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Structural Analysis of the Capsular Polysaccharide from *Acinetobacter lwoffii* F78

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The capsular polysaccharide from *Acinetobacter lwoffii* F78 was isolated and purified, and its structure was elucidated by chemical analyses, NMR spectroscopy, and mass spectrometry. The presence of a capsule on this bacterium was confirmed by transmission electron microscopy experiments, and the utilization of the antilipid A monoclonal antibody proved the nonendotoxin origin of the isolated material. The structure represents a novel nonbranched aminopolysaccharide with high heterogeneity of amino groups substituents: →3)-α-L-FucNAc-(1→3)-β-D-QuiNR^{1}4NR^2-(1→4)-β-L-GlcNR^{3}3NR^{4}A-(1→, where R^1 = 3-hydroxybutyric acid (3-HBA) or *N*-acetylated alanine (AlaNAc), R^2 = AlaNAc or 3-HBA, R^3 = acetyl or 3-HBA, and R^4 = 3-HBA or acetyl.

Introduction

Bacteria of the genus *Acinetobacter* (*Moraxellaceae*, γ subclass of Proteobacteria)[1] are rather ubiquitous microorganisms isolated from soil, water, sewage,[2–5] and human skin.[6] The growing incidence of nosocomial infections caused by some species has resulted in a significant increase in *Acinetobacter*-related studies.[7] The most common pathogens belong to the so-called *A. calcoaceticus–A. baumannii* complex;[8] however, other species including *A. lwoffii* have also been identified from clinical specimens.[9,10] The genus *Acinetobacter* represents a very complex taxonomic group,[1,11] and the search for a fast and reliable identification method for diagnosis is still not complete. Genotypization, however, which is rapid and relatively simple,[12] is very often not available for diagnostic laboratories owing to the high costs and equipment requirements. *Acinetobacter* are Gram-negative bacteria; thus, they possess lipopolysaccharides (LPSs) as the main constituents of the outer leaflet of the cell-wall outer membrane. In general, LPSs are built up of lipid A (lipid part anchoring the molecule into the membrane), a core oligosaccharide, and an O-antigen (O-chain), which is either a homo- or heterosaccharidic polysaccharide.[13] On the basis of differences in the LPS O-specific polysaccharide (OPS) structures, the phenotypization (O-serotyping) of species like *Salmonella enterica*[14] or *Escherichia coli*[15] was established and may be routinely applied in diagnosis. However, the O-serotyping of *Acinetobacter* faces some problems. Presently, 13 distinct chemical structures of O-antigens are described, which are correlated with species characterized by DNA–DNA hybridization as 13 different strains[16] and providing some basis for a possible *Acinetobacter* O-serotyping scheme. In addition to LPSs, other polysaccharides like capsules (capsular polysaccharides; CPSs) or slimes may be present in the bacterial cell envelope, mostly built up of heterosaccharidic repeating units. In some bacteria (e.g., strains of *Streptococcus* or *E. coli*) CPSs are responsible for pathogenicity.[17] On the basis of the CPS structures from *E. coli* strains, a serotyping scheme was established (K-serotyping), which is used in diagnostics.[15] To date, more than 10 surface polysaccharides were isolated from different *A. baumannii* reference strains;[16] however, it remained unclear whether they originated from CPSs, OPSs, or other polysaccharides. Presently, only one defined CPS of *Acinetobacter* was structurally characterized, namely, that from *A. calcoaceticus* BD4.[18] Few other surface polysaccharides of non-LPS origin were also described,[19,20] but little is known about their structures.

Here we report on the isolation, purification, and structural analyses of a CPS of *Acinetobacter lwoffii* F78, which is one of the bacteria isolated from a cowshed in Bavaria, Germany, in a study on allergy-protective microbial factors present in such environments.[21]

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Results and Discussion

Transmission Electron Microscopy (TEM)

Utilization of osmium tetroxide, uranyl acetate, or lysine-acetate-based ruthenium-red fixation protocols[22,23] resulted in the loss of the CPS when applying ultrathin sectioning for TEM experiments (Figure 1A). Also, the exchange of Epon (Serva, Germany) to LRWhite (Science Services, Germany) resin did not improve the preservation of the CPS. It is known that the expression of the CPS may depend on the growth phase of bacteria (exponential or stationary phase);[17] however, this was not the reason here, because independent to the growth phase, the use of any fixative agent led to complete loss of the capsule. Finally, simple dropping of the bacterial suspension onto Pioloform-coated grids (Serva, Germany) allowed the *A. lwoffii* F78 CPS to be observed. Incubation with cationic gold particles did not improve the picture, most probably due to a small overall charge of the molecule. Some improvement was achieved by incubation of already-air-dried samples in lead citrate vapor at 22 °C for 1 min (a Pioloform-coated grid with an air-dried sample was put in a Petri plate and a drop of lead citrate was placed next to the grid; then, the Petri plate was closed for about 1 min). Incubation times that were too long, however, led to a very strong contrast and decreased clarity of the pictures.

