Carbohydrate Research, 176 (1988) 160–163
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Structure of a glycan from the surface-layer glycoprotein of *Clostridium thermohydrosulfuricum* strain L111-69

RUDOLF CHRISTIAN,
Institut für Chemie der Universität für Bodenkultur, A-1180 Wien (Austria)

PAUL MESSNER*, CHRISTIAN WEINER, UWE B. SLEYTR,
Zentrum für Ultrastrukturforschung und Ludwig Boltzmann-Institut für Ultrastrukturforschung, Universität für Bodenkultur, A-1180 Wien (Austria)

AND GERHARD SCHULZ
Sandoz-Forschungsinstitut Wien, A-1235 Wien (Austria)

(Received August 15th, 1987; accepted for publication, October 14th, 1987)

Clostridium thermohydrosulfuricum strain L111-69 and *Clostridium thermosaccharolyticum* strain D120-70 were the first eubacteria for which crystalline surface layers (S-layers)[1] were shown to contain covalently linked carbohydrates[2]. Digestion of the purified *C. thermohydrosulfuricum* glycoprotein with pronase and gel-permeation chromatography[3] of the products on Bio-Gel P-30 yielded a single glycopeptide of apparent molecular weight ~20,000.

Carbohydrate analysis by g.l.c. of the alditol acetates after hydrolysis revealed mannose and rhamnose in the molar ratio 1:1.

The ^{1}H-n.m.r. spectrum of a solution of the glycan in D_2O contained signals for anomeric protons at 4.92 (s, 1 H) and 5.01 p.p.m. (s, 1 H), one signal for a CH_3 group of Rha at 1.27 p.p.m. (d, 3 H), and signals (10 H) in the range 3.4–4.2 p.p.m.

The proton-decoupled ^{13}C-n.m.r. spectrum of the glycan contained twelve signals corresponding to twelve carbons (Table I). Thus, the glycan was a regular polysaccharide with a disaccharide repeating-unit. There was one signal at 17.58 p.p.m. corresponding to C-6 of Rha, one at 61.34 p.p.m. (C-6 of Man), eight in the range 67.4–76.4 p.p.m., and two in the anomeric region (97.05 and 101.68 p.p.m.).

The proton-coupled ^{13}C-n.m.r. spectrum exhibited sufficient features to establish a hypothetical structure. The J value (172 Hz) of each of the C-1 signals

*Author for correspondence.

0008-6215/88/$ 03.50

TABLE I

^{13}C-CHEMICAL SHIFTS[a] AND $^{1}J_{C,H}$ VALUES (Hz) OF THE *Clostridium thermohydrosulfuricum* L111-69 GLYCAN AND SELECTED MODEL COMPOUNDS

Glycosyl residue	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
(1→3)-α-Rha*p*-(1→4)	101.68	67.35	75.25	71.13	70.04	17.58
	172	148	142	144	(144)	127
α-Rha*p*[b]	94.70	71.54	70.70	72.91	68.99	17.59
	170	149	142	143	145	128
(1→4)-α-Man*p*-(1→3)	97.05	71.53	70.08	76.44	72.39	61.34
	172	150	(144)	148	(142)	144
α-Man*p*[b]	94.66	71.46	71.02	67.68	73.20	61.81
	170	149	145	146	142	144

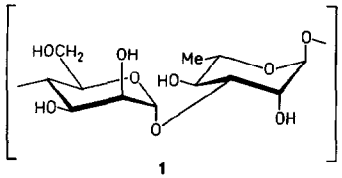
[a]In p.p.m. downfield from the signal of Me_4Si (67.40 p.p.m. upfield from the signal of 1,4-dioxane in D_2O). Couplings were measured by hand on enlarged 62.9-MHz spectra, and those from overlapping signals shown in brackets were determined from the doubled difference between the centre of the signal and one part each of the coupled signals. [b]Assignment from ref. 4.

at 101.68 and 97.05 p.p.m. (Table I) indicated[5] the pyranosyl residue to be α. Since $^{1}J_{C,H}$ values for monosaccharides are not significantly different from those in the corresponding glycosyl units of oligo-[6] or poly-saccharides[7], three resonances were selected [67.35 (*J* 148 Hz), 71.53 (*J* 150 Hz), and 76.44 p.p.m. (*J* 148 Hz)] as candidates for C-2 of Rha and Man. However, the first two lacked large two- or three-bond C–H couplings, a feature which is diagnostic of the C-2 resonances of Rha or Man. From the chemical shifts, glycosylation at either C-2 was excluded. Since no additional signal upfield of 70 p.p.m. was observed (unsubstituted C-4 of Man), Man is glycosylated in position 4. Because of the relatively large $^{1}J_{C,H}$ value (similar to that of the monosaccharide), the signal at 76.44 p.p.m. (*J* 148 Hz) is assigned to C-4 of Man.

