Carbohydrate Research, 253 (1994) 323–327
Elsevier Science B.V., Amsterdam

Note

Structure of the capsular K96 polysaccharide (K96 antigen) from *Escherichia coli* O77:K96:H$^-$ and comparison with the capsular K54 polysaccharide (K54 antigen) from *Escherichia coli* O6:K54:H10

Barbara Jann, Helga Kochanowski and Klaus Jann *
Max-Planck-Institut für Immunbiologie, D-7800 Freiburg (Germany)

(Received March 30th, 1993; accepted August 11th, 1993)

The capsular polysaccharides (K antigens) of *Escherichia coli* have been divided into two groups, differing mainly in their genetic determination, temperature regulation of expression, and substitution of the reducing end by lipid[1]. Evidence was found for the existence of a third group of capsular polysaccharides (provisionally termed I/II)[2] which, like group I polysaccharides, are not temperature-regulated, but which are, like group II polysaccharides, substituted with phospho-lipid[3] at the reducing end. The K54 polysaccharide, a representative of group I/II, has been analysed and its structure was reported[4] to consist of → 3)-β-D-glucosyluronic acid-(1 → 3)-α-L-rhamnosyl-(1 → repeating units. About 85% of the glucuronic acid residues were substituted, in a molar ratio of 6:1, with L-threonine and L-serine amidically linked to the carboxyl group. The amide substituents could be removed with alkali, without alteration of the carbohydrate chain. It has been reported previously[5] that the K54 antigen showed serological cross-reactivity with the K96 antigen of *E. coli*. The K96 polysaccharide, which also belongs to group I/II[2], was found to have the same sugar composition as the K54 polysaccharide. We now present the structure of the K96 polysaccharide, using NMR spectroscopy, and compare it with that of the K54 polysaccharide.

The polysaccharides were precipitated[6] from liquid cultures of *E. coli* strains A12b (O6:K54:H10) and E10 (O77:K96:H$^-$), together with the bacterial cells, using hexadecyltrimethylammonium bromide (CTAB), and extracted from the precipitates with calcium chloride. After repeated precipitation from aqueous solutions with ethanol, extraction with cold phenol at pH 6.5, and dialysis, the polysaccharides were obtained by lyophilisation.

* Corresponding author.

SSDI 0008-6215(93)E0299-G

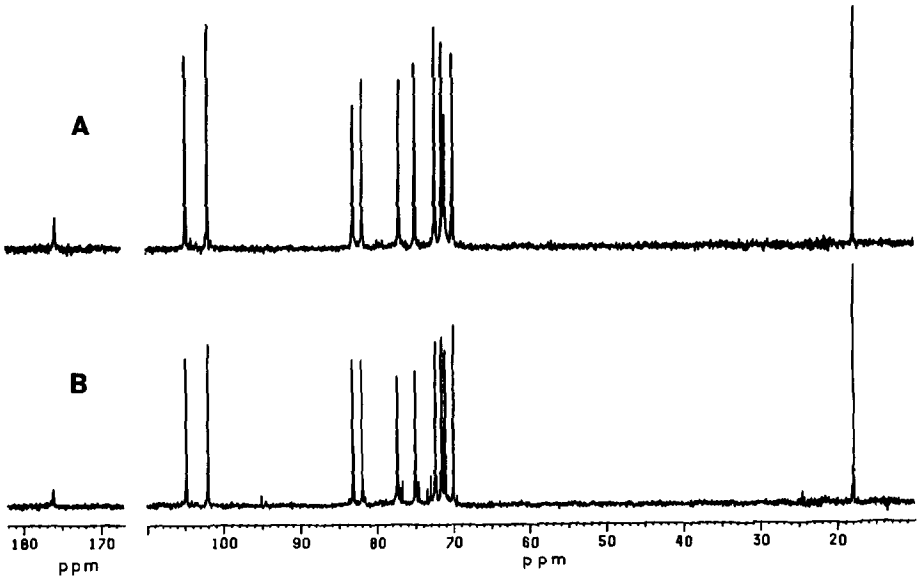

Fig. 1. 75-MHz ^{13}C NMR spectrum (δ 182–166 and 110–5) of the K96 polysaccharide (A) and the deaminoacylated K54 polysaccharide (B), recorded in D_2O (70°C) with acetone (δ_C 31.45) as internal standard.

Both polysaccharides consisted of equimolar amounts of D-glucuronic acid and L-rhamnose. The K96 polysaccharide did not contain amino acids, whereas the K54 polysaccharide contained threonine and serine, which were removed by treatment with M sodium hydroxide[4].

The ^{13}C NMR spectra of the K96 polysaccharide and the amino acid-free derivative of the K54 polysaccharide (Fig. 1) were identical, both containing twelve signals. The presence of two signals in the region of anomeric carbon atoms (δ 104.7 and 101.9), one signal due to a carboxyl group (δ 175.5–176.1), and one signal due to a C-6 methyl group (δ 17.9) were indicative of a disaccharide repeating unit containing glucuronic acid and rhamnose.

