2OH groups, and were assigned to C-1 and C-6 of the fructose residue and to C-6 of the glucose residues. The chemical shifts indicated that no CH_2OH group was substituted. There were no signals in the region δ 70–71, characteristic of glucose residues with C-4 unsubstituted. Thus, each glucose residue of the K11 antigen was 4-substituted.

The anomeric configurations of the glucose residues were determined using gated decoupling[19,20]. The signal at δ 103.5 had a $J_{C\text{-}1,H\text{-}1}$ value of 165 Hz and was assigned to a β residue, that at δ 95.9 had $J_{C\text{-}1,H\text{-}1}$ 175 Hz, indicating the α configuration, and the chemical shift and C–P coupling (5.7 Hz) showed this signal to be due to α-D-glucopyranose 1-phosphate. The β configuration of the D-fructofuranose

TABLE II

G.L.C.–M.S.[a] OF THE PRODUCTS OF METHYLATION ANALYSIS OF THE K11 ANTIGEN

Retention time (s)	*Fragments* (m/z)											*Interpretation*
	101	*102*	*113*	*118*	*129*	*145*	*146*	*161*	*162*	*173*	*233*	
10.72	+	+	–	–	–	+	+	+	+	–	–	2,5-Di-*O*-acetyl-1,3,4,6-tetra-*O*-methylglucitol
10.93	+	+	–	–	–	+	+	+	+	–	–	2,5-Di-*O*-acetyl-1,3,4,6-tetra-*O*-methylmannitol
14.08	–	–	+	+	+	–	–	–	+	+	+	1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol
15.34	–	–	–	+	+	–	–	–	–	–	–	1,3,4,5-Tetra-*O*-acetyl-2,6-di-*O*-methylglucitol

[a]Retention times were obtained on a CBCP-Sil 5 column with a temperature program of 140→250° at 5°/min.

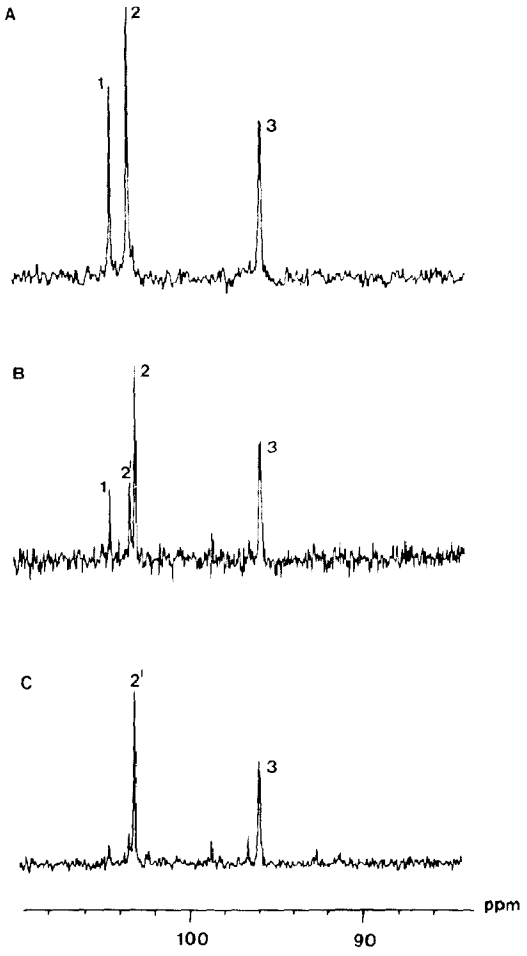

Fig. 1. ^{13}C-N.m.r. spectra (δ 86–108) of *A*, K11 antigen; *B*, the partially defructosylated K11; and *C*, defructosylated (~90%) K11 in D_2O at 25°.

residue was indicated by the signal at δ 104.4. The position of the fructose substitution could be derived from changes in the ^{13}C-n.m.r. spectrum during defructosylation of the K11 antigen. As shown in Fig. 1, defructosylation did not influence the chemical shift of the resonance of C-1 of the α-D-glucopyranosyl phosphate residue at δ 95.9. However, concomitant with a decrease in the intensity of the signal for C-2 of the β-D-fructose residue at δ 104.5, there was a decrease in that of C-1 of the β-D-glucopyranose residue and a new signal at δ 103.2 appeared. This indicated the β-D-fructofuranose residue to be linked to the β-D-glucopyranose residue of the main chain. The spectrum of the completely defructosylated K11 antigen contained no signals at δ 70–71, indicating each glucose residue of the main chain to be 4-linked.

