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Structural Analysis of the O-Antigen Side Chain Polysaccharides in the Lipopolysaccharides of *Klebsiella* Serotypes O2(2a), O2(2a,2b), and O2(2a,2c)

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The lipopolysaccharide (LPS) of *Klebsiella* serotype O2 is antigenically heterogeneous; some strains express multiple antigenic factors. To study this heterogeneity, we determined the structure of the O-antigen polysaccharides in isolates belonging to serotypes O2(2a), O2(2a,2b), and O2(2a,2c), by using composition analysis, methylation analysis, and both ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. The repeating unit structure of the 2a polysaccharide was identified as the disaccharide [→3)-β-D-Gal*f*-(1→3)-α-D-Gal*p*-(1→] and was identical to D-galactan I, one of two O polysaccharides present in the LPS of *Klebsiella pneumoniae* serotype O1 (C. Whitfield, J. C. Richards, M. B. Perry, B. R. Clarke, and L. L. MacLean, J. Bacteriol. 173:1420–1431, 1991). LPS from serotype O2(2a,2b) also contained D-galactan I as the only O polysaccharide, suggesting that the 2b antigen is not an O antigen. The LPS of serotype O2(2a,2c) contained a mixture of two structurally distinct O polysaccharides and provides a second example of this phenomenon in *Klebsiella* spp. One polymer was identical to D-galactan I, and the other polysaccharide, the 2c antigen, was a polymer with a disaccharide repeating unit structure, [→3)-β-D-Glc*p*NAc-(1→5)-β-D-Gal*f*-(1→]. The 2c structure does not resemble previously reported O polysaccharides from *Klebsiella* spp. Periodate oxidation confirmed that D-galactan I and the 2c polysaccharide are distinct glycans, rather than representing domains within a single polysaccharide chain. Monoclonal antibodies against the 2c antigen indicated that only LPS molecules with the longest O-polysaccharide chains contained the 2c epitope.

The genus *Klebsiella* contains a number of important pathogens which cause bacteremia, pneumonia, and urinary tract infections in humans. *Klebsiella* spp. generally produce large mucoid colonies because of the synthesis of capsular polysaccharides. Seventy-seven different capsular (K^-) antigens are now recognized (21). Fewer lipopolysaccharide (LPS) O antigens have been identified. Initially, 12 serotypes of O antigen were reported, but it was subsequently suggested that the actual numbers of unique structures may be as few as 8, on the basis of chemical analysis (15). Serotyping to subdivide isolates of *Klebsiella* spp. has focused primarily on the K antigens because the range of O serotypes is relatively limited and because the large amount of capsular polysaccharide provides practical difficulties in O serotyping.

Recent studies in this laboratory have been focused on the serotype O1 LPS of *Klebsiella* spp. The O side-chain polysaccharide of the O1 LPS molecule was shown to contain a mixture of two structurally distinct D-galactan polysaccharides (31). D-Galactan I chains were heterogeneous in size and contained the repeating structure [→3)-β-D-Gal*f*-(1→3)-α-D-Gal*p*(1→]. In contrast, D-galactan II was confined to higher-molecular-weight molecules and comprised [→3)-α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→]. A subsequent report by Kol et al. (16) confirmed these structural data. The structures of the O polysaccharides from serotypes O1 and O6 are identical (30). D-Galactan II is an important virulence determinant in *Klebsiella pneumoniae* O1, since mutants which synthesize only D-galactan I are no longer able to withstand complement-mediated serum killing (19).

As a result of these studies, we have become interested in the possibility that other *Klebsiella* O serotypes may also contain more than one type of O side-chain polysaccharide. One candidate for this phenomenon is serotype O2. Isolates originally designated as serotype O2 were found to be antigenically heterogeneous in serotyping, giving rise to O2 subgroups containing factors 2a, 2b, 2c, and 2d. The 2a and 2c antigens were reported to be dominant, with the minor 2b and 2d antigens being identified in cross-absorbed sera (13, 14). Subsequent studies by Ørskov (20) suggested further complexity, with as many as eight different antigens (designated 2a to 2h) being identified in 10 serotype O2 isolates. All 10 strains contained the 2a antigen, either in isolation or in conjunction with additional antigens. More recently, it was reported that serotype O2 strains contain polysaccharides which are structurally identical to the O side chains of either serotype O9 or O8, and, on the basis of these results, it was suggested that the O2 designation be eliminated (15). The O8 and O9 polysaccharides were reported to be composed of tri- and pentasaccharide galactan repeating units, respectively (15). We have reinvestigated these structures and found that the O8 polysaccharide is actually identical to serotype O1 (30). Although our analysis confirmed that the O9 repeating unit is a pentasaccharide, some linkage differences were detected. The O9 polysaccharide consists of a D-galactan I backbone with an α-D-Gal*p* residue linked 1→2 to every second α-D-Gal*p* in the backbone (18). We have also shown that strains carrying only the 2a antigen are recognized by monoclonal antibodies (MAbs) against D-galactan I (31). Taken together, these observations indicate that the status of

