Biochemistry (Moscow), Vol. 70, No. 9, 2005, pp. 1046-1054. Translated from Biokhimiya, Vol. 70, No. 9, 2005, pp. 1267-1276.

Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM04-266, February 20, 2005.

Anionic Polymers of the Cell Wall of *Brevibacterium linens* VKM Ac-2159

N. V. Potekhina[1]*, A. S. Shashkov[2], G. M. Streshinskaya[1], S. N. Senchenkova[2], and L. I. Evtushenko[3]

[1]Faculty of Biology, Lomonosov Moscow State University, 119992 Moscow, Russia; fax: (7-095) 939-4309; E-mail: potekchina@hotbox.ru
[2]Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky pr. 47, 117913 Moscow, Russia; fax: (7-095) 135-5328
[3]All-Russian Collection of Microorganisms (VKM), Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, 142292 Pushchino, Moscow Region, Russia; fax: (7-095) 923-3602

Received September 15, 2004
Revision received November 15, 2004

Abstract—Unsubstituted 1,3-poly(glycerol phosphate) and two sugar-1-phosphate polymers were identified in the cell wall of *Brevibacterium linens* VKM Ac-2159 by NMR spectroscopy and chemical methods. A monomer of one of the sugar-1-phosphate polymers has the branched repeating unit of the following structure: -4)-[β-D-Glc*p*NAc-(1→3)]-α-D-Glc*p*-(1-*P*-. The repeating unit of another sugar-1-phosphate polymer has a linear structure consisting of alternating β- and α-N-acetylglucosamine residues: -4)-β-D-Glc*p*NAc-(1→6)-α-D-Glc*p*NAc-(1-*P*-. Some part of the β-N-acetylglucosaminyl residues bear *O*-ester-bound succinic acid residues at C-3. The identified sugar-1-phosphate polymers have not been described earlier in cell walls of other bacteria.

Key words: *Brevibacterium*, teichoic acids, sugar-1-phosphate polymer, succinic acid, NMR spectroscopy

Data on the cell wall teichoic acids of *Brevibacterium linens* were first reported by Fiedler et al. [1, 2]. This species was shown to be genetically heterogeneous and to include strains different in the content of teichoic acids. As found by chemical analysis of the cell wall of a typical strain of *B. linens* ATCC 9172, it contained glycerol teichoic acid substituted by α-glucosyl and α-glucosaminyl residues [1]; however, the structure of this polymer was not determined completely. Recently we reported data on the simultaneous presence of two teichoic acids—1,3-poly(glycerol phosphate) and poly(glycosylglycerol phosphate)—in the cell wall of *B. linens* VKM Ac-2119 and *B. linens* VKM Ac-2120 (=Gk-3) [3, 4].

The goal of the present work was to study anionic polymers of *B. linens* VKM Ac-2159 earlier identified by morphological and physiological properties. The VKM Ac-2159 strain is shown to contain two sugar-phosphate polymers along with 1,3-poly(glycerol phosphate), and thus it significantly differs from other strains of this species studied earlier.

MATERIALS AND METHODS

Brevibacterium linens VKM Ac-2159 culture was grown as described by Naumova et al. [5]. The cell wall preparation was obtained by cell disintegration using a UZDN-1 ultrasonic disintegrator as described in [6]. Chromatography and electrophoresis were performed according to [3]. Succinic acid was identified as described in [7]. Teichoic acids were extracted from the cell wall with 10% TCA (1 : 10 w/v) at 4°C. After 24 h, the mixture was centrifuged and cell walls were extracted once more under the same conditions. The supernatants were pooled, and the carbohydrate-containing fractions were precipitated with two volumes of ethanol (fraction I). The

* To whom correspondence should be addressed.

0006-2979/05/7009-1046

precipitate was isolated by centrifugation at 1000 rpm for 15 min, and the supernatant was precipitated by two more volumes of ethanol (fraction II). The precipitates were dissolved in water, and their solutions were dialyzed against distilled water and lyophilized.

Reagents for detection of the products of teichoic acids degradation and conditions for acidic hydrolysis of the cell wall and anionic polymers are described in [3].

Oligomers were separated on a column (32 × 150 mm) with TSK 40 S gel in 1% AcOH; elution was monitored using a differential refractometer from Knauer (Germany). NMR spectra were recorded for solutions in 99.92% D_2O at 303 K using acetone as internal standard (δ_H 2.225 ppm and δ_C 31.45 ppm for ^1H and ^{13}C, respectively) and 80% H_3PO_4 as external standard (δ_P 0.0 ppm for ^{31}P). The 2D NMR spectra were recorded using a DRX-500 spectrometer from Bruker (Germany) according to the standard Bruker procedures. The spin lock time for TOCSY was 0.2 sec and mixing time for ROESY 0.1 sec. The $^1H/^{13}C$ HMBC and $^1H/^{31}P$ HMQC spectra were optimized for spin-coupling constants $J_{H,C}$ 8 Hz and $J_{H,P}$ 10 Hz.

RESULTS AND DISCUSSION

Glucose, glycerol, and also glycerol mono- and diphosphates and inorganic phosphate were detected in the acidic hydrolyzates of *B. linens* VKM Ac-2159 cell walls; this indicates the possible presence of glycerol teichoic acid in the cell wall.

The two preparations of carbohydrate-containing fractions isolated from the cell wall differed in content. Glycerol mono- and diphosphates, glucose and glucosamine were detected in the acidic hydrolyzates of the first fraction. Along with hydrolyzates typical of the first fraction, succinic acid was present in the second fraction. In addition, somewhat larger quantities of glucosamine were also revealed.

