N-3-Hydroxypropionyl- α -D-perosamine homopolymer constituting the O-chain of lipopolysaccharides from *Vibrio* bioserogroup 1875 possessing antigenic factor(s) in common with O1 *Vibrio cholerae*

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A structural study was performed by ^{13}C -n.m.r. spectroscopy and methylation analysis of the O-chain of lipopolysaccharide (LPS) from *Vibrio* bioserogroup 1875 possessing antigenic factor(s) in common with O1 *Vibrio cholerae*. It was demonstrated to contain a linear homopolymer of (1 $\rightarrow$ 2)-linked N-3-hydroxypropionyl- α -D-perosamine [4-(3-hydroxypropanamido)-4,6-

dideoxy- α -D-mannopyranose], which is very similar to, but not identical with, both (1 $\rightarrow$ 2)-linked linear N-3-deoxy-L-glycero-tetronyl(S-2,4-dihydroxybutyryl)- α -D-perosamine homopolymer and (1 $\rightarrow$ 2)-linked linear N-acetyl- α -D-perosamine homopolymer which constitute the O-chains of O1 *V. cholerae* and non-O1 *V. cholerae* bioserogroup Hakata LPS respectively.

INTRODUCTION

In recent years, three groups of non-cholera vibrio possessing antigenic factor(s) in common with O1 *Vibrio cholerae* have been isolated in Japan, i.e. *Vibrio* bioserogroup 1875 [1], *Vibrio fluvialis* Kobe [2] and non-O1 *V. cholerae* bioserogroup Hakata [3]. Isolation and chemical as well as serological characterizations, in particular, compositional sugar analysis, of their lipopolysaccharides (LPS) were carried out by our group [4–7]. Of particular interest is the fact that the O-chain of LPS from non-O1 *V. cholerae* Hakata contains a linear (1 $\rightarrow$ 2)-linked N-acetyl- α -D-perosamine (4-acetamido-4,6-dideoxy- α -D-mannopyranose) homopolymer [7] which is very similar to, but not identical with, a linear (1 $\rightarrow$ 2)-linked N-3-deoxy-L-glycero-tetronyl(S-2,4-dihydroxybutyryl)- α -D-perosamine homopolymer which constitutes the O-chain of O1 *V. cholerae* LPS [8,9].

O1 *V. cholerae* O-antigen consists of three antigenic factors: group somatic antigen A, Ogawa antigen factor B and Inaba antigen factor C [10,11]. *Vibrio* bioserogroup 1875 (referred to as 1875 Original) was isolated from estuarine seawater in Osaka Bay, Japan. The O-antigen of this group is composed of at least its own antigen factor (referred to as D) and the B and a small quantity of the C factors of O1 *V. cholerae* [1]. An antigenic variation similar to that found in O1 *V. cholerae* occurred in 1875 Original resulting in a variant (referred to as 1875 Variant) which possessed only factor C in addition to factor D [1].

In the present study, an unknown sugar, previously named NS II [4] and found in LPS of 1875 Original and its Inaba variant (1875 Variant), was identified as N-3-hydroxypropionyl-D-perosamine [4-(3-hydroxypropanamido)-4,6-dideoxy-D-mannopyranose]. Furthermore, the occurrence of a (1 $\rightarrow$ 2)-linked linear homopolymer of N-3-hydroxypropionyl- α -D-perosamine was demonstrated for the first time in the O-chain of both 1875 Original and Variant.

MATERIALS AND METHODS

Bacterial strains and LPS

Strains of *Vibrio* bioserogroup 1875 Original and Variant were provided by Dr. T. Shimada and Dr. R. Sakazaki, National Institute of Health, Tokyo, Japan. The bacteria were cultivated in nutrient broth supplemented with 3% (w/v) NaCl (pH 7.2) at 28 °C for 16 h with vigorous aeration. After being killed by heating at 120 °C for 20 min, the cells were collected by centrifugation (15000 g), washed with distilled water and acetone-dried. LPS was extracted from the acetone-dried cells by the hot phenol/water technique [12] and purified by repeated ultracentrifugation (105000 g, 3 h) and RNAase treatment as described previously [4].