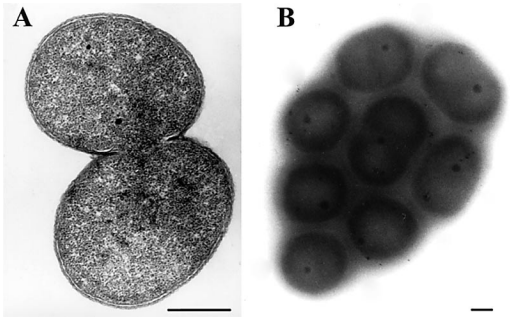

Figure 1. TEM micrographs of *A. lwoffii* F78: (A) ultrathin cut after osmium tetroxide fixation; (B) air-dried bacterial suspension (bar = 0.5 μm).

Isolation and Chemical Analyses of CPS

The purified CPS was obtained in a yield of 1.4% from the dry biomass. The high amounts of nucleic acids in the crude extract (after phenol/water extraction and ultracentrifugation) hampered the chemical analyses of the CPS. Most probably due to the high viscosity of the aqueous CPS solutions, enzymatic treatment did not remove the nucleic acids completely, even when the procedure was repeated twice. It was also not possible to remove the nucleic acids by HPLC or other chromatography methods. Ethanol dehydrates nucleic acids and exposes the negatively charged phosphate groups. Positively charged ions like sodium can thus bind to the charged groups, which leads to precipitation of the nucleic acids as a result of reduced repulsive forces.[24] This purification step for recovery of DNA/RNA is common in molecular biology and turned out to be a very useful purification method; thus, after ethanol precipitation the CPS was free of any residual nucleic acids.

In order to exclude the possibility that the isolated polysaccharide might originate from a LPS, the CPS preparation was subjected to SDS-PAGE and silver stain and to a Western blot test developed after mild acid hydrolysis with mAb A6, which specifically recognizes the free lipid A moiety.[25] This antibody was successfully applied in the identification of the nonstainable smooth form LPS from various *Acinetobacter* strains.[16] The CPS isolated from *A. lwoffii* F78 gave no positive reaction with mAb A6, except for a faint smear when applied in very high amounts (15 μg and higher), which is in contrast to that obtained for the purified LPS from *A. lwoffii* F78 (Hanuszkiewicz et al., in press), which reacted with less than 1.5 μg (Figure 2). Because mAb A6 detects free lipid A very sensitively (20 ng is enough to obtain a positive result),[25] the weak staining of the CPS samples in the Western blot test could be assigned to trace amounts of LPS; however, the lack of any reaction in the high-molecular mass region excluded the O-specific polysaccharide (OPS) character of the isolated polysaccharide. Additionally, the assays for quantification of Kdo and organic-bound phosphates as well as fatty acids gave negative results.

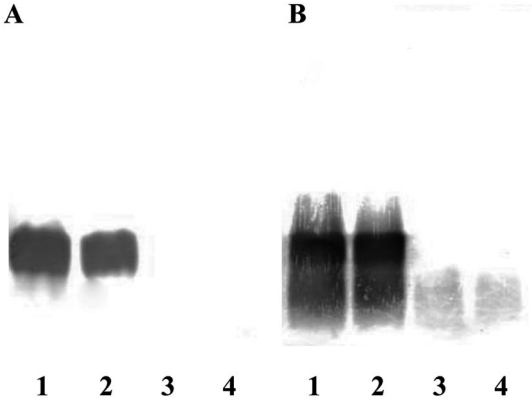

Figure 2. SDS-PAGE (A, stained with silver nitrate; B, Western blot developed with mAb A6) of *A. lwoffii* F78 LPS (line 1: 2.5 μg, line 2: 1.5 μg) and CPS (line 3: 25 μg, line 4: 15 μg).