Both preparations were studied by NMR spectroscopy.

Fraction I. In the ^{13}C-NMR spectrum of polymeric fraction I (Fig. 1) there were two intense signals in the resonance area of anomeric carbon atoms (δ 102.7 and 96.6), two signals of unsubstituted hydroxymethyl groups of pyranoses (δ 62.3 and 61.8), a signal of the carbon atom bound to a nitrogen atom (δ 57.4), and signals of CH_3CON groups (δ 176.0 and 23.5). The data indicated the presence of a regular polymer containing two pyranose residues, one of them being 2-acetamido-2-deoxypyranose. Two intense signals (δ 68.0 and 71.0) typical of 1,3-poly(glycerol phosphate) (polymer 1) were also detected in the spectrum of this fraction along with the minor signals.

The presence of at least two polymers in fraction I was proved by the ^{31}P-NMR spectrum with two main sig-

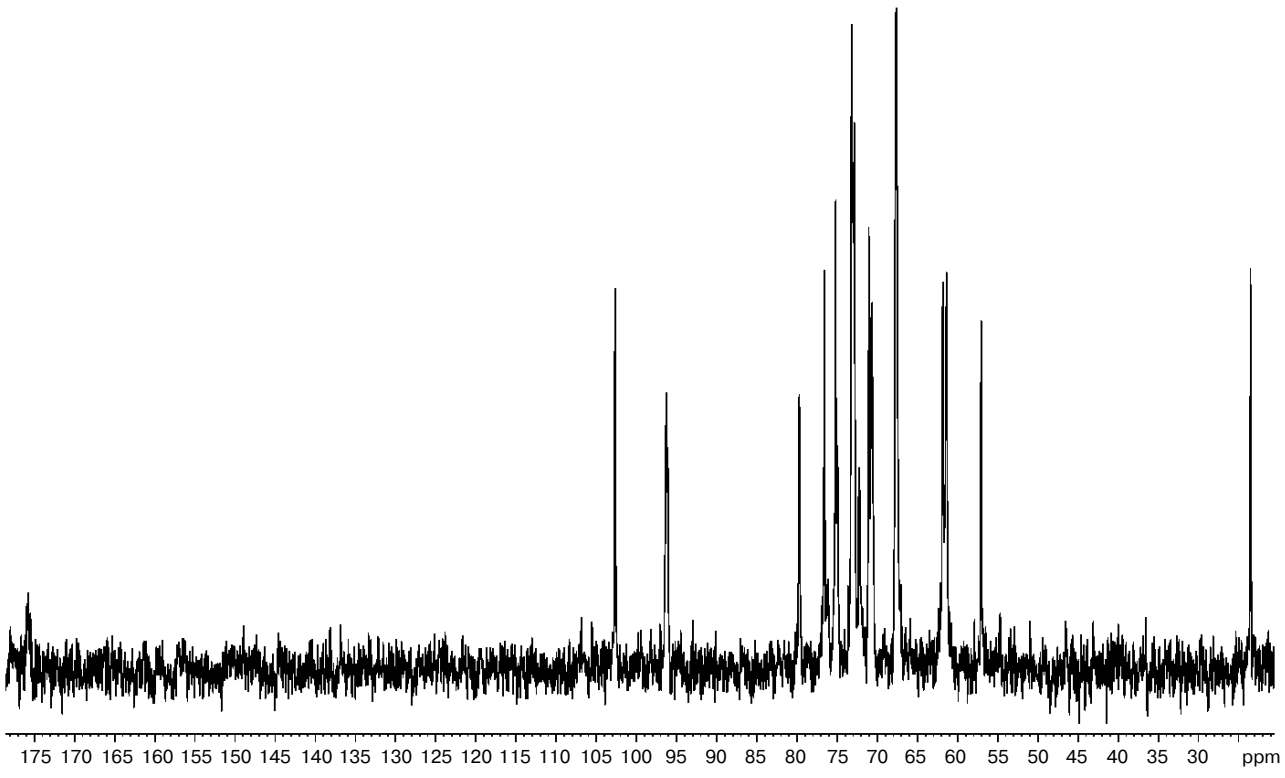


Fig. 1. ^{13}C-NMR spectrum of fraction I.

Table 1. ^{13}C-NMR spectra (125 MHz) of cell wall polymers of *B. linens* VKM Ac-2159 (δ, ppm)[a]