Isolation of the O-chain of LPS

LPS (800 mg) was hydrolysed in 5% (v/v) acetic acid at 100 °C for 2 h, and precipitated free lipid A was removed by centrifugation (12000 g, 15 min). The supernatant was, after evaporation to dryness, subjected to gel chromatography on a column (2.6 cm $\times$ 100 cm) of Sephadex G-50 (Pharmacia, Uppsala, Sweden) which was eluted with water. The eluate was monitored by refractometry (RID-6A, Shimadzu, Kyoto, Japan), and fractions (5 ml) were collected. Fractions eluted in the first peak were combined and freeze-dried yielding O-chain [4] (330 mg and 384 mg from 1875 Original and Variant LPS respectively).

Deacylation and N-acetylation of O-chain

O-chain was deacylated by the method of DiFabio *et al.* [13] using thiophenol at 100 °C for 20 h. The reaction mixture was neutralized with 2 M HCl, dialysed against water, N-acetylated [14] and freeze-dried.

Isolation and purification of the unknown sugar, NS

For isolation and purification of NS, the O-chain (100 mg) isolated from 1875 Original LPS was hydrolysed in 10 M HCl (2 ml) at 90 °C for 15 min, and the hydrolysate was diluted with 10 ml of water and then evaporated to dryness. The residue was dissolved in water (5 ml) and passed through a column (1.2 cm × 20 cm) of Dowex 50 (H⁺) (The Dow Chemical Co.), the resin being washed with water (200 ml). The non-adsorbed materials were evaporated to dryness, acetylated in 2 ml of pyridine/acetic anhydride (1:1, v/v) at 100 °C for 40 min and dried under a flow of N₂. The product was dissolved in 2 ml of benzene/acetone (20:1, v/v) and partially purified using a Kieselgel 60 (Merck, Darmstadt, Germany) column (1.2 cm × 20 cm) by elution with 100 ml of benzene/acetone (1:1, v/v), yielding acetylated NS (70.2 mg). The acetylated NS was further purified by h.p.l.c. using a column of Nucleosil (50 µm; 2 cm × 25 cm; Chromato Packing Center, Kyoto, Japan) and the solvent system benzene/acetone (10:1, v/v; 8 ml/min). The eluate (5 ml fractions) was analysed by t.l.c. on Kieselgel 60 F₂₅₄ (Merck) using benzene/acetone (2:1, v/v). Two substances were obtained which co-migrated on t.l.c. ($R_F = 0.42$) but were distinguishable by g.l.c. (temperature programme A, see below). The first material eluted with a retention time $t = 16.83$ min is referred to as NS1 (5.0 mg) and the second one with retention time $t = 17.44$ min as NS2 (4.9 mg).

Methylation analysis of O-chain

O-chain (2 mg) was methylated by the method of Hakomori [15]. The methylated O-chain was treated with 1 ml of anhydrous HF [16] at room temperature for 3 h. After complete removal of HF under reduced pressure, the residue was reduced with NaBH₄ and acetylated by the method of Stelner et al. [17].

Analytical methods

Neutral sugars were estimated by g.l.c. as their alditol acetates [18] after hydrolysis in 2 M trifluoroacetic acid at 120 °C for 1 h. Heptose was also estimated by the colorimetric method of Osborn [19]. Amino sugars, except for perosamine, were analysed by g.l.c. as their *N*-acetylditol acetates [20]. For estimation of perosamine and NS, samples were subjected to solvolysis with anhydrous HF at room temperature for 3 h. After removal of HF the products were reduced with NaBH₄ at room temperature for 16 h and acetylated with pyridine/acetic anhydride (1:1) at 100 °C for 30 min. The perosamine and NS contents were determined on the basis of their peak areas compared with that of xylose used as an internal standard. 3-Deoxy-D-manno-2-octulosonate was determined by the periodate/thiobarbituric acid reaction as described by Weissbach and Hurwitz [21]. The absolute configuration of perosamine was determined using the de-*N*-acylated and *N*-acetylated O-chain by g.l.c. and g.l.c.-m.s. of its acetylated (*S*)-(+)- and (*R*)-(-)-*sec*-butyl glycosides prepared by the method of Gerwig et al. [22]. *V. cholerae* NIH 41 (Ogawa) LPS was used as a source of standard D-perosamine [8].