Sugar analysis by GC and GC–MS identified 2-amino-2,6-dideoxygalactose (FucN), 2,3-diamino-2,3-dideoxyglucuronic acid (GlcN3NA), and 2,4-diamino-2,4,6-trideoxyglucose (QuiN4N; bacillosamine).[26] The absolute configuration assignment identified L-FucN and D-Ala. GC–MS analyses of the CPS after weak methanolysis proved that the amino groups of GlcN3NA were substituted by 3-hydroxybutyric acid (3-HBA) at the 2- and 3-positions and that the amino groups of QuiN4N were substituted at the 2-position by 3-HBA and at the 4-position by alanine (Ala) or at the 2-position by Ala and at the 4-position by 3-HBA. The presence of amino sugars in surface polysaccharides is a common feature amongst *Acinetobacter*[16] and QuiN4N as well as 3-HBA are known *Acinetobacter* polysaccharides

residues. 3-HBA had been identified as a constituent of *Acinetobacter* OPS before, in LPS of *A. baumannii* strain 24,[27] and in *A. haemolyticus* strains 57 and 61[28] it substituted QuiN4N at the 4-position, and in *A. baumannii* O23,[29] in which it was linked to the amino group of Qui3N. In the OPSs of *Acinetobacter* strain 90,[30] *A. baumannii* O2,[31] and *Acinetobacter* strain 108,[32] 3-HBA substituted the amino groups of Fuc4N in the first and Fuc3N in the two latter ones. Also, in *A. haemolyticus* strain ATCC 17906,[33] a QuiN4N residue was identified; however, in this case both amino groups were substituted by acetyl groups. Additionally, in this LPS a D-Ala residue was identified substituting D-GalNAcA at the 6-position. Fucosamine residues are also common in *Acinetobacter* polysaccharides,[32,34] also as Fuc3N[31,32,35] or Fuc4N.[30] The GlcN3NA was not identified in *Acinetobacter* yet and is generally not common in OPS/CPS structures; however, it was already found in a few, for example, *Vibrio ordalii*,[36] *Listonella anguillarum*,[37] *Thiobacillus* sp. IFO 14570,[38] *Pseudoalteromonas* sp. KMM 634,[39] and *Pseudomonas aeruginosa* O:6,[40] where it was described for the first time. However, in none of these species was GlcN3NA substituted by 3-HBA.

NMR Spectroscopic Studies of CPS

The ^{1}H NMR spectrum (Figure 3) contained three anomeric signals at δ = 5.07 ($J_{H1,C1}$ = 176 Hz), 4.65 ($J_{H1,C1}$ = 164 Hz), and 4.35 ppm ($J_{H1,C1}$ = 163 Hz) corresponding to a trisaccharide repeating unit containing one α- and two β-linked pyranoside residues. The ^{1}H NMR spectrum also suggested the presence of seven methyl groups belonging to three 3-HBA, one alanine residue, and three *N*-acetyl residues. In addition, two methyl signals were present (δ = 1.19 and 1.17 ppm) originating from C6 of two 6-deoxyhexoses. Because of overlapping signals at 2.36 and 2.51 ppm, only one proton multiplet of 3-HBA could be assigned, and in case of the other two, only one proton signal was identified. Integration analyses showed that the overall number of 3-HBA residues in one repeating unit should be only two; thus, the three distinct signals were due to high heterogeneity of the amino group substitution pattern (see below). Analysis of the 2D spectra (TOCSY, COSY, HMQC) allowed all proton and carbon chemical shifts to be assigned (Table 1). Analysis of the NMR spectra identified the presence of one *galacto*- and two *gluco*-configured sugars. Six upfield-shifted carbon signals at 48.83, 57.47, 57.30, 54.90, 54.03, and 50.53 ppm were assigned to amino substituted C2 of residue A, C2 and C4 of residue B, C2 and C3 of residue C, and C2 of alanine, respectively. Interresidual NOE signals observed in the ROESY spectrum showed connectivities between protons A1 and B3, C1 and A3, and B1 and C4, which was confirmed by downfield shifts of the corresponding carbon atoms, and by the HMBC experiment (long-range interresidual correlation signals between H1 of residue A and C3 of residue B, H1 of residue B and C4 of residue C, and H1 of residue C and C3 of residue A). As a result of the high heterogeneity in the substitution pattern, it was not possible to assign the amino group substituents by the HMBC spectrum alone; yet, the positions of 3-HBA and the alanine and *N*-acetyl residues were determined by GC–MS and ESI MS analyses (Figure 4; see also below). However, the HMBC experiment proved that one acetyl residue substituted the amino group of alanine (long-range correlation signal between H2 of alanine and C1 of *N*-acetyl X; Table 1), identifying AlaNAc in CPS of *A. lwoffii* F78, which is unique for *Acinetobacter* polysaccharides and in general not common in CPS/OPS structures. The AlaNAc as a constituent of polysaccharides of Gram-negative bacteria was already reported, for example, in a few *Pseudoalteromonas* species,[39] *Aeromonas salmonicida*[41] and *Proteus penneri*.[42] In addition, the HMBC spectrum contained eight carbonyl signals, three of which (δ = 174.48, 175.16, 175.38 ppm) were assigned to 3-HBA residues, another three (two overlapping signals at δ = 175.54 ppm and one at δ = 174.84 ppm) to three *N*-acetyl groups, one at δ