The ^1H NMR spectra of the deaminoacylated K54 polysaccharide and the native K96 polysaccharide each contained one signal for a β-anomeric proton of the *gluco/galacto* configuration (δ 4.69; $J_{1,2}$ 7.5 Hz; 7.7 Hz) and one signal for an anomeric proton of the *manno* configuration (δ 5.13 and 5.14; $J_{1,2} < 2$ Hz). A signal at δ 1.26 and 1.27 was indicative of a methyl group from a 6-deoxy sugar. Assignments of the signals (Table I) were obtained using 2D COSY one- and two-step H-relayed coherence transfer (COSYRCT) 2D spectra[7,8]. The visual multiplicities and coupling constants of the signals were determined with the help of 1D homonuclear double resonance in the difference mode[9]. The results showed that the repeating unit consisted of 3-α-Rha and 3-β-GlcA.

The signals of the ^{13}C NMR spectra were assigned (Table I) with a heteronuclear COSY spectrum (XHCORRD) (Fig. 2). For the determination of the

TABLE I

^{1}H and ^{13}C NMR data of the K96 polysaccharide and the deaminoacylated K54 (K54-alk) polysaccharide. The visible multiplicities of the C-4 signals of glucuronic acid in both polysaccharides could not be determined because of overlap of signals

PS	Residue	Proton	δ (ppm)	Visible multiplicity	$J_{H,H}$	Hz	Carbon	δ (ppm)	$J_{C\text{-}1,H\text{-}1}$ (Hz)
K96	→ 3)-β-D-GlcA-(1 →	H-1	4.69	d	$J_{1,2}$	7.5	C-1	104.7	163
		H-2	3.54	dd	$J_{2,3}$	10	C-2	74.9	
		H-3	3.62	t	$J_{3,4}$	8.5	C-3	83.15	
		H-4	3.63		$J_{4,4}$	9.5	C-4	71.5	
		H-5	3.8	d	$J_{5,4}$		C-5	77.3	
							C-6	176.1	
	→ 3)-α-L-Rha-(1 →	H-1	5.14	bs	$J_{1,2}$	< 2	C-1	101.9	173
		H-2	4.29	dd	$J_{2,3}$	3	C-2	71.1	
		H-3	3.94	dd	$J_{3,4}$	9.5	C-3	81.9	
		H-4	3.61	t	$J_{4,5}$	9.5	C-4	72.3	
		H-5	4.06	dq	$J_{5,6}$	6.5	C-5	70.0	
		H-6	1.27	d	$J_{6,5}$		C-6	17.9	
K54-alk	→ 3)-β-D-GlcA-(1 →	H-1	4.69	d	$J_{1,2}$	7.7	C-1	104.7	161
		H-2	3.52	dd	$J_{2,3}$	10	C-2	74.9	
		H-3	3.62	t	$J_{3,4}$	8.5	C-3	83.05	
		H-4	3.63		$J_{4,5}$	9.4	C-4	71.4	
		H-5	3.84	bd	$J_{5,4}$		C-5	77.0	
							C-6	175.6	
	→ 3)-α-L-Rha-(1 →	H-1	5.13	bs	$J_{1,2}$	< 2	C-1	101.9	173
		H-2	4.27	bd	$J_{2,3}$	2.5	C-2	71.1	
		H-3	3.92	dd	$J_{3,4}$	9.5	C-3	81.9	
		H-4	3.60	t	$J_{4,5}$	9.5	C-4	72.3	
		H-5	4.04	dq	$J_{5,6}$	6.5	C-5	70.0	
		H-6	1.26	d	$J_{6,5}$		C-6	17.9	