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TABLE 1. *Klebsiella* strains used in this study

Strain	Serotype	O2 subtype formula[a]		Reference
		Extended	Simplified	
2482	O2:K43	2a	2a	20
CWK49	O2:K$^-$	2a	2a	This study
5758	O2:K28	2a	2a	20
7380	O2:K$^-$	2a,2b	2a+[b]	20
5053	O2:K$^-$	2a,2c	2a+[b]	20
D5050	O2:K4	2a,2c,2d	2a±[c]	20
7444	O2:K35	2a,2e	2a±[c]	20
2212/52	O2:K59	2a,2e,2h	2a±[c]	20
6613	O2:K27	2a,2f,2g	2a±[c]	20
E5051	O2:K6	Unknown		
C5046	O2:K3	Unknown		
NCTC8172	O6:K64			
CWK2 (KD2)	O1:K$^-$			31
889	O8:K69			
1205	O9:K72			

[a] According to Ørskov (20).
[b] 2a+, strain contains the 2a antigen plus an additional antigen(s).
[c] 2a±, strain contains part of the 2a antigen and an additional antigen(s).

Klebsiella strains originally placed in serotype O2 is unclear. To resolve this question, we determined the structure of the LPS O side-chain polysaccharides from *Klebsiella* isolates representing serotypes O2(2a), O2(2a,2b), and O2(2a,2c).

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *Klebsiella* strains used in this study are listed in Table 1. The O-antigen status is from the results of Ørskov (20). With the exception of *K. pneumoniae* CWK2, the species of the isolates have not been identified. The prototype strains used for LPS extraction were *Klebsiella* strains CWK49 [serotype O2(2a):K$^-$], 7380 [O2(2a,2b):K$^-$], and 5053 [O2(2a,2c):K$^-$]. *Klebsiella* strain CWK49 was isolated as a spontaneous, unencapsulated mutant from *Klebsiella* strain 2482 (O2:K43); the LPS profiles of the parent and mutant were identical on the basis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Strains were routinely grown and maintained by using Luria broth, and incubation was at 37°C. For the large-scale preparation of LPS, bacteria were grown in a fermentor (28 liters; Microfirm; New Brunswick Scientific) by using a medium of 3.7% (wt/vol) brain heart infusion (Difco) at 37°C and 200 rpm with aeration at 25 liters/min for 18 h.

LPS extraction and purification of O polysaccharides. Details of the LPS preparation procedures are reported elsewhere. Briefly, cells were washed in 2% (wt/vol) saline; digested with lysozyme, ribonuclease, and deoxyribonuclease (12); and then extracted with hot aqueous phenol (29). LPS was recovered from the dialyzed aqueous layer by repeated ultracentrifugation at 105,000 × *g* (12 h at 4°C). To isolate the O polysaccharides, 700-mg samples of LPS were resuspended in 300 ml of 1.5% acetic acid and the lipid A was released by hydrolysis at 100°C for 2 h; the resulting precipitate containing lipid A was removed by centrifugation. The supernatant was lyophilized, and the O polysaccharides were then isolated by chromatography on a Sephadex G-50 column (2.5 by 90 cm), which was eluted with pyridinium acetate (0.05 M, pH 4.7). Fractions were monitored for hexoses, amino sugars, KDO, and phosphate.

SDS-PAGE and Western blotting. LPS obtained by phenol extraction or by the SDS-proteinase K method (11) was analyzed by SDS-PAGE. The conditions for electrophoresis (7) and silver staining (28) have been reported previously. Western blotting (immunoblotting) was performed by using modifications (4) of the original method of Towbin et al. (27). Immunoblots were developed with alkaline phosphatase-conjugated goat anti-mouse F(ab)$_2$ (Jackson Laboratory). The color reagent was nitroblue tetrazolium with 5-bromo-4-chloro-3-indolyl phosphate (Sigma).