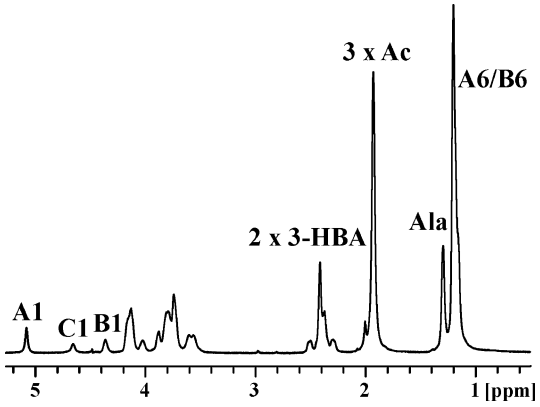

Figure 3. ^{1}H NMR spectrum of the CPS from *A. lwoffii* F78 recorded at 50 °C (signals B1 and C1 are slightly decreased due to the water suppression).

Table 1. ^{1}H and ^{13}C NMR chemical shifts (in ppm) of the CPS from *A. lwoffii* F78 (bolded values state the substitution). Spectra were recorded with acetone as internal standard (δ_H = 2.225 ppm and δ_C = 31.45 ppm) at 50 °C.

Residue	H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	H6/C6
A	5.07	4.11	3.77	3.73	3.78	1.19
α-Fuc*p*N	97.43	48.83	**77.84**	70.41	68.03	16.59
B	4.36	3.72	3.82	3.60	3.55	1.17
β-Qui*p*N4N	101.85	57.47	**75.00**	57.30	71.51	18.39
C	4.65	3.72	4.03	3.81	3.88	–
β-Glc*p*N3NA	102.31	54.90	54.03	**77.44**	77.61	172.89
3-HBA	–	2.39	4.16	1.20		
	174.48	45.62	66.12	23.10		
3-HBA	–	2.29/ 2.51	4.16	1.15		
	175.16	46.67	66.12	22.58		
3-HBA	–	2.37	4.14	1.20		
	175.38	46.42	66.12	23.10		
Alanine	–	4.36	1.29			
	176.13	50.53	17.60			
N-Ac	–	1.93				
(× 2)	175.54	23.40				
N-Ac	–	1.91				
(X)	174.84	23.40				

= 176.13 ppm to C1 of alanine, and one at δ = 172.89 ppm to C6 of hexosuronic acid (residue C). On the basis of the absolute configuration of L-Fuc*p*N proved by GC analyses and on the glucosylation effect on ^{13}C chemical shifts and by using the published rules and NMR spectroscopic data,[40,43–45] the absolute configuration D was assigned for β-Qui*p*N4N and L was assigned to the β-Glc*p*N3NA residues. In particular, the observed chemical shifts of C-1 of β-L-GlcNR^{3}3NR^{4}A and C-2, C-3, and C-4 of α-L-FucNAc in the GlcNR^{3}3NR^{4}A-(1→3)-α-L-FucNAc moiety, C-1 of α-L-FucNAc and C-3, and C-4 of β-D-QuiNR^{1}4NR^2 in α-L-FucNAc-(1→3)-β-D-QuiNR^{1}4NR^2, and C-1 of β-D-QuiNR^{1}4NR^2 and C-3, and C-4 of β-L-GlcNR^{3}3NR^{4}A in the β-D-QuiNR^{1}4NR^2-(1→4)-β-L-GlcN3NA moiety, were compared with the corresponding calculated chemical shifts in the respective disaccharides with the D or L configuration.