G.l.c. and g.l.c.-m.s.

G.l.c. was carried out on a Shimadzu 14A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a fused silica capillary column (0.25 mm × 35 m) coated with HR52 (Chromato Packing Center), with temperature programmes of (A) 200 °C (3 min)

increased to 300 °C at 5 °C/min, (B) 150 °C (3 min) increased to 300 °C at 5 °C/min and (C) 150 °C (3 min) increased to 240 °C at 3 °C/min. G.l.c.-m.s. was performed on a DX-300 (Nihon Denshi, Tokyo, Japan) instrument using the column described above. Electron-impact m.s. (e.i.-m.s.) was recorded at 70 eV, and isobutane was used as reactant gas in chemical ionization m.s. (c.i.-m.s.).

N.m.r. spectroscopy

¹H- (400 MHz) and ¹³C- (100.6 MHz) n.m.r. spectra were recorded on a Varian VRX400S spectrometer in benzene-²H₆ (for NS) or ²H₂O (for O-chain). Tetramethylsilane (0.00 p.p.m.) was used as an internal standard for calibration of the chemical shifts of NS. The chemical shifts of O-chain were calibrated relative to that of HO²H (4.65 p.p.m. for ¹H-n.m.r.) and internal dioxane (67.40 p.p.m. for ¹³C-n.m.r.). Assignments were made by ¹H, ¹H homonuclear and ¹H, ¹³C heteronuclear shift-correlated n.m.r. spectroscopy (COSY)-n.m.r. experiments. The anomeric configuration was determined by gated ¹³C-n.m.r. experiments.

RESULTS

Sugar composition of O-chain

Table 1 shows the sugar composition of the O-chain isolated from 1875 Original and Variant LPS. The two O-chains revealed quite similar (qualitatively and quantitatively) sugar compositions; they contained glucose, mannose, *L*-glycero-D-mannoheptose, glucosamine, quinovosamine, perosamine and NS. 3-Deoxy-D-manno-2-octulosonate was not detectable in either O-chain by the usual periodate/thiobarbituric acid reaction [21]. As we reported previously [4], hydrolysis of the O-chain of 1875 strains in HCl or trifluoroacetic acid yielded perosamine and two kinds of unknown sugars (formerly referred to as NS I and NS II). However, when the O-chains were treated with anhydrous HF, very small amounts of perosamine and only one unknown sugar (NS) were detected. G.l.c. and g.l.c.-m.s. of NS revealed the retention time, e.i.- and c.i.-mass spectra which were indistinguishable from those obtained for NS II. The sugar compositions of these O-chains except for perosamine and NS were almost identical with that reported previously [4].

Identification of NS

HF solvolysis of the O-chain of 1875 Original after de-*N*-acylation with thiophenol and *N*-acetylation released large amounts (1.5 µmol/mg) of perosamine and a very small amount (less than 0.01 µmol/mg) of NS. No peak corresponding to NS I was observed by g.l.c. even after hydrolysis of the de-*N*-acylated and *N*-acetylated O-chain in 10 M HCl by which NS I was produced. These results together with the fact that NS I was not detectable in the O-chain after HF solvolysis, as stated above, suggested that the previously reported NS I is an artifact produced by acid hydrolysis of the O-chain and also that NS is perosamine carrying an *N*-acyl group.