MAbs. The procedures used for the production of MAbs are described elsewhere (19), as is the reactivity of MAb O1-2.6, which recognizes the D-galactan I component of serotype O1 LPS (31). MAb O2-67.1 was obtained by the fusion of spleen cells from a mouse immunized with formalin-killed *Klebsiella* strain 5053 cells. Both MAbs were class immunoglobulin M (IgM).

NMR spectroscopy. Spectra were obtained at 30°C with polysaccharide solutions in deuterium oxide, by using a Bruker AM-500 spectrometer equipped with an Aspect 3000 computer and by using standard Bruker software. Proton spectra were obtained by using a spectral width of 2.5 kHz, a 16,000 data block, and a 90° pulse. Chemical shifts are expressed relative to that of internal acetone (δ, 2.225 ppm). Broadband, proton-decoupled ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 125 MHz by using a spectral width of 31 kHz, a 32,000 data block, and a 90° pulse, employing WALTZ decoupling (26). Heteronuclear $^1J_{C,H}$ couplings were measured by using gated decoupling. Two-dimensional, ^1H-detected carbon proton multiple-bond correlation spectra were acquired by using the protocol of Bax and Summers (3) on a Bruker AM-500 spectrometer fitted with a 5-mm inverse probe.

Analytical methods. The amounts of neutral sugars were estimated by the phenol-sulfuric acid method (8), 3-deoxy-D-*manno*-octulosonic acid was analyzed by the method of Aminoff (2), phosphate was analyzed by the method of Chen et al. (5), and aminodeoxyglycoses were analyzed by the method of Gatt and Berman (9). Quantitative analyses of glycoses were made by the gas-liquid chromatographic alditol acetate method (10), and their configurations were determined by capillary gas-liquid chromatography of their 2-(−)-butyl glycoside derivatives (17). The conditions for polysaccharide hydrolysis, methylation analysis, and periodate oxidation are described in detail elsewhere (1). Methylated derivatives were analyzed by gas-liquid chromatography–mass spectrometry, by using a Hewlett-Packard 5885SB gas chromatograph equipped with a flame ionization detector and a fused silica OV-17 capillary column (0.32 mm by 25 m; Quadrex Corporation). The temperature program was 200°C for 2 min and was increased to 240°C at a rate of 1°C per min. Optical rotations were determined by using a Perkin-Elmer 243 polarimeter.

RESULTS AND DISCUSSION

Structure of the O polysaccharide in the LPS of *Klebsiella* sp. strain CWK49 [serotype O2(O2a)]. The purified 2a polysaccharide from *Klebsiella* strain CWK49 had an $[\alpha]_D$ of +61° (c 4.4, water). Acid hydrolysis gave D-galactose as the sole constituent. The ^1H and ^{13}C NMR spectra of the purified 2a O polysaccharide showed two major anomeric signals, indicating a disaccharide repeating-unit structure. The signals in the spectra were identical to those previously reported for D-galactan I, one of two O polysaccharides in the LPS of *K. pneumoniae* serotype O1 (31), and to those reported for the identical structure in the O polysaccharides of serotype 4 and 10 LPS from *Pasteurella haemolytica* (23,

TABLE 2. Methylation analysis of *Klebsiella* serotype O2(2a) and O2(2a,2c) O polysaccharides

Methylated glycose	T_{GM}[a]	Molar ratio[b] of methylated glycose in the following polysaccharide:		
		2a	Oxidized 2a	2a,2c
1,3,4-tri-*O*-acetyl-2,5-di-*O*-methyl-D-arabinitol	0.84		0.94	
1,3,4-tri-*O*-acetyl-2,5,6-tri-*O*-methyl-D-galactitol	1.35	0.96		0.95
1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-galactitol	1.38			0.49
1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-galactitol	1.46	1.00	1.00	1.00
1,3,5-tri-*O*-acetyl-2-deoxy-(*N*-methylacetamido)-4,6,di-*O*-methyl-D-GlcNAc	3.79			0.44

[a] Retention time (T_{GM}) figures are quoted relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (1.00).
[b] Molar ratios are relative to 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-galactitol.