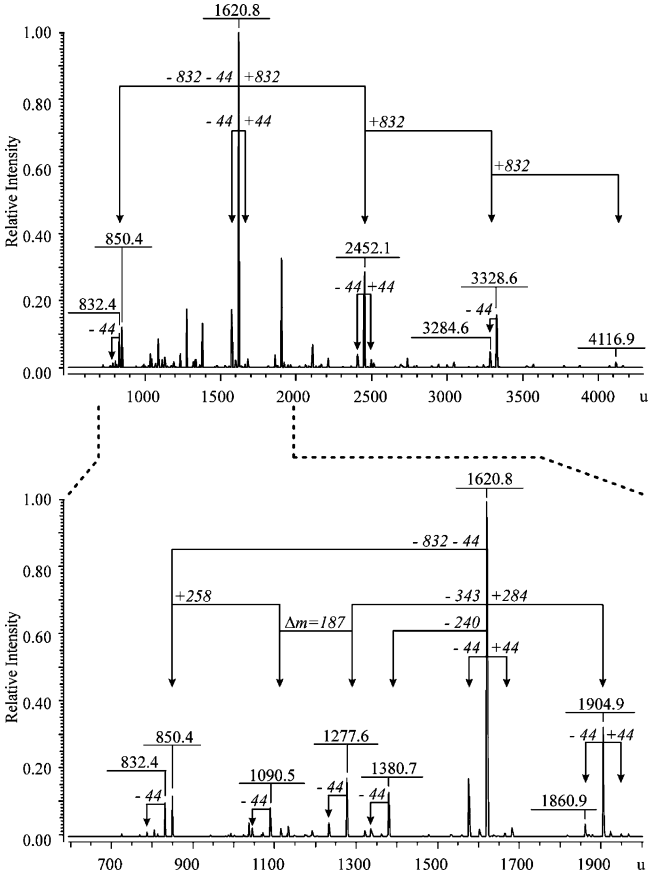

Figure 4. ESI FT-ICR MS CSD spectrum of the CPS from *A. lwoffii* F78. The mass difference of 832 u corresponds to one repeating unit containing one dHexNAc, one dHexNN, one HexNNA, one AlaNAc, two 3-HBA, and one *N*-acetyl group. For fragmentation details see Table 2.

Taken together, the data elucidated the novel structure of the trisaccharide backbone of the repeating unit of the CPS from *A. lwoffii* F78 (Figure 5). Only one other fully characterized CPS is known from *Acinetobacter*, namely, that of *A. calcoaceticus* BD4,[18] which contained no amino sugars but L-Rha, D-Man, D-Glc, and D-GlcA in an approximate molar ratio of 4:1:1:1. Another CPS was isolated from *A. venetianus* RAG-1, but only chemical composition analyses were performed to identify L-GalNAcA, D-GalNAc, and D-QuiNAc4NAc.[19] Preliminary chemical analyses of CPS from *A. radioresistence* KA53 proved the presence of L-Ala and sugars like Glc, Gal, GalNAc, and GlcNAc; however, neither a complete structure nor the character of an additional aminuronic acid was described.[20]

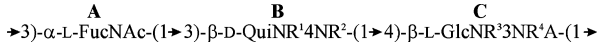

Figure 5. Structure of one repeating unit of the CPS from *A. lwoffii* F78: R^1 = 3-HBA or alanine; R^2 = alanine or 3-HBA; R^3 = acetyl or 3-HBA; R^4 = 3-HBA or acetyl.

Mass Spectrometry

Mass spectrometric analyses confirmed both the overall structure and the substitution heterogeneity in the CPS. Owing to the big size of the molecule it was not possible to record ion peaks of intact molecular species under normal soft ionization conditions. However, capillary skimmer dissociation led to unspecific fragmentation induced by collisions in the ion source and thus enabled the analysis of fragment ion mass spectra, which proved the structure of the CPS repeating unit (Figure 4). The mass difference of 832 u corresponded to a molecule consisting of one dHexNAc (*N*-acetyldeoxyhexosamine; here FucNAc), one dHexNN (QuiN4N), one HexNNA (GlcN3NA), and as additional substituents, one AlaNAc, two 3-HBA, and one *N*-acetyl residue. Each molecular ion possessed two accompanying ions, that is, $\Delta m/z = \pm 44$, corresponding to an exchange between one 3-HBA and one *N*-acetyl group. The most prominent ions (like the one at m/z = 1620.8, corresponding to two repeating units with one additional acetyl group instead of one 3-HBA) possessed additional accompanying ions of $\Delta m/z = \pm 44$, indicating further exchange of substituents. The fragmentation observed in the spectra (Table 2) also confirmed the substitutions of particular sugar residues, proving the presence of AlaNAc and 3-HBA as substituents of dHexNN (QuiN4N) and 3-HBA and the *N*-acetyl group as substituents of HexNNA (GlcN3NA), and the presence of dHexNAc (FucNAc). Thus, the MS analyses confirmed the overall heterogeneity in the amino group substitution pattern as well as the constituents of one repeating unit of CPS from *A. lwoffii* F78.

Table 2. Fragmentation pattern observed in ESI CSD FT-ICR MS spectrum in one repeating unit of *A. lwoffii* F78 CPS.