Considering that C-6 (61.34 p.p.m.) of Man is unsubstituted, C-4 (76.44 p.p.m.) is substituted, both C-2's (67.35 and 71.53 p.p.m.) are unsubstituted, and one C-2 (67.35 p.p.m.) is adjacent to a glycosidic linkage, the following two structures are possible: (*a*) a (1→4)-mannan having rhamnosyl branches at positions 3, or (*b*) →4)-α-Man*p*-(1→3)-α-Rha*p*-(1→ repeating-units (**1**).

Structure (*a*) was ruled out by a Smith-degradation experiment since only the Rha units remained intact and low-molecular-weight fragments were obtained.

Next, the C-1 signals (101.68 and 97.05 p.p.m.) were assigned. No large upfield shift is to be expected[8] for an $\alpha D_{2a}\rightarrow 4D_{2a}$ ($\alpha L_{2a}\rightarrow 4L_{2a}$)-linkage[9] or an $\alpha L_{2a}\rightarrow 4D_{2a}$



1

TABLE II

^{1}H-CHEMICAL SHIFTS[a] OF THE *Clostridium thermohydrosulfuricum* L111-69 GLYCAN DETERMINED WITH CHORTLE[13]

Glycosyl residue	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*
(1→3)-α-Rha*p*-(1→4)	4.92	4.19	3.8	3.51	4.03	1.27
(1→4)-α-Man*p*-(1→3)	5.01	3.9	3.9	3.8	3.9	3.7[b]

[a]Shifts are in p.p.m. downfield from the signal of sodium 3-(trimethylsilyl)propionate-d_4. Shifts determined from the proton spectrum directly are given with two decimals. [b]CHORTLE affords only the mean of the chemical shifts of both the H-6 resonances. These signals are located at ~3.45 and ~3.95 p.p.m.

($\alpha D_{2a}\rightarrow 4L_{2a}$)-linkage[10]. Thus, the signal at 101.68 p.p.m. corresponds to C-1 of Rha. On the other hand, for an $\alpha D_{2a}\rightarrow 3L_{2a}$-linkage[11], an upfield shift is to be expected in contrast to an $\alpha L_{2a}\rightarrow 3L_{2a}$-linkage[12]. Therefore, the signal at 97.05 p.p.m. corresponds to C-1 of Man. The remaining signals were assigned by application of the reported substitution shifts[11].

The ^{1}H-n.m.r. spectrum was assigned using the CHORTLE (*c*arbon–*h*ydrogen correlations from *o*ne-dimensional pola*r*ization-*t*ransfer spectra by *l*east-squares analysis)[13] method (Table II). The observed 0.4-p.p.m. downfield shift of the Rha H-5 resonance agrees with the structure, because H-5 of Rha is close to the HO-3 of Man in the glycan. Similar effects are observed if H-1 is close to an oxygen[14]. The same deshielding (0.4 p.p.m.) of H-5 of Man (compared to methyl α-D-mannopyranoside) is observed, because H-5 is close to HO-4 of the Rha.

The $[\alpha]_D$ value of the polysaccharide based on those for monosaccharides was calculated according to Klyne's rule[15] (Table III). The observed $[\alpha]_D^{20}$ value of +12° (*c* 1, water) agrees well with the value (+11°) calculated for the D-Man*p*/L-Rha*p* combination.

TABLE III

$[M]_D$ VALUES CALCULATED ACCORDING TO KLYNE'S RULE[15] FOR POLYSACCHARIDES COMPOSED OF Man–Rha DISACCHARIDE REPEATING-UNITS

Compound	$[\alpha]_D^{20}$ *(degrees)*	$[M]_D$ *(degrees)*
Methyl α-D-mannopyranoside[16]	+79.2	+154
Methyl α-L-mannopyranoside[16]	−79.4	−154
Methyl α-D-rhamnopyranoside	+67.2	+120
Methyl α-L-rhamnopyranoside[17]	−67.2	−120
Polysaccharides (calc.)		
(D-Man*p* + D-Rha*p*)	+89	+274
(D-Man*p* + L-Rha*p*)	+11	+34
(L-Man*p* + D-Rha*p*)	−11	−34
(L-Man*p* + L-Rha*p*)	−89	−274
C. thermohydrosulfuricum L111-69 glycan, observed	+12.3	+38