To determine the chemical structure of NS, purified NS1 and NS2 were examined by ¹H- and ¹³C-n.m.r. spectroscopy. The ¹H-n.m.r. spectra (Table 2) of peracetylated NS1 and NS2 contained the signals of pyranose ring protons at the chemical shifts shown and a 5-methyl group. The $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$ values (3.30, 10.99 and 9.77 Hz respectively) determined for NS1 indicated that it has the mannopyranosyl configuration. Similarly, NS2 was also shown to have the same configuration. As significant additional signals, an amide proton, cross-coupling with H-4 in two-dimensional n.m.r., was observed at 4.58 p.p.m. (NS1) and

Table 1 Sugar composition of O-chain isolated from *Vibrio* bioserogroup 1875 Original and Variant LPSValues are expressed as $\mu\text{mol/mg}$.

O-chain from 1875	Glucose	Mannose	L-Glycero-D-mannoheptose	Glucosamine	Quinovosamine	Perosamine	NS*
Original	0.12	0.12	0.24	0.13	0.12	0.03	1.67
Variant	0.17	0.11	0.30	0.18	0.13	0.03	1.98

* Identified as *N*-3-hydroxypropionyl-D-perosamine and estimated on the basis of its M_r .**Table 2** ^1H -n.m.r. (400 MHz, in C_6D_6) data of peracetylated NS1 and NS2 isolated from *Vibrio* bioserogroup 1875 Original O-chain of its LPSOther signals: OCOCH_3 , 1.56–1.74 p.p.m.

Compound	Chemical shift (p.p.m.)*									Coupling constant (Hz)								
	H-1	H-2	H-3	H-4	H-5	H-6	H-2'	H-3'	NH	H-1	H-2	H-3	H-4	H-5	H-6	H-2'	H-3'	NH
NS1	6.37	5.45	5.28	4.56	3.63	1.23	1.98	4.23	4.58	2.20	3.30	10.99	9.77	6.23	6.23	6.23	6.23	—†
NS2	5.71	5.57	4.80	4.47	2.95	1.23	1.98	4.21	4.55	1.10	3.12	10.80	9.88	6.23	5.86	6.23	6.23	9.52

* Spectra were measured at 25 °C.

† Not measured.

4.55 p.p.m. (NS2), and two kinds of methylene protons (one of which was hydroxylated) derived from the *N*-acyl group were also detected (4.23 and 1.98 p.p.m. for NS1 and 4.21 and 1.98 p.p.m. for NS2 respectively). The $J_{\text{C-1-H-1}}$ value (176.25 Hz) obtained by a gated ^{13}C -n.m.r. spectrum of NS1 showed the anomeric configuration to be α and that (161.75 Hz) of NS2 to be β . These results indicate that NS1 is 4(3-hydroxypropanamido)-4,6-dideoxy- α -mannopyranose (*N*-3-hydroxypropionyl- α -perosamine) and NS2 is the β -anomer. This was also supported by the ^{13}C -n.m.r. data in which C-1 to C-6 signals were observed for NS1 and NS2 at 91.41 and 90.95 p.p.m. (C-1), 70.75 and 73.00 p.p.m. (C-5), 68.91 and 71.01 p.p.m. (C-3), 68.53 and 68.52 p.p.m. (C-2), 51.09 and 50.94 p.p.m. (C-4) and 17.95 and 17.82 p.p.m. (C-6) respectively. The signals originating from the *N*-acyl group (3-hydroxypropionyl) of NS1 and NS2 were observed at 167.63 and 168.32 p.p.m. (C-1'), 60.63 and 60.62 p.p.m. (C-3') and 36.06 and 36.06 p.p.m. (C-2') respectively.

The acetylated (*S*)-(+)- and (*R*)-(–)-butyl glycosides of perosamine prepared from the O-chain of 1875 Original and Variant gave retention times (t_r = 19.63 and 19.76 min respectively, temperature programme C) and e.i.-mass spectra (results not shown) identical with those obtained for the respective derivatives of D-perosamine isolated from O1 *V. cholerae* NIH 41 (Ogawa) LPS [8], indicating that the perosamine present in the O-chain of 1875 Original and Variant had the D configuration. On the basis of the results described above, NS was identified as *N*-3-hydroxypropionyl-D-perosamine.