Fragment (m/z)	Residue
187	dHexNAc – H_2O
240	HexNNA + 2 × Ac – H_2O
258	HexNNA + 2 × Ac
284	HexNNA + Ac + 3-HBA – H_2O
343	dHexNN + AlaNAc + 3-HBA – H_2O

Conclusions

In this study we present the structural analysis of the capsular polysaccharide (CPS) of *Acinetobacter lwoffii* F78. The bacterium was isolated from a cowshed in Bavaria (Germany) in the course of investigating allergy-protective microbial components present in farming environments. Hot phenol/water extraction of the dry biomass, followed by enzymatic digestion, ultracentrifugation, and ethanol precipitation of nucleic acids allowed the purification of *A. lwoffii* F78 CPS, and its structure was established on the basis of NMR spectroscopy, GC, GC–MS, and ESI FT-ICR MS analyses. One repeating unit of *A. lwoffii* F78 CPS consisted of a trisaccharide backbone [→3)-α-L-FucNAc-(1→3)-β-D-QuiN4N-(1→4)-β-L-GlcN3NA-(1→], where the amino groups of QuiN4N were substituted by AlaNAc or 3-HBA, or both, and the amino groups of GlcN3NA were substituted by 3-HBA and an acetyl group. High heterogeneity in the substitution pattern was resolved by a CSD experiment in ESI FT-ICR MS and by GC–MS. Besides presenting a novel structure, we were also able to prove the capsular origin of the isolated polysaccharide, which in case of some other *Acinetobacter* polysaccharides is still an open question. Although TEM experiments utilizing fixation protocols did not show any CPS, simple dropping of bacterial suspension onto the grids showed the presence of capsular polysaccharide surrounding *A. lwoffii* F78 cells. In addition, Western blot developed with mAb A6 excluded the lipopolysaccharide-related origin of the isolated molecule.

Experimental Section

Bacteria and Isolation of the CPS: Strain *A. lwoffii* F78 was isolated, characterized, and cultivated as described.[21] The dry bacterial biomass was extracted with hot phenol/water,[46] and the CPS-containing water phase was dialyzed and further purified by enzymatic treatment (RNase A, Sigma–Aldrich, USA; DNase I, Roche, Germany; Proteinase K, Roche, Germany). The nucleases were applied at 37 °C in a buffer consisting of 0.01 M $MgCl_2$, 0.05 M NaCl, and 0.1 M Tris/Cl (pH 7.5) for 12 h, and Proteinase K was subsequently utilized at 56 °C for 1 h. After dialysis, the sample was ultracentrifuged (105000 × g, 4 °C, 24 h) and the CPS-containing supernatant was ultracentrifuged again (500000 × g, 4 °C, 48 h). The supernatant was collected, and residual nucleic acids were precipitated with 95% ethanol at –80 °C for 1 h in the presence of 0.3 M sodium acetate buffer (pH 6.5).[24] The remaining CPS was further purified on a column (2.5 × 80 cm) of Biogel P60 in acetic acid/pyridine/water (10:4:986).

Transmission Electron Microscopy (TEM): Bacteria were grown in SuperBroth (SB, 200 mL)[21] for 4.5 h to OD_{600} of 0.8 or at 37 °C for 12 h and centrifuged (5500 × g, 4 °C, 30 min). The pellet was then prepared for microscopy as described previously.[22,23] To visualize the CPS, bacteria were grown in SB (4 mL) to OD_{600} of 0.4, centrifuged, resuspended in PBS (500 μL), and dropwise transferred directly onto the Pioloform-coated copper nets. The excessive liquid was removed with filter paper and samples were air dried prior to observation.

Chemical Analyses of CPS: The CPS was subjected to SDS-PAGE (12% acrylamide in the separating gel, silver stain) and Western blot by utilizing antilipid A monoclonal antibody (mAb) A6 as described previously.[25] Qualitative analyses of sugars were performed with GC and GC–MS after methanolysis or hydrolysis with 2 or 4 M trifluoroacetic acid.[47] Quantitative estimation of fatty acids, 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo), and organic-bound phosphate, and the determination of the absolute configurations of FucNAc and alanine, were performed as described previously.[48]

Mass Spectrometry: Electrospray ionization Fourier-transform ion cyclotron resonance mass spectrometric (ESI FT-ICR MS) analyses were performed with a 7 T Apex II (Bruker Daltonics, USA). For the negative-ion mode the samples were dissolved in a water/2-propanol/triethylamine mixture (50:50:0.001). The samples were sprayed at a flow rate of 2 μL min^{-1}. Capillary entrance voltage was set to 3.8 kV and dry gas temperature to 150 °C. Capillary skimmer dissociation (CSD) was induced by increasing the capillary exit voltage from –100 to –350 V. The spectra, which showed several charge states for each component, were charge deconvoluted, and mass numbers given refer to the monoisotopic molecular masses.[49]

NMR Spectroscopy: The 1D and 2D [correlation spectroscopy (^1H,^1H COSY), total correlation spectroscopy (TOCSY), rotating nuclear Overhauser effect spectroscopy (ROESY), ^1H,^{13}C heteronuclear multiple bond correlation (HMBC), ^1H,^{13}C heteronuclear multiple quantum coherence (HMQC), and ^1H,^{31}P HMQC] NMR spectra were recorded for D_2O solutions with a Bruker AMX 600 spectrometer. The resonances were measured relative to external 85% phosphoric acid (δ_P = 0 ppm) and internal acetone (δ_H = 2.225 ppm and δ_C = 31.45 ppm) at 50 °C.