25). Methylation analysis of the native polysaccharide yielded equimolar amounts of 2,5,6-tri-*O*-methyl-D-galactose and 2,4,6-tri-*O*-methyl-D-galactose (Table 2). Following periodate oxidation, the methylation products were 2,5-di-*O*-methyl-L-arabinose and 2,4,6-tri-*O*-methyl-D-galactose (Table 2), confirming both the structure and the linkage of the residues. The 2a antigen first described by Kauffmann (13) and subsequently by Ørskov (20) therefore comprises the following repeating-unit structure: [→3)-β-D-Gal*f*-(1→3)-α-D-Gal*p*-(1→] (D-galactan I). The same polysaccharide structure has also been identified in *Serratia marcescens* serotypes O16 and O20 (22).

We have previously reported that the SDS-PAGE profile of the LPS of *Klebsiella* sp. strain 2482 (O2:K43) was identical to that of mutant strains of *K. pneumoniae* serotype O1 in which only D-galactan I was synthesized. The LPS of *Klebsiella* strain 2482 also reacted with MAbs specific for D-galactan I (31). The presence of the identical D-galactan I structure in both O1 and O2a LPS therefore clarifies our previous immunochemical analyses.

Structure of the O polysaccharide in the LPS of *Klebsiella* sp. strain 7380 [serotype O2(O2a,2b)]. The purified 2a,2b O polysaccharide from *Klebsiella* sp. strain 7380 had an $[\alpha]_D$ of +62° (c 2.5, water). The polysaccharide yielded only D-galactose following acid hydrolysis. The results of ^1H and ^{13}C NMR spectroscopy and methylation analyses showed that only one repeating-unit structure was present, that of D-galactan I (30). Previously, we did not detect D-galactan I in the *Klebsiella* strain 7380 LPS in Western blots probed with MAb specific for D-galactan I (31). Following the determination of the structure, we reexamined the immunoreactivity in both Western blots and enzyme-linked immunosorbent assays (ELISAs). Although the Western blots were reproducibly negative, a positive result was obtained by using the ELISAs. The reasons for the different results obtained by these techniques are unclear. We conclude that the only true O antigen in *Klebsiella* sp. strain 7380 is the 2a structure and that the 2b antigen described by Kauffmann (13, 14) and Ørskov (20) arises from a different cellular component.

Structure of the O polysaccharides in the LPS of *Klebsiella* sp. strain 5053 [serotype O2(2a,2c)]. The O-polysaccharide fraction from *Klebsiella* strain 5053 had an $[\alpha]_D$ of +26° (c 5.62, water). Quantitative analysis of the component sugars showed the fraction to contain D-galactose and 2-acetamido-2-deoxy-D-glucose (*N*-acetyl[NAc]-D-glucosamine) in the molar ratio of 5:1. The ^1H NMR spectrum (Fig. 1A) showed four anomeric proton signals with chemical shifts at 5.23, 5.09, 5.01, and 4.72 ppm (integrated ratio, 2:2:1:1). An additional signal at 2.09 ppm (integrated ratio of 3H) was attributed to the methyl resonance of the *N*-acetyl substituent of the 2-amino-2-deoxy-D-glucose component. Consistent with the ^1H NMR spectrum, the ^{13}C NMR spectrum (Fig. 1B) of the O-polysaccharide fraction showed four characteristic anomeric carbon signals. The resonances at 110.30 ($^1J_{C,H}$, 180 Hz) and 109.24 ($^1J_{C,H}$, 174 Hz) ppm had chemical shifts and $J_{C,H}$ coupling constants indicative of the presence of two unique β-D-galactofuranose structural units (Table 3). The remaining two anomeric resonances at 101.59 ($^1J_{C,H}$, 163 Hz) and 100.49 ($^1J_{C,H}$, 173 Hz) ppm are indicative of hexopyranose residues with β- and α-anomeric configurations, respectively. Characteristic signals for the acetamido substituent were seen at 21.4 ($\underline{C}H_3CONH$) and 175.9 ($CH_3\underline{C}ONH$) ppm, in addition to a signal at 56.2 ppm, characteristic of the C-2 resonance of a 2-acetamido-2-deoxyglycose residue.