Table 3 shows the results of g.l.c.–m.s. of the various derivatives prepared from purified NS1 shown in Figure 1, i.e. fully acetylated (A), reduced and acetylated (B), fully methylated (C) and reduced and methylated (D). The M_r values (403, 447, 305 and 321 for A, B, C and D respectively) estimated by g.l.c.–m.s. (c.i. mode) were in good agreement with those calculated for the respective derivatives. In e.i.-mass spectra of each derivative, the fragment ions (m/z 115 for A and B, m/z 87 for C and D) corresponded to 3-hydroxypropionyl residues of each derivative. The fragment

Table 3 G.l.c.–m.s. data of derivatives A, B, C and D of *N*-3-hydroxypropionyl-D-perosamine

Derivative	M_r^*	Characteristic fragments (Intensity, %) [†]
A	403	72 (68.72), 100 (39.0), 115 (58.2), 126 (25.3), 153 (41.1), 173 (57.4), 186 (100.0), 202 (11.9), 212 (20.4), 215 (21.6), 228 (40.6), 241 (17.7), 257 (8.6), 271 (7.3), 284 (6.4), 300 (7.3), 316 (1.4), 344 (1.4)
B	447	84 (30.6), 110 (100.0), 115 (24.3), 126 (14.6), 132 (12.7), 138 (18.3), 152 (10.7), 165 (18.4), 170 (7.8), 180 (18.3), 186 (12.3), 198 (10.1), 225 (6.2), 230 (2.0), 240 (15.0), 258 (8.2), 300 (20.8), 318 (1.77), 360 (5.5)
C	305	71 (37.9), 87 (73.7), 88 (100.0), 101 (16.7), 118 (16.0), 125 (14.0), 128 (15.8), 142 (7.8), 156 (27.0), 157 (12.2), 173 (22.1), 186 (10.3), 214 (11.0), 230 (1.9), 242 (5.8), 274 (7.7), 290 (1.8)
D	321	88 (43.1), 89 (27.6), 102 (100.0), 142 (6.3), 144 (6.4), 156 (2.8), 174 (99.0), 188 (38.0), 200 (7.8), 230 (4.9), 232 (10.8), 262 (34.4)

* Determined by c.i.-m.s. on the basis of peaks at m/z for ($M+1$)⁺.

† Estimated by e.i.-m.s. at 70 eV.

ion at m/z 228 (A) was assigned to the fragment produced by loss of acetic acid (60) from the perosamine residue (m/z 288) of A. The fragment ions m/z 360 ($\rightarrow$ 300) and 230 ($\rightarrow$ 170) detected for B were assigned to C-1–C-4 and C-4–C-6 fragments respectively, both of which carried a 3-hydroxypropionyl residue on its amino group. The fragment ion m/z 186 (C) was assigned to the fragment derived by loss of methanol (32) from the perosamine residue (m/z 218) of C. The fragment ions at m/z 262 ($\rightarrow$ 230) and 174 ($\rightarrow$ 142) (D) were assigned to C-1–C-4 and C-4–C-6 fragments respectively of D. The M_r values and e.i.-mass spectra obtained for the corresponding derivatives prepared from NS2 were identical with those obtained for A, B, C and D, confirming

Figure 1 Structures and partial fragmentation patterns of (A) peracetylated, (B) reduced and peracetylated, (C) permethylated and (D) reduced and permethylated derivatives of *N*-3-hydroxypropionyl- α -D-perosamine isolated from O-chain of *Vibrio* bioserogroup 1875 Original LPS

that NS2 is the β -isomer of NS1. These results were compatible with those obtained by ^1H and ^{13}C n.m.r.

Since acid treatment (10 M HCl, 90 °C, 15 min) of purified NS1 and NS2 gave NS I, which was formerly reported [4] to give a high yield (approx. 70 %), and it is known [23,24] that 4-amino sugars are unstable, forming ring structures involving C-1 and the amino group and/or *N*-acyl group, if present, we concluded that NS I is an artifact produced by acid hydrolysis of the O-chain, although its structure has not been elucidated.