The tentative assignments of the ^{13}C signals (Table III) of the K11 antigen, its defructosylated form, and the trisaccharide derived therefrom were achieved by comparison with those for methyl α- and β-D-glucopyranoside, α- and β-D-glucopyranosyl phosphate, methyl α- and β-D-fructofuranoside, and 4-linked α- and β-D-glucopyranose. The interpretation of the ^{13}C-n.m.r. spectra accords with the results of methylation analysis.

The above results indicate the repeating unit of the K11 antigen to be **1**.

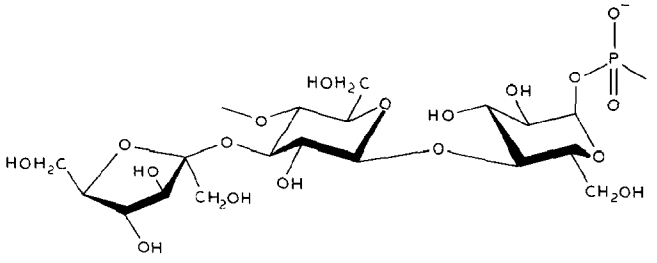

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TABLE III

^{13}C-N.M.R. DATA (δ IN p.p.m., *J* IN Hz)

	Atom	*K11-dF*[a]		*K11-TS*[b]		*K11*			*α-Glc-1-P* δ	*4-α-Glc* δ	*4-β-Glc* δ
		δ	$J_{C,P}$	δ	δ	δ	*APT*[c]	$J_{C,P}$			
α-Glc	C-1	96.2	6.2	92.0	96.7	95.9	+	5.7	96.3	92.4	96.4
	C-2	71.9	10.0	72.7	74.7	71.8	+	9.8	72.9	71.9	74.5
	C-3	72.3	—	73.9	75.1	72.3	+	—	74.3	72.1	75.1
	C-4	78.6	—	77.7	77.7	78.0	+	—	70.9	79.9	79.9
	C-5	72.1	—	70.9	76.2	72.0	+	—	74.1	70.8	75.5
	C-6	60.5	—	60.8	60.8	60.5	−	—	61.9	60.8	60.8
		δ	$J_{C,P}$	δ		δ	*APT*	$J_{C,P}$	*β-Glc-OMe* δ		
β-Glc	C-1	103.2	—	103.0		103.5	+	—	104.0		
	C-2	73.8	—	73.8		73.8	+	—	74.1		
	C-3	75.3	—	78.8		75.3	+	—	76.8		
	C-4	74.4	6.3	70.3		75.2	+	5.5	70.6		
	C-5	75.8	4.0	75.5		76.6	+	1.2	76.8		
	C-6	61.2	—	60.9		60.7	—	—	61.8		
				δ		δ	*APT*	$J_{C,P}$	*β-Fru-OMe* δ		
β-Fru	C-1			61.4		61.3	−	—	60.7		
	C-2			104.4		104.4	−	—	104.7		
	C-3			79.8		80.1	+	—	77.7		
	C-4			75.6		75.3	+	—	75.9		
	C-5			81.8		81.1	+	—	82.1		
	C-6			63.2		63.0	−	—	63.6		

[a]Defructosylated K11. [b]Trisaccharide from K11. [c]Sign of signals in the attached-proton test.