Residue	Chemical shift					
	C-1	C-2	C-3	C-4	C-5	C-6
Polymer 1						
-1)-sn-Gro-(3-*P*-	68.0	71.0	68.0			
Polymer 2						
-4)-α-D-Glc*p*-(1-*P*- (A)	96.6	73.45	80.1	73.5	73.5	61.8
3) ↑						
β-D-Glc*p*NAc-(1	102.7	57.4	75.7	71.5	77.0	62.3
-4)-α-D-Glc*p*-(1-*P*- (B)	96.4	72.6	73.5	75.3	73.5	61.9
Fragments of oligomers of polymer 2						
-4)-α-D-Glc*p*-(1-*P*-	96.4	73.5	80.2	73.5	73.3	61.7
3) ↑						
β-D-Glc*p*NAc-(1	103.1	57.3	75.5	71.3	76.9	62.2
-4)-α-D-Glc*p*-(1-*P*-	96.4	72.6	73.5	75.3	73.5	61.9
-*P*-4)-D-Glc*p*-OH (α) (C)	93.4	72.8	73.5	75.3	73.2	61.7
(β)	97.4	75.5	76.4	75.6	76.7	61.9
-*P*-4)-D-Glc*p*-OH (α) (D)	93.35	73.5	80.2	73.5	72.3	61.7
3) (β)	97.5	78.5	82.7	73.5	76.7	62.0
↑						
β-D-Glc*p*NAc-(1	102.9[α] 102.8[β]	57.4	75.5	71.3	77.0	62.2
Polymer 3						
→6)-α-D-Glc*p*NAc-(1-*P*-	95.9	55.1	72.25	70.8	73.3	69.2
-4)-β-D-Glc*p*NAc-(1→	103.0	56.8	74.45	75.7	76.5	61.9
→6)-α-D-Glc*p*NAc-(1-*P*-	95.9	55.1	72.25	70.8	73.3	69.2
-4)-β-D-Glc*p*NAc-(1→	103.2	55.3	75.5	73.3	76.6	61.9
3)		(−1.5)[b]	(+1.1)	(−2.4)		
|						
Suc	n.i.	30.7	30.0	n.i.		

Note: n.i., signal is not identified.

[a] Signals for NAc at δ 23.3-23.7 (Me) and 175.7-176.1 (CO).

[b] The acylation effect is given in brackets.

[α,β] For C-1 β-D-Glc*p*NAc glycosylating α- or β-D-Glc*p*-OH, respectively.

nals at δ −1.1 and +0.3. A minor signal at δ −0.6 was also present in the spectrum.

In the ^{1}H-NMR spectrum of freshly precipitated fraction I there were two main signals at δ 5.49 and 4.86 in the resonance area of the protons at the anomeric carbon atoms. Minor signals at δ 4.6-5.3 and δ 5.52 were also observed. The ^{1}H-NMR spectrum was partly attributed by the 2D ^{1}H/^{1}H COSY, TOCSY, ROESY, and ^{1}H/^{31}P HMQC spectra. Analysis of spectra demonstrated that some of the most intense signals belong to α-glucopyranose and 2-acetamido-2-deoxy-β-glucopyranose residues. The ^{1}H/^{31}P HQMC spectrum indicated correlations of the anomeric protons of the α-Glc*p* residue and H-4 protons of the same residue with the phosphorus atoms resonating at δ −1.1. In the ROESY spectrum an inter-residual correlation peak H-1 β-Glc*p*NAc with H-3 α-Glc*p* was observed. Based on the data, we concluded that a sugar-phosphate polymer (polymer 2) with branched repeating unit of the following structure was present in fraction I:

-4)-α-D-Glc*p*-(1-*P*-
3)
↑
β-D-Glc*p*NAc-(1 .

The structure of repeating unit of this polymer was proved by analysis of the 2D ^{1}H/^{13}C HSQC and HMBC heteronuclear spectra. The HSQC spectrum demonstrated the absence of substituents in the β-D-Glc*p*NAc residue and the presence of substituents at positions 3 and 4 in the α-D-Glc*p* residue (Table 1). In the HMBC, we found a correlation peak H-1 β-D-Glc*p*NAc and C-3 α-D-Glc*p*; this proved substitution of the second residue by the first at position 3. The HSQC spectrum demonstrated the position of the protons of 1,3-poly(glycerol phosphate) and as a consequence, we unequivocally attributed a signal of the phosphorous atom at δ +0.3 in the ^{1}H/^{31}P HMQC spectrum as belonging to the repeating unit of this polymer.

Fraction Ia. Recording the 2D NMR spectra, we detected a significant increase in intensities of the minor signals in ^{1}H,^{13}C- and ^{31}P-NMR spectra; these data are attributable to lability of the phosphodiester bond in sugar-1-phosphate polymers [8]. However, analysis of the minor peaks in 1D and 2D spectra of the partly degraded fraction I reliably demonstrated the presence of free D-Glc*p*NAc residues, and this indicates the lability of also 1→3 glycoside bond spatially close to the phosphodiester bond. To study the autohydrolyzates of polymer 2 in