Methylation analysis (Table 2) of the O-polysaccharide fraction gave 2,5,6-tri-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-galactose, 2,4,6-tri-*O*-methyl-D-galactose, and 2-deoxy-4,6-di-*O*-methyl-2-(*N*-methylacetamido)-D-glucose in the molar ratio 2:1:2:1. Since the ^{13}C NMR spectrum indicated the presence of two distinct β-D-galactofuranose residues, it can be concluded that the 2,5,6-tri-*O*-methyl-D-galactose arises from a 1→3-linked β-D-galactofuranose residue and the 2,3,6-tri-*O*-methyl-D-galactose arises from a 1→5-linked β-D-galactofuranose residue. The integrated ratio of the anomeric proton signals in the ^1H NMR spectrum of the O-polysaccharide fraction is consistent with the above conclusions. The methylation evidence also indicated that the fraction contains 1→3-linked D-galactopyranose residues and 1→3-linked 2-acetamido-2-deoxy-D-glucopyranose residues, present in the molar ratio 2:1.

An inspection of the chemical shifts of 12 resonances in the ^{13}C and ^1H NMR spectra of the O-polysaccharide fraction suggested that the major (ca. 65%) component could be a homogeneous linear polymer of a repeating disaccharide unit [→3)-β-D-Gal*f*-(1→3)-α-D-Gal*p*-(1→], previously characterized and defined as D-galactan I (31). In order to determine whether the O-polysaccharide fraction was homogeneous or was a mixture of two or more distinct polysaccharides, two-dimensional NMR COSY and NOESY analyses were made as previously described (1). The residues in the ^1H NMR spectrum of the O-polysaccharide fraction were labeled *a* to *d*, according to the decreasing order of the chemical shifts of their H-1 resonances, and the ring proton resonances for each residue were assigned through their cross-peaks in the COSY spectra (Tables 3 and 4). From the NOESY experiments, it was found that the β-D-Gal*f* residue *a* showed connectivity to H-3 of residue *b*, identifying it as a 1→3-linked α-D-Gal*p* residue. Since irradiation of H-1*b* showed enhancement on H-3*a*, it was determined that residue *b* is linked 1→3 to the β-D-Gal*f* residue *a*. The major