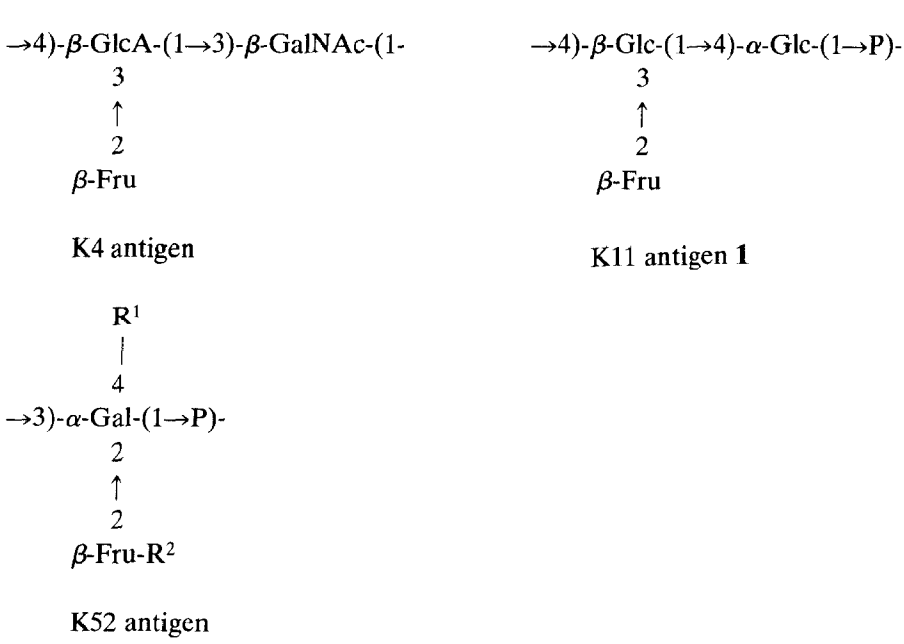

Fig. 2. Primary structure of the fructose-containing K antigens of *E. coli* O5:K4:H4, O13:K11:H11, and O4:K52:H⁻: R^1 = OAc, R^2 = OAc and OPr.

The K11 antigen is the third capsular polysaccharide of *E. coli* in which terminal β-D-fructofuranosyl units have been found, the others being the K4 antigen from *E. coli* O5:K4:H4[10] and the K52 antigen from O4:K52:H[8] (Fig. 2).

Preparations of the capsular K11 antigen that were isolated from bacteria cultivated at 20° or 37° were linked to phosphatidylglycerol which is characteristic for K-antigens of group II of *E. coli*.

Serological analysis. — In an enzyme-linked immunoabsorbent assay (ELISA)[21,22] using a homologous antiserum (rabbit), the native K11 antigen was strongly reactive, with a reciprocal titre of 25 000. The defructosylated polymer, which contained only 5% of fructose, gave a reciprocal titre of 800, and the periodate-oxidized polymer, in which all of the D-fructofuranose and all of the α-D-glucopyranose were destroyed, had a titre of 200. These results showed the β-D-fructofuranose to be the immunodominant sugar in the K11 antigen. A comparison of the serology of the fructose-containing capsular K4, K11, and K52 antigens (Table IV) showed that, although the fructofuranose residue is the immunodominant sugar in each polymer, they are only weakly cross-reactive, indicating that the fructose residues are presented in different manners at the surface of the polymers.

TABLE IV

RECIPROCAL ELISA TITRES

Antigen	*Reciprocal ELISA titres*		
	K4-serum	*K11-serum*	*K52-serum*
K4	100 000	3200	6500
K11	6500	25 000	6500
K52	6500	6500	100 000

EXPERIMENTAL

Bacteria and cultivation. — *E. coli* Su4321-41 (O13:K11:H11) was obtained from Drs. I. and F. Ørskov (Copenhagen). The bacteria were grown to the late logarithmic phase in a fermenter in 10-L-batches, which contained per L: $K_2HPO_4 \cdot 3\ H_2O$ (9.7 g), KH_2PO_4 (2 g), sodium citrate $\cdot 5\ H_2O$ (0.5 g), $MgSO_4 \cdot 7\ H_2O$ (0.1 g), casamino acids (1 g), ammonium sulfate (20 g), D-glucose (2 g), and the dialysable part of yeast (100 mL from 500 g in 5 L of deionised water).

Isolation and purification of the polymers. — The polymer and the bacterial cells were precipitated from the liquid cultures by the addition of 1 vol. of aq. 2% cetyltrimethylammonium bromide (Cetavlon). Each of the following operations was carried out at 4°.