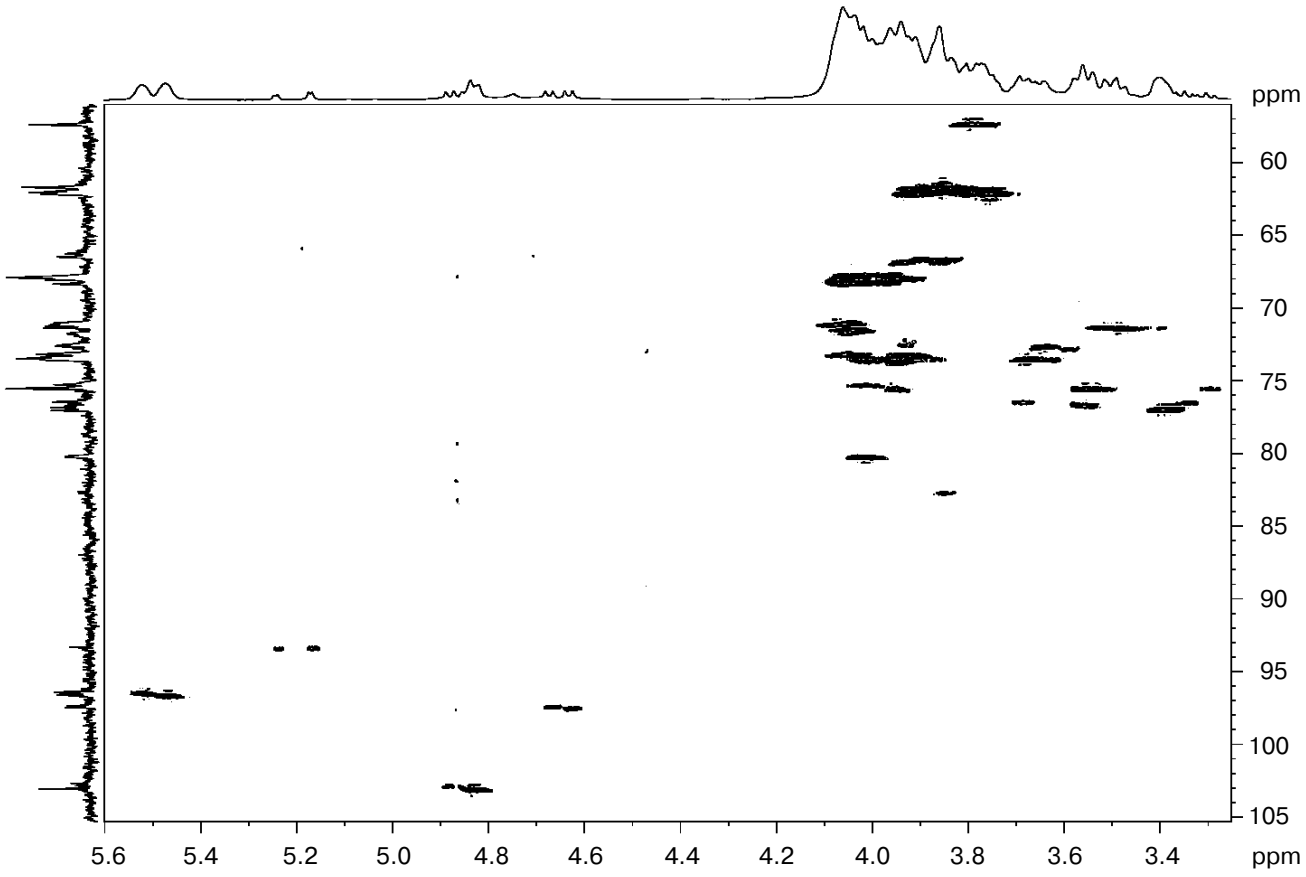


Fig. 2. Part of the HSQC spectrum of fraction Ia polymers. At the top, ^{1}H-NMR spectrum; at the left, ^{13}C-NMR spectrum, the signals of N-acetyl groups being not shown.