Acknowledgments

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[1] S. Misbah, H. Hassan, M. Y. Yusof, Y. A. Hanifah, S. Abu-Bakar, *Singapore Med. J.* **2005**, *46*, 461–464.
[2] P. Baumann, *J. Bacteriol.* **1968**, *96*, 39–42.
[3] E. L. Carr, P. Kampfer, B. K. Patel, V. Gurtler, R. J. Seviour, *Int. J. Syst. Evol. Microbiol.* **2003**, *53*, 953–963.
[4] M. Gennari, M. Parini, D. Volpon, M. Serio, *Int. J. Food Microbiol.* **1992**, *15*, 61–75.
[5] M. Gennari, P. Lombardi, *Zentralbl. Bakteriol.* **1993**, *279*, 553–564.
[6] H. Seifert, L. Dijkshoorn, P. Gerner-Smidt, N. Pelzer, I. Tjernberg, M. Vaneechoutte, *J. Clin. Microbiol.* **1997**, *35*, 2819–2825.
[7] E. Bergogne-Berezin, K. J. Towner, *Clin. Microbiol. Rev.* **1996**, *9*, 148–165.
[8] L. Dijkshoorn, A. Nemec, H. Seifert, *Nat. Rev. Microbiol.* **2007**, *5*, 939–951.
[9] T. Mori, T. Nakazato, R. Yamazaki, Y. Ikeda, S. Okamoto, *Intern. Med.* **2006**, *45*, 803–804.
[10] M. Perilli, A. Felici, A. Oratore, G. Cornaglia, G. Bonfiglio, G. M. Rossolini, G. Amicosante, *Antimicrob. Agents Chemother.* **1996**, *40*, 715–719.
[11] P. J. Bouvet, P. A. D. Grimont, *Int. J. Syst. Bacteriol.* **1986**, *36*, 228–240.
[12] J. E. Clarridge III, *Clin. Microbiol. Rev.* **2004**, *17*, 840–862.
[13] O. Holst, S. Müller-Loennies in *Comprehensive Glycoscience: From Chemistry to Systems Biology* (Eds.: J. P. Kamerling, G.-J. Boons, Y. C. Lee, A. Suzuki, N. Taniguchi, A. G. J. Voragen), Elsevier, Oxford, UK, **2007**, vol. 1, pp. 123–178.