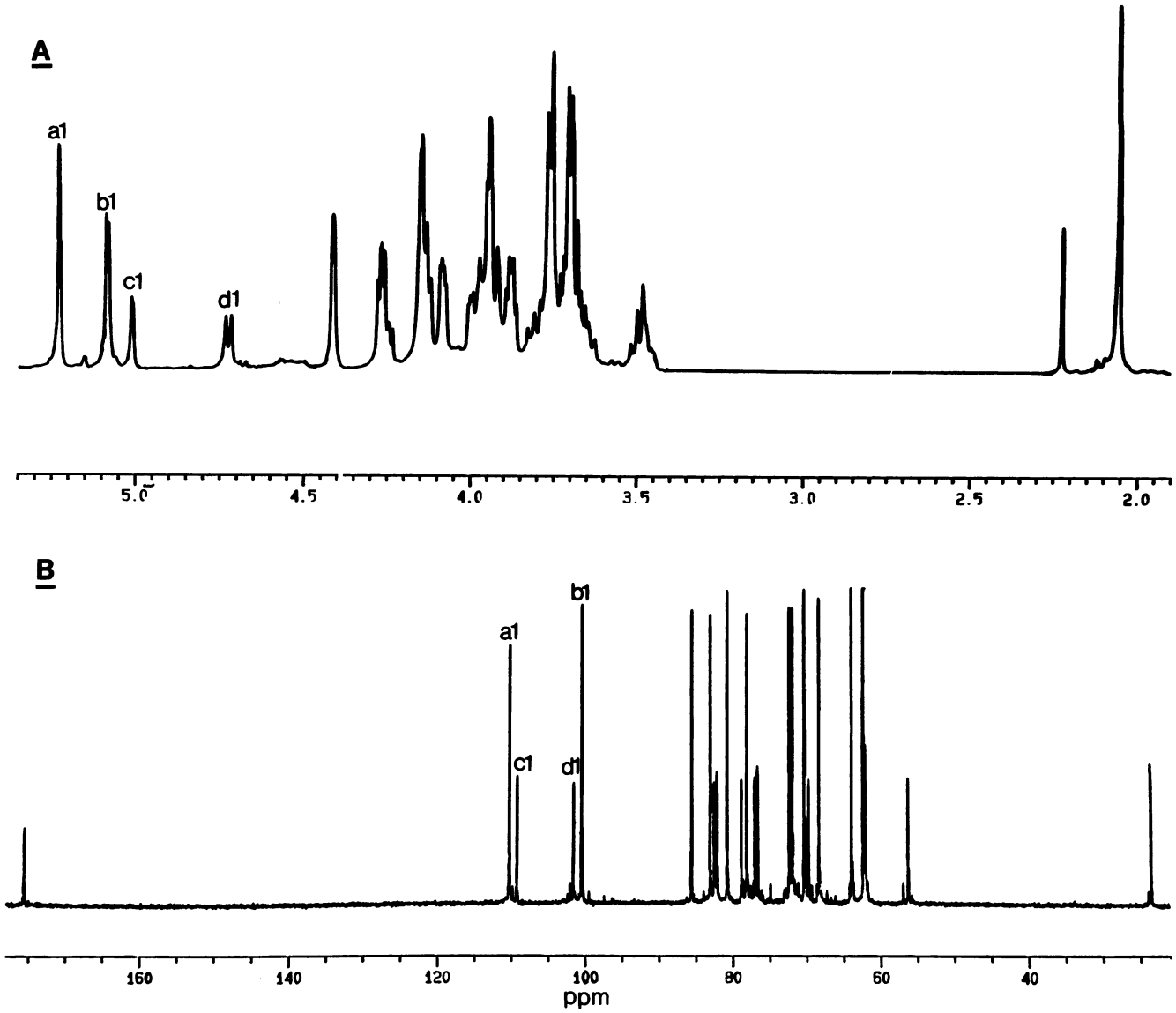

FIG. 1. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of the LPS O polysaccharides from *Klebsiella* sp. strain 5053 [serotype O2(2a,2c)].

component of the O-polysaccharide fraction is therefore a linear polymer of a repeating disaccharide structure [→3)-β-D-Gal*f*-(1→3)-α-D-Gal*p*-(1→], identified as D-galactan I in *K. pneumoniae* serotype O1 (31) and as the 2a antigen in *Klebsiella* sp. strains CWK49 and 7380 described above.

The minor (ca. 35%) second component of the O-polysaccharide fraction was suspected to be a polymer of a repeating disaccharide composed of 1→5-linked β-D-Gal*f* and 1→3-linked β-D-Glc*p*NAc residues. This latter conclusion was also supported by NOESY experiments. Irradiation of H-1*c*

TABLE 3. ^{13}C NMR chemical shifts and $J_{C,H}$ coupling constants of the glycose units present in the O-polysaccharide fraction of *Klebsiella* serotype O2(2a,2c)

Residue	Chemical shift (ppm) (coupling constant) in the O-polysaccharide fraction[a]						
	C-1	C-2	C-3	C-4	C-5	C-6	C-6′
a →3)-β-D-Gal*f*-(1→	110.30 (180)	80.71	85.52	83.02	71.89	62.31	62.31
b →3)-α-D-Gal*p*-(1→	100.49 (173)	68.25	78.06	70.27	72.29	63.82	63.82
c →5)-β-D-Gal*f*-(1→	109.24 (174)	82.34	76.96	82.49	78.79	62.22	62.22
d →3)-β-D-Glc*p*NAc-(1→	101.59 (163)	56.22	82.11	69.71	76.59	62.22	62.03

[a] Spectrum measured at 30°C in deuterium oxide; values are quoted in parts per million relative to that of internal acetone (31.07 ppm). Values in parentheses are $J_{C,H}$ coupling constants expressed in hertz.

TABLE 4. ^{1}H NMR chemical shifts and $J_{H,H}$ coupling constants of the glycose units present in the O-polysaccharide fraction of *Klebsiella* serotype O2(2a,2c)

Residue	Chemical shift (ppm) (coupling constant) in the O-polysaccharide fraction[a]						
	H-1	H-2	H-3	H-4	H-5	H-6	H-6′
a →3)-β-D-Gal*f*-(1→	5.23 (1.0)	4.42	4.08	4.27	3.88	3.68[c]	3.70[c]
b →3)-α-D-Gal*p*-(1→	5.09 (3.1)	3.94[b]	3.94[b]	4.14	4.14	3.77	3.77
c →5)-β-D-Gal*f*-(1→	5.01 (1.6)	4.00	4.25	4.15	3.97	3.70	3.70
d →3)-β-D-Glc*p*NAc-(1→	4.73 (8.2)	3.81	3.63	3.50	3.47	3.77	3.92

[a] Spectrum measured at 30°C in deuterium oxide; values are quoted in parts per million relative to that of internal acetone (2.225 ppm). Values in parentheses are $J_{H,H}$ coupling constants expressed in hertz.
[b] Correct shift value, misprinted as 4.97 ppm in a previous analysis of the O1 LPS of *K. pneumoniae* (29).
[c] Chemical shifts may be interchanged.