Structural analysis of the O-chain

The high content of perosamine (*N*-3-hydroxypropionylated) in the O-chain of 1875 Original and Variant suggested the presence of perosamine homopolymer in both O-chains. As expected, nine strong signals were observed in ^{13}C -n.m.r. spectra (Table 4) of 1875 Original and Variant O-chain, indicating the presence of a simple regular structure in the O-chain. The chemical shifts in the spectra were closely similar to those observed for the O-chain consisting of a linear (1 $\rightarrow$ 2)-linked homopolymer of *N*-acetyl- α -

Figure 2 Structure of (1 $\rightarrow$ 2)-linked *N*-3-hydroxypropionyl- α -D-perosamine homopolymer constituting the O-chain of *Vibrio* bioserogroup 1875 Original and Variant LPS

The homopolymer consists of 15–20 residues of *N*-3-hydroxypropionyl- α -D-perosamine.

D-perosamine [7] which was found in *V. cholerae* bioserogroup Hakata LPS or prepared by the selective *N*-acetylation of *N*-deacetylated O-chain of O1 *V. cholerae*. Similar spectra were also reported [8,9] for O-chain isolated from O1 *V. cholerae*. In the ^{13}C -n.m.r. spectra of 1875 Original O-chain, the shifted resonance signal of C-2 (78.2 p.p.m.) to lower field compared with that (around 69.64–70.96 p.p.m.) observed for unsubstituted methyl glycosides of α -D-perosamine carrying various *N*-acyl groups [25] indicated the 2-substitution of perosamine residues in the O-chain. Only one significant signal at 101.8 p.p.m. detected in the anomeric region and the $J_{\text{C-1-H-1}}$ value of 173.2 Hz estimated in a gated ^{13}C -n.m.r. spectrum showed the presence of a single type of α -glycosyl linkage of the perosamine residues. Furthermore, the signals originating from the *N*-acyl group (3-hydroxypropionyl) were detected at 176.2 p.p.m. (C-1'), 59.1 p.p.m. (C-3') and 40.0 p.p.m. (C-2'). An almost identical result was obtained by ^{13}C -n.m.r. for 1875 Variant O-chain (Table 4). These results indicate that a (1 $\rightarrow$ 2)-linked *N*-3-hydroxypropionyl- α -D-perosamine homopolymer (Figure 2) was present in the O-chain of 1875 Original and Variant. The following ^1H -n.m.r. data of the O-chain of 1875 Original and Variant confirm the structure. ^1H -n.m.r. data of 1875 Original O-chain: 5.08 p.p.m. (1 H, broad s, $J_{1,2}$ low, H-1), 4.07 p.p.m. (1 H, broad d, $J_{2,3}$ low, H-2), 4.00 p.p.m. (1 H, broad d, $J_{3,4}$ 10.26 Hz, H-3), 3.87 p.p.m. (1 H, t, $J_{4,5}$ 10.07 Hz, H-4), 3.78 p.p.m. (3 H, m, H-5 and H-3'), 2.46 p.p.m. (2 H, t, $J_{2,3}$ 5.86 Hz, H-2') and 1.11 p.p.m. (3 H, d, $J_{6,5}$ 5.86 Hz, H-6). ^1H -n.m.r. data of 1875 Variant O-chain: 5.06 p.p.m. (1 H, broad s, $J_{1,2}$ low, H-1), 4.05 p.p.m. (1 H, broad

Table 4 ^{13}C -n.m.r. (100.6 MHz, in $^2\text{H}_2\text{O}$) data of O-chain isolated from *Vibrio* bioserogroup 1875 Original and Variant LPS

Origin of O-chain	Chemical shift (p.p.m.)*									Coupling constant ($J_{\text{C-1-H-1}}$, Hz)
	C-1	C-2	C-3	C-4	C-5	C-6	C-1'	C-2'	C-3'	
Original	101.8	78.2	69.0	54.3	69.7	18.1	176.2	40.0	59.1	173.2
Variant	101.7	78.3	69.0	54.3	69.7	18.1	176.2	40.0	59.1	173.9

* Spectra were measured at 25 °C.

s, $J_{2,3}$ low, H-2), 3.98 p.p.m. (1 H, broad d, $J_{3,4}$ 10.98 Hz, H-3), 3.85 p.p.m. (1 H, t, $J_{4,5}$ 10.08 Hz, H-4), 3.75 p.p.m. (3 H, m, H-5 and H-3'), 2.43 p.p.m. (2 H, t, $J_{2,3}$ 5.49 Hz, H-2') and 1.09 p.p.m. (3 H, d, $J_{6,5}$ 5.49 Hz, H-6).