[14] M. Y. Popoff, J. Bockemuhl, L. L. Gheesling, *Res. Microbiol.* **2004**, *155*, 568–570.
[15] F. Orskov, I. Orskov, *Can. J. Microbiol.* **1992**, *38*, 699–704.
[16] R. Pantophlet in *Acinetobacter, Molecular Microbiology* (Ed.: U. Gerischer), Caister Academic Press, Norfolk, UK, **2008**, pp. 61–98.
[17] I. W. Sutherland in *Comprehensive Glycoscience: From Chemistry to Systems Biology* (Eds.: J. P. Kamerling, G.-J. Boons, Y. C. Lee, A. Suzuki, N. Taniguchi, A. G. J. Voragen), Elsevier, Oxford, UK, **2007**, vol. 2, pp. 521–558.
[18] N. Kaplan, E. Rosenberg, B. Jann, K. Jann, *Eur. J. Biochem.* **1985**, *152*, 453–458.
[19] D. Nakar, D. L. Gutnick, *Microbiology* **2001**, *147*, 1937–1946.
[20] S. Navon-Venezia, Z. Zosim, A. Gottlieb, R. Legmann, S. Carmeli, E. Z. Ron, E. Rosenberg, *Appl. Environ. Microbiol.* **1995**, *61*, 3240–3244.
[21] J. Debarry, H. Garn, A. Hanuszkiewicz, N. Dickgreber, N. Blümer, E. von Mutius, A. Bufe, S. Gatermann, H. Renz, O. Holst, H. Heine, *J. Allergy Clin. Immunol.* **2007**, *119*, 1514–1521.
[22] K. Dittmar, U. Mamat, M. Whiting, T. Goldmann, K. Reinhard, S. Guillen, *Mem. Inst. Oswaldo Cruz* **2003**, *98 Suppl 1*, 53–58.
[23] S. Hammerschmidt, S. Wolff, A. Hocke, S. Rosseau, E. Müller, M. Rohde, *Infect. Immun.* **2005**, *73*, 4653–4667.
[24] J. Sambrook, D. W. Russel in *Molecular Cloning* (Eds.: J. Argentine, N. Irwin), Cold Spring Harbor Laboratory Press, New York, USA, **2001**, vol. 3, pp. A8.12–A8.18.
[25] R. Pantophlet, L. Brade, H. Brade, *J. Endotoxin. Res.* **1997**, *4*, 89–95.
[26] N. Sharon, *Glycobiology* **2007**, *17*, 1150–1155.
[27] E. V. Vinogradov, L. Brade, H. Brade, O. Holst, *Carbohydr. Res.* **2003**, *338*, 2751–2756.
[28] R. Pantophlet, S. R. Haseley, E. V. Vinogradov, L. Brade, O. Holst, H. Brade, *Eur. J. Biochem.* **1999**, *263*, 587–595.
[29] S. R. Haseley, W. H. Traub, S. G. Wilkinson, *Eur. J. Biochem.* **1997**, *244*, 147–154.
[30] S. R. Haseley, O. Holst, H. Brade, *Eur. J. Biochem.* **1997**, *245*, 470–476.
[31] S. R. Haseley, S. G. Wilkinson, *Eur. J. Biochem.* **1995**, *233*, 899–906.
[32] E. V. Vinogradov, R. Pantophlet, L. Dijkshoorn, L. Brade, O. Holst, H. Brade, *Eur. J. Biochem.* **1996**, *239*, 602–610.
[33] S. R. Haseley, O. Holst, H. Brade, *Eur. J. Biochem.* **1997**, *244*, 761–766.
[34] S. R. Haseley, S. G. Wilkinson, *Eur. J. Biochem.* **1996**, *237*, 229–233.
[35] S. R. Haseley, O. Holst, H. Brade, *Eur. J. Biochem.* **1997**, *247*, 815–819.
[36] I. Sadovskaya, J. R. Brisson, N. H. Khieu, L. M. Mutharia, E. Altman, *Eur. J. Biochem.* **1998**, *253*, 319–327.
[37] I. Sadovskaya, J. R. Brisson, E. Altman, L. M. Mutharia, *Carbohydr. Res.* **1996**, *283*, 111–127.
[38] A. S. Shashkov, S. Campos-Portuguez, H. Kochanowski, A. Yokota, H. Mayer, *Carbohydr. Res.* **1995**, *269*, 157–166.
[39] E. L. Nazarenko, N. A. Komandrova, R. P. Gorshkova, S. V. Tomshich, V. A. Zubkov, M. Kilcoyne, A. V. Savage, *Carbohydr. Res.* **2003**, *338*, 2449–2457.
[40] Y. A. Knirel, N. A. Kocharova, A. S. Shashkov, B. A. Dmitriev, N. K. Kochetkov, *Carbohydr. Res.* **1981**, *93*, C12–C13.
[41] Z. Wang, S. Larocque, E. Vinogradov, J. R. Brisson, A. Dacanay, M. Greenwell, L. L. Brown, J. Li, E. Altman, *Eur. J. Biochem.* **2004**, *271*, 4507–4516.
[42] E. V. Vinogradov, A. S. Shashkov, Y. A. Knirel, N. K. Kochetkov, Z. Sidorczyk, A. Swierzko, *Carbohydr. Res.* **1991**, *219*, c1-c3.
[43] P. E. Jansson, L. Kenne, G. Widmalm, *Carbohydr. Res.* **1989**, *188*, 169–191.
[44] Y. A. Knirel, E. V. Vinogradov, N. A. Kocharova, A. S. Shashkov, B. A. Dmitriev, N. K. Kochetkov, *Carbohydr. Res.* **1983**, *122*, 181–188.
[45] G. M. Lipkind, A. S. Shashkov, Y. A. Knirel, E. V. Vinogradov, N. K. Kochetkov, *Carbohydr. Res.* **1988**, *175*, 59–75.
[46] O. Westphal, K. Jann, *Methods Carbohydr. Chem.* **1965**, *5*, 83–91.
[47] J. P. Kamerling, G. J. Gerwig in *Comprehensive Glycoscience: From Chemistry to Systems Biology* (Eds.: J. P. Kamerling, G.-J. Boons, Y. C. Lee, A. Suzuki, N. Taniguchi, A. G. J. Voragen), Elsevier, Oxford, UK, **2007**, vol. 2, pp. 1–67.
[48] C. Oertelt, B. Lindner, M. Skurnik, O. Holst, *Eur. J. Biochem.* **2001**, *268*, 554–564.
[49] A. N. Kondakova, E. V. Vinogradov, Y. A. Knirel, B. Lindner, *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2343–2349.

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