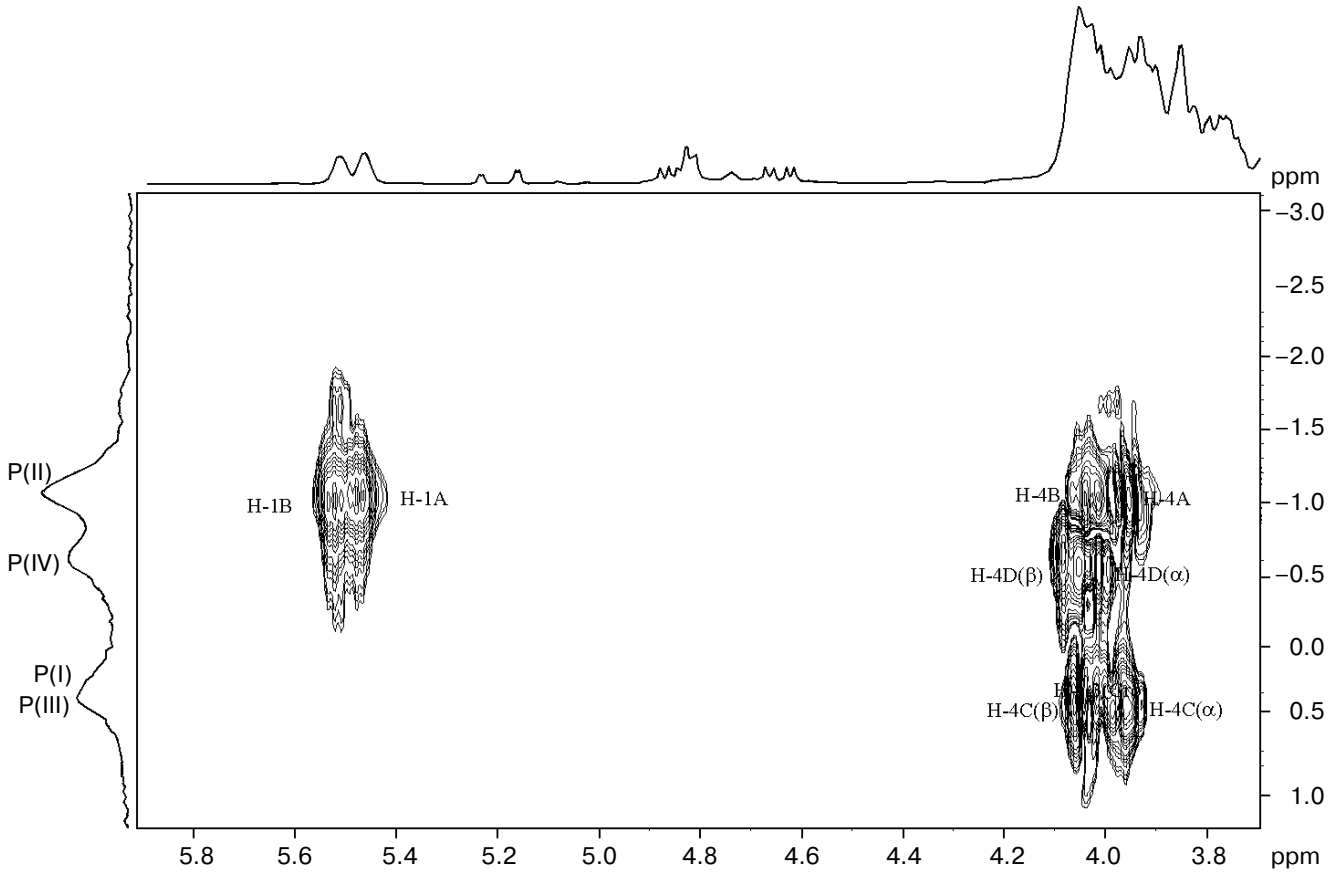


Fig. 3. 2D $^{1}H/^{31}P$ HMQC spectrum of fraction Ia. At the top, part of the ^{1}H-NMR spectrum; at the left, the ^{31}P-NMR spectrum.

detail, the partially degraded fraction I was chromatographically purified from the monomers; the resulting fraction Ia containing oligomers and polymers was neutralized with aqueous ammonia and studied by the NMR methods described above.

Four signals of various intensities for residues with α-configuration of the glycoside center (δ 5.52, 5.49, 5.24, and 5.17) and six signals for residues with β-configuration (δ 4.885, 4.85, 4.835, 4.825, 4.67, and 4.63) were observed in the ^{1}H-NMR spectrum of fraction Ia in the resonance area of the protons at the anomeric carbon atoms (Fig. 2). Eight signals of various intensities at δ 93.4-103.0 were observed in the anomeric area of the ^{13}C-NMR spectrum, and there were three signals (δ −1.1, −0.6, and +0.4) in the ^{31}P-NMR spectrum (Fig. 3). Analysis of all the 2D homo- and heteronuclear spectra allowed the attribution of signals in the 1D spectra (Tables 1 and 2) and revealing of the fragments not typical of the initial polymer 2, including the α-D-Glc*p* residues at the reducing end of oligomeric units. The dimensions of the oligomeric chain—three α-D-Glc*p* residues with 1.5 β-D-Glc*p*NAc residues on average—were found via intensities of the signals of α-D-Glc*p* residues. Taking into account the equimolar ratio of these residues in the undegraded polysaccharide 2, a phenomenon of unusual acidic degradation of polymer 2 with a break in the glycoside bond was proved.

Fraction II. In the ^{13}C-NMR spectrum of polymers of fraction II (Fig. 4), we observed all signals of polymers 1 and 2, signals of the partly degraded polymer 2, and some new intense and minor signals of polymer 3. Two additional peaks in the resonance area of anomeric carbon atoms (δ 103.0 and 95.9) and two peaks in the resonance area of the nitrogen-bound atoms (δ 56.8 and 55.7) should be noted among the intense signals; this indicates the presence of two aminosaccharides in the repeating unit of the polymer. Two new minor signals in the high-field area with chemical shifts (δ 30.7 and 30.0) typical of the succinic acid residue [7] should be also noted.

In the ^{31}P-NMR spectrum (Fig. 5) we also observed an intense additional signal at δ −1.8 that was absent from the spectrum of polymers of fraction I. The high-field position of the new signal suggested that it is attributable to the phosphorus atom of the phosphodiester group at the anomeric carbon atom and thus, an additional polymer is sugar-phosphate. This suggestion is proved by the presence of a correlation peak of a proton in the anomer-

Table 2. ^{1}H-NMR spectra (500 MHz) of cell wall polymers of *B. linens* VKM Ac-2159 (δ, ppm)[a]

Residue	Chemical shift						
Polymer 1	H-1	H-1'	H-2	H-3	H-3'		
-1)-sn-Gro-(3-*P*^{I}-	4.05	3.98	4.06	4.05	3.98		
Polymer 2	H-1	H-2	H-3	H-4	H-5	H-6	H-6'
-4)-α-D-Glc*p*-(1-*P*^{II}- (A) 3) ↑	5.49	3.69	4.04	3.95	4.05	3.855	3.855
β-D-Glc*p*NAc-(1	4.86	3.78	3.57	3.48	3.41	3.92	3.75
-4)-α-D-Glc*p*-(1-*P*- (B)	5.52	3.645	3.92	4.015	4.05	3.86	3.86
Fragments of oligomers of polymer 2							
-4)-α-D-Glc*p*-(1-*P*- 3) ↑	5.49	3.68	4.02	3.96	4.05	3.85	3.85
β-D-Glc*p*NAc-(1	4.835 4.825[b]	3.79	3.55	3.50	3.40	3.92	3.755
-4)-α-D-Glc*p*-(1-*P*-	5.52	3.645	3.92	4.015	4.05	3.86	3.86
-*P*^{III}-4)-D-Glc*p*-OH (α) (C) (β)	5.24 4.67	3.30 3.59	3.89 3.94	4.02 3.96	4.04 3.58	3.83 3.90	3.83 3.78
-*P*^{IV}-4)-D-Glc*p*-OH (α) (D) 3) (β) ↑	5.17 4.63	3.63 3.345	4.02 3.86	4.02 4.06	3.92 3.57	3.88 3.92	3.83 3.78
β-D-Glc*p*NAc-(1	4.85[α], 4.885[β]	3.79	3.55	3.51	3.40	3.92	3.755
Polymer 3							
→6)-α-D-Glc*p*NAc-(1-*P*^{V}- (E)	5.515 5.44[c]	3.94	3.755	3.55	3.91	4.01	3.95
-4)-β-D-Glc*p*NAc-(1→ (F)	4.625	3.81	3.77	3.99	3.555	3.80	3.93
-*P*^{VI}-4)-β-D-Glc*p*NAc-(1→ (F') 3) |	4.77	3.85	5.19	4.24	3.65	3.80	3.93
Suc		2.47	2.67				