The (1→2) linkage of the perosamine residues in the O-chain of 1875 Original and Variant was further confirmed by methylation analysis of the O-chain. G.l.c. and g.l.c.-m.s. of the products obtained from the O-chain of 1875 Original and Variant showed the presence of a large amount of 1,2,5-tri-*O*-acetyl-3-*O*-methyl-4-*N*-[methoxy(3-*O*-methyl)propanamido]-4,6-dideoxymannitol derived from 2-substituted *N*-3-hydroxypropionylperosamine and a small amount of 1,5-di-*O*-acetyl-2,3-di-*O*-methyl-4-*N*-[methoxy(3-*O*-methyl)propanamido]-4,6-dideoxymannitol derived from *N*-3-hydroxypropionylperosamine at the non-reducing terminus. The amount of 2-substituted perosamine relative to that of the terminal one was calculated to be 15:1 and 14:1 for 1875 Original and Variant O-chain respectively, based on the peak areas in g.l.c. These results and that obtained by compositional analysis of the O-chain (Table 1) demonstrated that the O-chain of 1875 Original and Variant comprised a (1→2)-linked 3-hydroxypropionyl- α -D-perosamine homopolymer consisting of approximately 15–20 perosamine residues (Figure 2).

DISCUSSION

In the present study, a structural analysis has been carried out on the O-chain of LPS isolated from *Vibrio* bioserogroup 1875 Original and Variant which possess antigen factor(s) in common with *V. cholerae*. The unknown sugar NS, which was previously described (NS II) [4] as being present in the O-chain of 1875 Original and Variant, has been identified as *N*-3-hydroxypropionyl-D-perosamine. NS I, which was also considered to be a constituent of the O-chain, has been shown to be an artifact produced by acid hydrolysis of the O-chain, because NS I was not detectable in the products obtained by HF solvolysis of the O-chain and, moreover, the acid hydrolysis of purified NS1 and NS2 produced NS I in high yield.

Structural analysis by means of n.m.r. and methylation analysis demonstrated that the O-chain of 1875 Original and Variant LPS comprised a (1→2)-linked *N*-3-hydroxypropionyl- α -D-perosamine homopolymer. No structural difference was found in the O-chains of the two strains examined in the present study. This structure is closely related to, but not identical with, that of O-specific polysaccharide portions of LPS isolated from O1 *V. cholerae* [8,9] and non-O1 *V. cholerae* bioserogroup Hakata [7] which consists of a (1→2)-linked *N*-3-deoxytetronyl(*S*-2,4-dihydroxybutyryl)- α -D-perosamine homopolymer and an *N*-acetyl- α -D-perosamine homopolymer respectively, and also to that of *Yersinia enterocolitica* O9 [26] and *Brucella abortus* [27], both of which consist of an *N*-formyl- α -D-perosamine homo-

polymer. 1875 Original possesses Ogawa antigen factor B and Inaba antigen factor C of O1 *V. cholerae*, and its Inaba variant 1875 Variant possesses Inaba antigen factor C. It is also known that non-O1 *V. cholerae* bioserogroup Hakata [3], *Y. enterocolitica* O9 [28] and *B. abortus* [29] share Inaba antigen factor C with O1 *V. cholerae*. The serological cross-reactivities with O1 *V. cholerae* expressed by these bacteria may be attributed to the similarities of the O-chains of their LPS which are composed of (1→2)-linked α -D-perosamine carrying different *N*-acyl groups.

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