(β-D-Gal*f*) resulted in enhancement on H-3*d* (β-D-Glc*p*NAc), indicating that it was 1→3 linked. Irradiation of H-1*d* produced enhancement on H-5*c*, confirming that the β-D-Gal*f* residue was 1→5 linked. Consequently, the second polysaccharide component of the O-chain fraction, representing the 2c antigen, was a polymer of a disaccharide containing the structure [→3)-β-D-Glc*p*NAc-(1→5)-β-D-Gal*f*-(1→]. This structure has not been previously reported in *Klebsiella* sp. O polysaccharides, and it does not resemble other known O-polysaccharide structures from this organism (15).

Confirmation of the presence of two distinct polysaccharides in the 2a,2c O-chain fraction from *Klebsiella* sp. strain 5053 was obtained through periodate degradation studies. Periodate oxidation of the O-chain fraction was followed by reduction with $NaBH_4$ and mild acid hydrolysis. Sephadex G-50 gel filtration chromatography was used to separate the products, which included a polysaccharide and an oligosaccharide. The void volume contained the polysaccharide (ca. 60% yield), having an $[\alpha]_D$ of −63° (c 0.9, water). This fraction was composed of L-arabinose and D-galactose in a molar ratio of 1:1. Methylation analysis identified 2,5-di-*O*-methyl-L-arabinose and 2,4,6-tri-*O*-methyl-D-galactose (1:1) (Table 2), and its ^{1}H and ^{13}C NMR spectra were identical to those of the previously characterized periodate oxidation product of D-galactan I (31). The product (ca. 30% yield) obtained from the oligosaccharide region of the Sephadex G-50 column had an $[\alpha]_D$ of −12° (c 1.0, water) and was composed of D-GlcNAc and threitol (1:1). Partial ^{1}H and ^{13}C NMR results (H-1, 4.65 ppm [8.5 Hz]; C-1, 101.4 ppm [162 Hz]) indicated that the oligosaccharide was the β-D-Glc*p*-NAc-(1→2)-threitol derivative expected from the oxidation of the 2c polysaccharide. Since the 2c polysaccharide was completely degraded by periodate oxidation, while the derivative form of D-galactan I remained as a high-molecular-weight polymer, it is clear that the 2a,2c O-chain fraction in *Klebsiella* sp. strain 5053 contains two structurally distinct polysaccharide chains, rather than single chains with distinct structural domains.

Distribution of 2c polysaccharide in the LPS of *Klebsiella* serotype O2. To determine the distribution of 2c epitopes, MAb O2-67.1 was isolated. This MAb was specific for the O2(2a,2c) LPS in *Klebsiella* sp. 5053 and showed no reaction in either Western blots (Fig. 2) or ELISAs (30) with LPS from serotype O1, O6, O8, or O9. There was also no reaction with O2 strains now known to synthesize only D-galactan I or with those reported to express the 2e, 2f, 2g, or 2h sub-antigens described by Ørskov (20). In addition to strain 5053, two other strains (D5050 and E5051) also contained the 2c epitope (Fig. 2). SDS-PAGE profiles indicate that the molecular weight ranges of the LPS molecules of these three strains are roughly similar. However, strain 5053 appeared to produce more low-molecular-weight O-substituted LPS in the region expected for D-galactan I, compared with strains E5051 and D5050 (Fig. 2). *Klebsiella* sp. strain D5050 was reported to express the 2c antigen in the original serological studies of Kauffmann (13, 14) and Ørskov (20). The serotype-distinctive (2c) antigen was not present throughout the SDS-PAGE LPS profile but was restricted to the high-molecular-weight range, i.e., those LPS molecules substituted with the longest O polysaccharides (Fig. 2). A similar phenomenon was observed in the distribution of D-galactan II in the LPS of *K. pneumoniae* serotype O1 (31). It was not possible to obtain samples of 2c LPS devoid of contaminating 2a polymer. Consequently, there are no direct chemical data concerning linkage of the 2c polysaccharide to the LPS lipid A core. Indirect evidence for the classification of the 2c polymer as an O antigen comes from its behavior in serotyping studies (13, 14, 20), its migration properties in SDS-PAGE, and the observation that LPS core mutations eliminate expression of both the 2a and 2c polysaccharides (30).