The polymer was extracted from the precipitates with M calcium chloride, purified by three cycles of precipitation from aqueous solution with ethanol (to 80% final concentration), followed by repeated extraction with cold aqueous 80% phenol (buffered to pH 6.5 with sodium acetate) to remove contaminating proteins[12–13]. The combined aqueous phases were centrifuged for 4 h at 100 000*g* and the supernatant solution was lyophilised. The residue was further purified by elution from Sephadex G-50.

Analytical methods. — Fructose was determined as peracetylated glucitol and mannitol by g.l.c.[23] after mild hydrolysis (0.1M trifluoroacetic acid, 30 min, 100°), and glucose after strong hydrolysis (0.1M HCl, 72 h, 100°) which destroyed the fructose. Glucose and fructose were also determined with the hexokinase–phosphoglucoisomerase–glucose-6-dehydrogenase–NADP[24] kit (Boehringer, Mannheim). The ketose-specific phenol–acetone–boric acid reagent[25] was used to determine the fructose. Phosphate was quantified by the method of Ames[26]. Protein was determined by the method of Bradford[27], and nucleic acid by spectrometry (258 nm) of samples in 10mM sodium hydroxide.

G.l.c. was performed on a column (15 m × 2 mm) of ECNSS-M at 190° with a Varian Aerograph series 1400 instrument equipped with an autolinear temperature programmer and a Hewlett–Packard 3380 integrator. G.l.c.–m.s. (70 eV) was performed with a Finnigan MAT 1020 B automatic system on a column (25 m × 0.25 mm) of CBCP SIL5 with helium as the carrier gas and with a temperature

gradient of 2°/min from 140°. N.m.r. spectroscopy [external sodium 4,4-dimethyl-4-sila(2,2,3,3-$^2H_4$)pentanoate] was performed with a Bruker WM 300 spectrometer in the F.t. mode at 33°. The chemical shifts are related to the signal for Me_4Si. For the ^{31}P resonances, phosphoric acid was used as external standard.

Defructosylation of the polymer. — An aqueous solution of the K11 antigen (100 mg in 20 mL) at pH 5.0 was stirred for 4 h at 70°. The fructose liberated was removed by dialysis against water and the dialysate was lyophilized to yield ~70 mg of polymeric material.

Isolation of the trisaccharide. — A solution of K11 polymer (100 mg) in 0.05M NaOH (100 mL) was kept for 4 h at 4°, then neutralised with Dowex 50 (H^+) resin, incubated for 12 h at room temperature with alkaline phosphatase (EC 3.1.3.1; 100 μL, 35 U) in 50mM sodium carbonate buffer (pH 9.6). The trisaccharide was purified by elution from a column (1.5 × 80 cm) of Biogel P-1 with water (yield 30%, based on the K11 antigen).

Periodate oxidation. — Preparative periodate oxidation has been described[28]. A solution of the polysaccharide preparation (10 mg) in phosphate-buffered saline (10 mL, pH 7.4) was incubated at room temperature with an equal volume of 0.1M sodium metaperiodate. At intervals, aliquots were diluted with water (1:1000) and the decrease in absorbance at 223 nm, which indicated the consumption of periodate[29], was measured. Erythritol was used as the reference substance.

Methylation analysis. — The K11 polymer (5 mg) was methylated with methyl sulfoxide–potassium hydride–methyl iodide in a modification of the procedure described by Hakomori[14–16]. After hydrolysis, reduction, and acetylation, the partially methylated alditol acetates were analyzed by g.l.c.–m.s.

Antisera. — Antisera against *E. coli* O13:K11:H11 (Su4321-41), O5:K4:H4 (U1-41), and O4:K52:H^- (A 103) were obtained by immunization of rabbits with formalin-killed bacteria. Bacterial suspensions (2 × 10^7, 5 × 10^7, 10^8, and 10^9 cells/animal) were injected at intervals of 4 days; 5 days after the last injection, the animals were bled and the serum was prepared. Normal (non-immune) serum was taken from the animal prior to immunization. The specificities of the sera were determined with the ELISA[21,22] technique, using K11-, K4-, and K52-specific polymers as antigens.

Enzyme-linked immunosorbent assay (ELISA). — A modified method[10] was used.

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