EXPERIMENTAL

General. — Hexose and rhamnose were determined colorimetrically or by g.l.c.[3]. The $[\alpha]_D^{20}$ value was determined with a Perkin–Elmer model 141 polarimeter. ^1H- and ^{13}C-n.m.r. spectra were recorded[3] with a Bruker WM 250 instrument at 298 K for solutions in D_2O. The CHORTLE experiment[13] was performed using the pulse sequence described at 298 K for a solution of the glycan in D_2O.

Isolation of the glycopeptide. — *Clostridium thermohydrosulfuricum* strain L111-69 was grown[2] under anaerobic conditions in TS-medium (Becton Dickinson) in 4-L bottles at 65°. The isolation of the S-layer glycoprotein and degradation into the glycopeptide with pronase followed described methods[3]. During the purification, chromatography on Dowex 50W-X8 (H^+) resin was omitted. After chromatography on a column (1 × 113 cm) of Bio-Gel P-4, the final purification was performed on a calibrated column (1 × 113 cm) of Bio-Gel P-30 equilibrated with aqueous 1% acetic acid. Appropriate fractions were collected and lyophilised, and the glycopeptide was stored at −20°.

Smith degradation[3]. — The glycopeptide (18 mg) was oxidised for 48 h at 4° in the dark, then reduced with sodium borohydride, hydrolysed with aqueous 5% acetic acid (1 mL) for 1.5 h at 100°, and applied to a column (1 × 113 cm) of Bio-Gel P-2 equilibrated with water. Fractions (1 mL) were tested[3] for rhamnose.

ACKNOWLEDGMENTS

We thank Dr. Frank M. Unger for critical reading of the manuscript, and Klaus Jaeger for skilful technical assistance. This work was supported in part by the "Fonds zur Förderung der wissenschaftlichen Forschung in Österreich" project 5290 and the "Österreichisches Bundesministerium für Wissenschaft und Forschung".

REFERENCES

1 U. B. Sleytr and P. Messner, *Annu. Rev. Microbiol.*, 37 (1983) 311–339.
2 U. B. Sleytr and K. J. I. Thorne, *J. Bacteriol.*, 126 (1976) 377–383.
3 P. Messner, U. B. Sleytr, R. Christian, G. Schulz, and F. M. Unger, *Carbohydr. Res.*, 168 (1987) 211–218.
4 P. A. J. Gorin and M. Mazurek, *Can. J. Chem.*, 53 (1975) 1212–1223.
5 K. Bock, I. Lundt, and C. Pedersen, *Tetrahedron Lett.*, (1973) 1037–1040.
6 R. Christian, G. Schulz, and F. M. Unger, *Tetrahedron. Lett.*, (1985) 3951–3954.
7 R. Christian, G. Schulz, F. M. Unger, P. Messner, Z. Küpcü, and U. B. Sleytr, *Carbohydr. Res.*, 150 (1986) 265–272.
8 N. K. Kochetkov, O. S. Chizhov, and A. S. Shashkov, *Carbohydr. Res.*, 133 (1984) 173–185.
9 L. V. Backinowsky, N. E. Balan, A. S. Shashkov, and N. K. Kochetkov, *Carbohydr. Res.*, 84 (1980) 225–235.
10 K. Bock and M. Meldal, *Acta Chem. Scand., Ser. B*, 37 (1983) 775–783.
11 A. Jaworska and A. Zamojski, *Carbohydr. Res.*, 126 (1984) 191–203.
12 C. Laffite, A. M. Nguyen Phuoc Du, F. Winternitz, R. Wylde, and F. Pratviel-Sosa, *Carbohydr. Res.*, 67 (1978) 105–115.
13 G. A. Pearson, *J. Magn. Reson.*, 64 (1985) 487–500.
14 R. U. Lemieux and K. Bock, *Arch. Biochem. Biophys.*, 221 (1983) 125–134.
15 W. Klyne, *Biochem. J.*, 47 (1950) xli–xlii.
16 E. Fischer and L. Beensch, *Ber.*, 29 (1896) 2927–2931.
17 E. Fischer, M. Bergmann, and A. Rabe, *Ber.*, 53 (1920) 2362–2388.