[a] Signals of NAc at δ 2.02-2.07.
[α,β] For H-1 of β-D-Glc*p*NAc glycosylating α- or β-D-Glc*p*-OH, respectively.
[b] Two signals, depending on the distance between the unit and the end of oligomeric chain.
[c] For the E residue bound to the F′ residue by a phosphodiester bond.

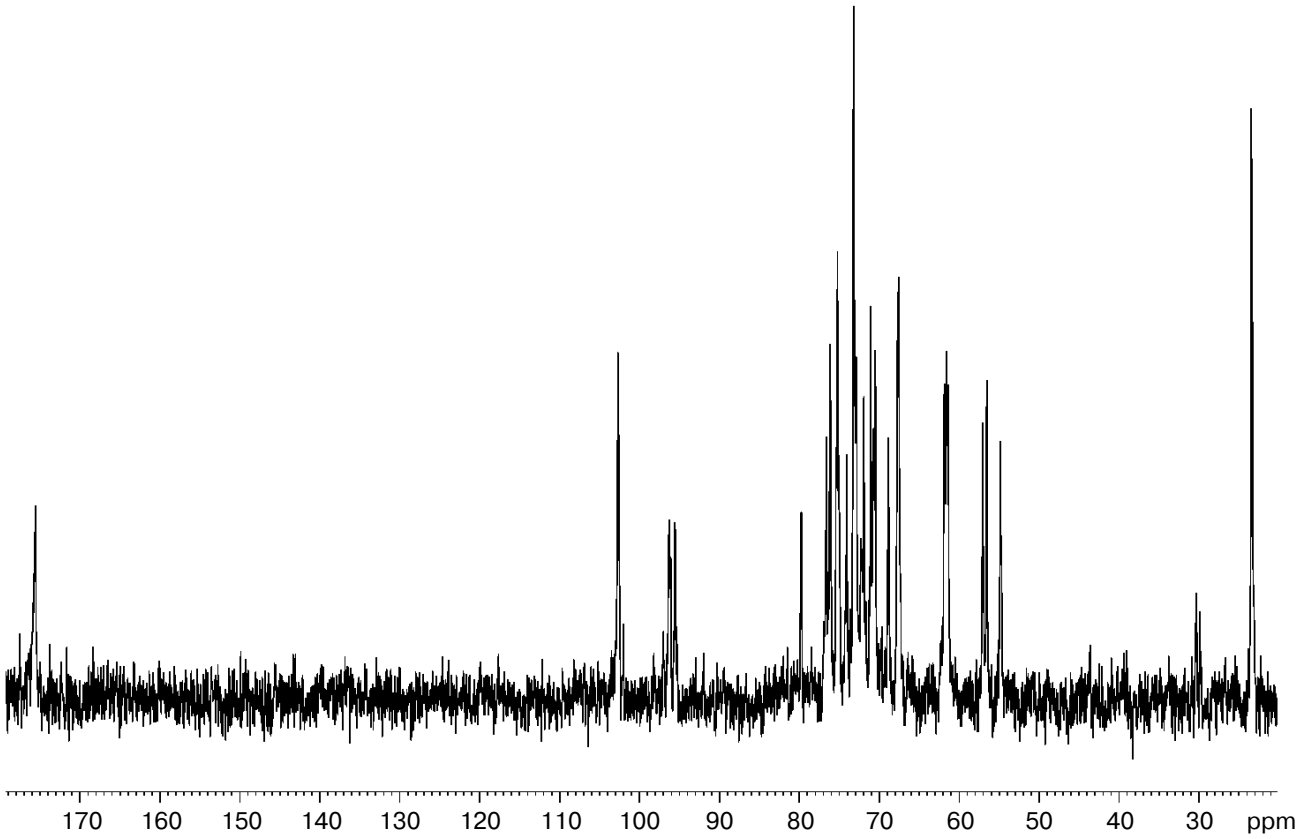


Fig. 4. ^{13}C-NMR spectrum of fraction II.

ic area (δ 5.515) and the above-mentioned phosphorus atom (δ −1.8) in ^{1}H/^{31}P HMQC (Fig. 5).

In the anomeric area of the ^{1}H-NMR spectrum, we observed additional very intense signals at δ 5.515 and 4.625 and also several minor signals; some of them are probably attributable to the products of partial hydrolysis of the additional sugar-phosphate polymer 3. However, two triplets in the high-field area (δ 2.47 and 2.67) suggested that appearance of several additional minor signals in the anomeric area may be due to the presence of saccharide residues acylated by succinic acid mentioned above.

The signals in the 1D spectra of fraction II were attributed by the 2D experiments described above and also by ^{1}H/^{13}C HMQC-TOCSY (Tables 1 and 2). As shown by the 2D data the repeating unit of polymer 3 has the following structure:

-4)-β-D-Glc*p*NAc-(1→6)-α-D-Glc*p*NAc-(1-*P*-.