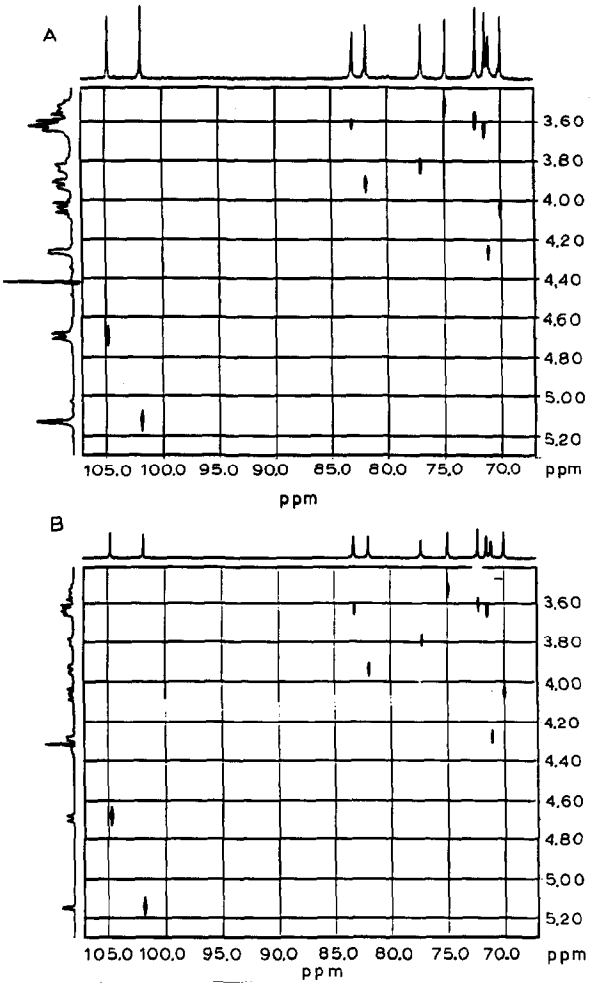

Fig. 2. 2D 300-MHz heteronuclear $^{13}C-^{1}H$ COSY spectrum of the K96 polysaccharide (A) and the deaminoacylated K54 polysaccharide (B).

absolute configurations of the sugar residues, use was made of the previous determination of D-GlcA and L-Rha in the K54 polysaccharide[4]. Using this information, the configurations could be verified from the glycosylation effects[10].

The results obtained show that the deaminoacylated K54 polysaccharide and the K96 polysaccharide both have structure **1**.

$$\rightarrow 3)\text{-}\beta\text{-D-GlcA-}(1 \rightarrow 3)\text{-}\alpha\text{-L-Rha-}(1 \rightarrow$$
$$\mathbf{1}$$

As reported previously[4], in the K54 polysaccharide ca. 85% of the glucuronic acid residues are substituted at the carboxyl group amidically with threonine and serine. Thus, the K54 polysaccharide appears as a substituted K96 polysaccharide. From the fact that the substitution is not complete, we assume that, during biosynthesis,

the K54 polysaccharide is first assembled and then modified by transfer of threonine and serine.

Whereas amide substitution of acidic polysaccharide has been reported before[11–13], the fact that the unsubstituted polysaccharide occurs as a surface antigen in its own right has hitherto not been described. In contrast, *O*-acetylation of a polysaccharide has not only been found as a result of form variation[14] where both forms occur together within the same bacterial culture. It has also been reported that a polysaccharide and its *O*-acetylated form occur as distinct antigens in different *E. coli* strains[15–17], a situation comparable to that described in this communication.

REFERENCES

1 B. Jann and K. Jann, *Curr. Top. Microbiol. Immunol.*, 150 (1990) 19–42.
2 A. Finke, B. Jann, and K. Jann, *FEMS Microbiol. Lett.*, 69 (1990) 129–134.
3 M.A. Schmidt and K. Jann, *FEMS Microbiol. Lett.*, 14 (1982) 69–78.
4 P. Hofmann, B. Jann, and K. Jann, *Carbohydr. Res.*, 13 (1985) 261–271.
5 I. Ørskov, F. Ørskov, B. Jann, and K. Jann, *Bacteriol. Rev.*, 41 (1977) 667–710.
6 W.F. Vann and K. Jann, *Infect. Immun.*, 25 (1979) 85–92.
7 J. Jeener, B.H. Meier, P. Bachmann, and E.E. Ernst, *J. Chem. Phys.*, 71 (1979) 4546–4553.
8 J. Dabrowski, *Methods Stereochem. Anal.*, 9 (1987) 349–386.
9 N.A. Kocharowa, Y.A. Knirel, A.S. Shashkov, N.K. Kochetkov, and G.B. Pier, *J. Biol. Chem.*, 263 (1988) 11291–11295.
10 G.M. Lipkind, A.S. Shashkov, Y.A. Knirel, E.V. Vinogradov, and N.K. Kochetkov, *Carbohydr. Res.*, 175 (1988) 57–75.
11 T. Dengler, B. Jann, and K. Jann, *Carbohydr. Res.*, 150 (1986) 233–240.
12 B.A. Dmitriev, V.L. L'vov, N.V. Tochtamysheva, A.S. Shashkov, N.K. Kochetkov, B. Jann, and K. Jann, *Eur. J. Biochem.*, 134 (1983) 517–521.
13 W. Gromska and H. Mayer, *Eur. J. Biochem.*, 62 (1976) 391–399.
14 F. Ørskov, I. Ørskov, A. Sutton, R. Schneerson, W. Lind, W. Egan, G.E. Hoff, and J.R. Robbins, *J. Exp. Med.*, (149) 669–685.
15 K. Jann and M.A. Schmidt, *FEMS Microbiol. Lett.*, 7 (1980) 79–81.
16 M.-L. Rodriguez, B. Jann, and K. Jann, *Carbohydr. Res.*, 173 (1988) 243–253.
17 A. Bax, M.F. Summers, W. Egan, N. Guirgis, R. Schneerson, J.B. Robbins, F. Ørskov, I. Ørskov, and W.F. Vann, *Carbohydr. Res.*, 173 (1988) 53–64.