No evidence of phase variation was detected in *Klebsiella* strain 5053 serotype O2(2a,2c), and all cells in the population express the 2c antigen (30). The 2a epitope was not accessible on the cell surface of strains which also express 2c, so it was not possible to determine whether all cells also produce the 2a polysaccharide. Similar results were reported for *K. pneumoniae* serotype O1 (31).

The O2(2a,2c) LPS, represented by *Klebsiella* sp. strain 5053, therefore joins serotype O1 and provides the second example in *Klebsiella* spp. of the simultaneous expression of two structurally distinct O polysaccharides. It appears that the D-galactan I structure is well distributed in *Klebsiella* strains belonging to serotypes O1, O6, and O8 (30, 31) and to serotype O2 isolates (13, 14, 20). We have recently determined that a DNA probe consisting of the *K. pneumoniae* O1 *rfb* gene cluster, which is responsible for the expression of D-galactan I, hybridizes to DNAs from strains belonging to serotypes O1, O2, O6, and O8 (6). Interestingly, the presence of a cross-reactive antigen in several isolates of *Klebsiella* spp. was reported by Pickett and Cabelli (24). The relationship between their Sm antigen and D-galactan I is unclear because the O-antigen serotypes of the strains examined were not reported. Although Pickett and Cabell: believed that the Sm antigen was distinct from LPS, our data on the presence of two O antigens in some *Klebsiella* spp. might explain their observations. It is clear from structural data on other O serotypes (15) that D-galactan I is not present in all *Klebsiella* spp. and is therefore not a true common antigen.

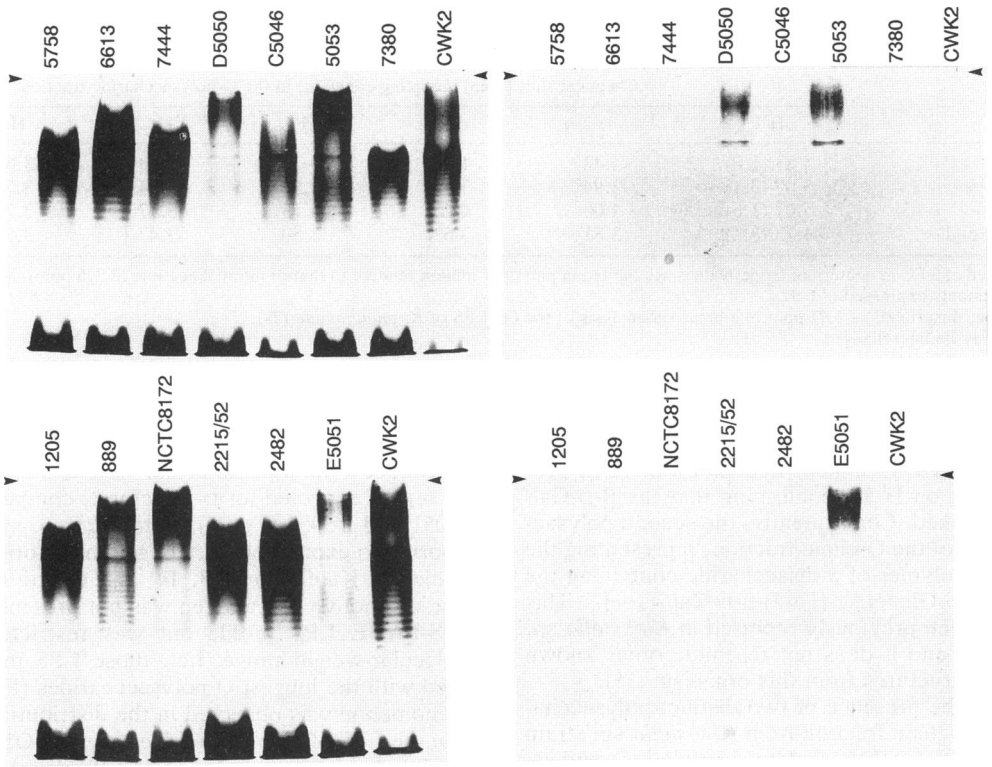

FIG. 2. Distribution of 2c epitopes in *Klebsiella* sp. isolates. LPS samples were analyzed by SDS-PAGE (left panels) and by Western blotting against MAb O2-67.1 (right panels). The strain designation is indicated above each lane, and details of the different serotypes are given in Table 1. Arrowheads indicate the tops of the resolving gels.

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