The presence of a phosphodiester bond between the C-1 of the residue with α-configuration of the glycoside center and C-4 of the residue with β-configuration was proved by the ^{1}H/^{31}P HMQC data: along with the above-mentioned correlation peak δ_H/δ_P 5.515/−1.8 (H-1α/P), there was a correlation peak 3.99/−1.8 (H-4β/P). The presence of β,1→6 glycoside bond between the residues was proved by ^{1}H/^{1}H ROESY (correlation peaks δ_H/δ_H 4.625/4.01, H-1β/H-6α and 4.625/3.95, H-1β/H-6′α) and ^{1}H/^{13}C HMBC (correlation peak δ_H/δ_C 4.625/69.2, H-1β/C-6α). The ^{1}H/^{13}C HSQC spectrum revealed the presence in the ^{1}H spectrum of a low-field signal (δ_H 5.19) attributable to a carbon atom with chemical shift δ_C 75.5 not typical of the anomeric carbon atoms. It was natural to assume that the low-field proton shift was caused by α-effect of acylation by succinic acid of the hydroxy group of any of the aminosaccharide residues of polymer 3. The acylation site (the hydroxy group at C-3 of the β-D-Glc*p*NAc residue) was determined by analysis of the minor correlation peaks in COSY and TOCSY and proved by the presence of typical α- (for the C-3) and β- (for the C-4 and C-2) acylation effects in the ^{13}C spectrum (Table 1).

Depending on the growth conditions, the set of polymers in the cell wall of *B. linens* VKM Ac-2159 remained the same, but their ratios changed: in some cases polymer 2 prevailed, whereas polymer 3 was minor with a low degree of acylation by succinic acid. Studying this bacterium, we obtained cell wall preparations (another growth experiment) with prevailing polymer 3, in which

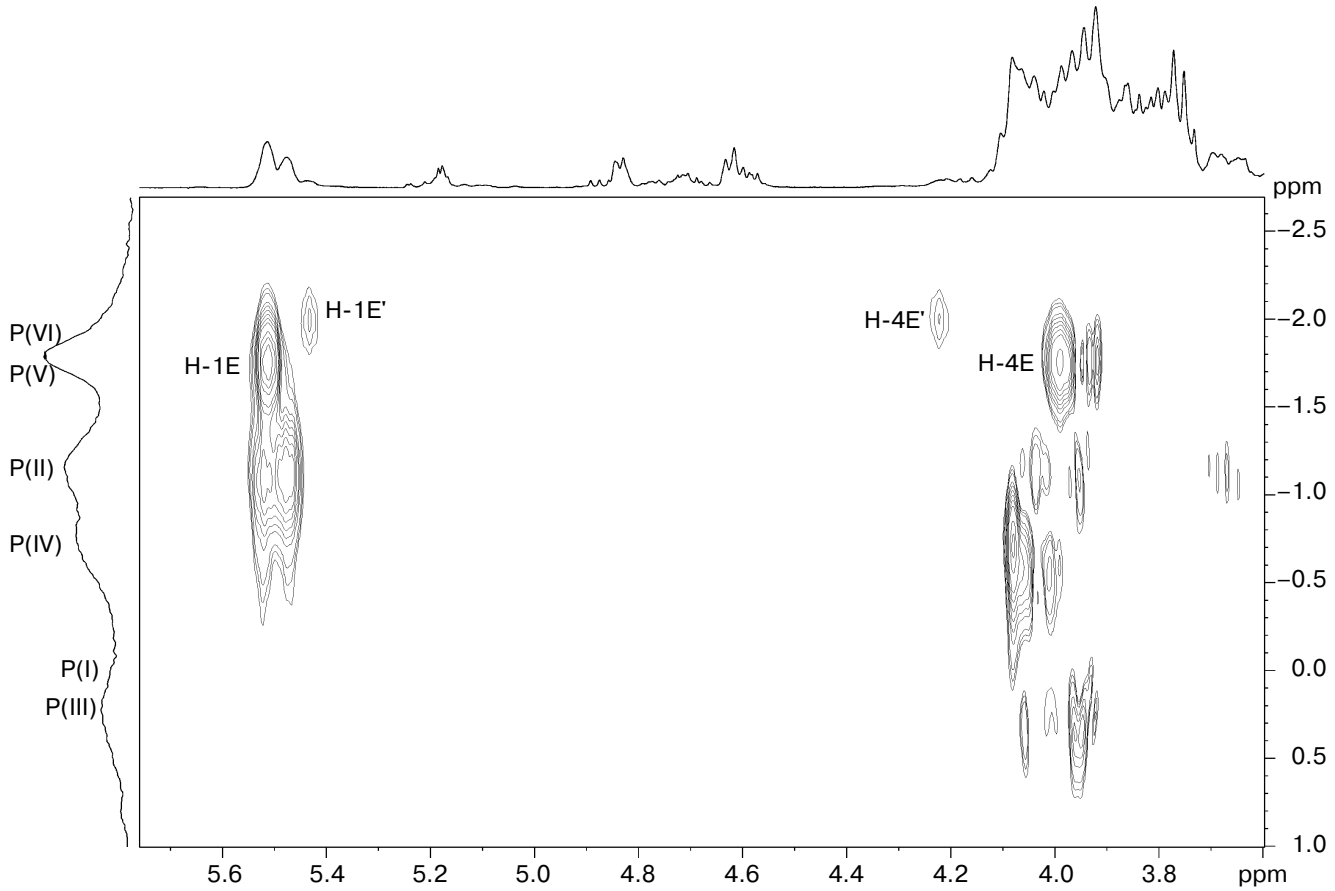
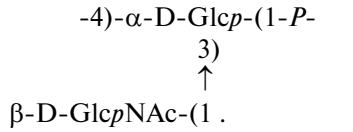


Fig. 5. 2D $^{1}H/^{31}P$ HMQC spectrum of fraction II. At the top, part of the ^{1}H spectrum; at the left, the ^{31}P spectrum. P^{V}, atom of the phosphoric acid residue between residues E and F′.

50% β-N-acetylglucosaminyl residues were substituted by succinate at C-3. The amount of polymer 1 remained constant in all cell wall preparations from *B. linens* VKM Ac-2159 studied by us.

So, we found three anionic polymers in the cell wall of *B. linens* VKM Ac-2159: 1,3-poly(glycerol phosphate) teichoic acid and two sugar-1-phosphate polymers not earlier described in bacteria. A monomer of one of the sugar-1-phosphate polymers has a branched repeating unit of the following structure (disaccharide):



Sugar-1-phosphate polymer with the branched trisaccharide as a monomer was described in *Spirilliplanes yamanashiensis* VKM Ac-1993^{T} [9]. The side glycosyl residues were found earlier in the sugar-phosphate polymers in streptococcus; the polymers had complex structure and contained from six to eight sugar residues in a monomer [10].

A repeating unit of another sugar-1-phosphate polymer of *B. linens* VKM Ac-2159 is also a disaccharide but of a linear structure consisting of alternating α- and β-N-acetylglucosamine. Some of the β-N-acetylglucosaminyl residues bear succinic acid residues bound by an ester bond. A structurally analogous polymer with disaccharide as the monomer but without the succinic acid residues →6)-α-D-Glc*p*NH_2-(1→6)-α-D-Glc*p*NAc-1-*P*- was found in the cell wall of several actinomycetes of *Streptomyces* genus earlier considered to belong to the genus *Streptoverticillium* [11]. Succinyl residues were earlier found in teichoic acids of *Nocardiopsis dassonvillei* subsp. *albirubida* VKM Ac-1882^{T} [7], *Streptomyces griseus* [12], and lipomannan of *Micrococcus luteus* (*"Micrococcus lysodeikticus"*) [13].

The cell wall teichoic acid of *B. linens* VKM Ac-2159 was identified as unsubstituted 1,3-poly(glycerol phosphate). As mentioned above, such a polymer is present in cell walls of two other strains—*B. linens* VKM Ac-2119 and VKM Ac-2120 [3]. Glycerol teichoic acid was also identified in a typical strain of *B. linens* ATCC 9172. However, a significant difference in VKM Ac-2159 from the above-mentioned organisms related with identifica-

tion of sugar-phosphate polymers may indicate that the strain more likely belongs to another species of *Brevibacterium*. Clarification of the species to which VKM Ac-2159 belongs is a subject for further taxonomic studies.

This work was financially supported by the Russian Foundation for Basic Research (grant No. 04-04-49096), INTAS (grant No. 01-2040), and Scientific Schools of the Russian Federation (grant NSh 1557.2003.3).

REFERENCES

1. Fiedler, F., Schaffler, M. J., and Stackebrandt, E. (1981) *Arch. Microbiol.*, **129**, 85-93.
2. Fiedler, F., and Bude, A. (1989) *J. Gen. Microbiol.*, **135**, 2837-2848.
3. Shashkov, A. S., Potekhina, N. V., Evtushenko, L. I., and Naumova, I. B. (2004) *Biochemistry* (Moscow), **69**, 658-664.
4. Gavrish, E. Yu., Krauzova, V. I., Potekhina, N. V., Karasev, S. G., Plotnikova, E. G., Korosteleva, L. A., and Evtushenko, L. I. (2004) *Mikrobiologiya*, **73**, 218-225.
5. Naumova, I. B., Kuznetsov, V. D., Kudrina, K. S., and Bezzubenkova, A. P. (1980) *Arch. Microbiol.*, **126**, 71-75.
6. Streshinskaya, G. M., Naumova, I. B., and Panina, L. I. (1979) *Mikrobiologiya*, **48**, 814-819.
7. Shashkov, A. S., Streshinskaya, G. M., Kozlova, Yu. I., Potekhina, N. V., Taran, V. V., Evtushenko, L. I., and Naumova, I. B. (1997) *Biochemistry* (Moscow), **62**, 1135-1139.
8. Archibald, A. R., Baddiley, J. G., and Button, D. (1968) *Biochem. J.*, **110**, 543-557.
9. Shashkov, A. S., Streshinskaya, G. M., Evtushenko, L. I., and Naumova, I. B. (2001) *Carbohydr. Res.*, **336**, 237-242.
10. Cisar, J. O., Sandberg, A. L., Reddy, G. P., Abeygunawardana, C., and Bush, C. A. (1997) *Infect. Immun.*, **65**, 5035-5041.
11. Kozlova, Yu. I., Streshinskaya, G. M., Shashkov, A. S., Evtushenko, L. I., and Naumova, I. B. (1999) *Biochemistry* (Moscow), **64**, 671-677.
12. Naumova, I. B. (1973) *Uspekhi Sovrem. Biol.*, **75**, 375-378.
13. Powell, D. A., Duckworth, M., and Baddiley, J. (1975) *Biochem. J.*, **151**, 378-397.