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The poly-α- and -β-1,4-glucuronic acid moiety of teichuronopeptide from the cell wall of the alkalophilic *Bacillus* strain C-125

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Teichuronopeptide is a structural component of the cell wall of alkalophilic *Bacillus* strain C-125 and is a complex composed of polyglutamate and polyglucuronate. A structural analysis of the polyglucuronic acid moiety was carried out. Periodate oxidation and Smith degradation of the moiety, and enzymic analysis after reduction of glucuronic acid to glucose, revealed that glucuronic acid bound together with alternately α- and β-1,4-linkages.

INTRODUCTION

Uronic acid is widely distributed as a component of various polysaccharides and glycoproteins and is found commonly in the Animal, Plant and Microbial Kingdoms. There are, however, a few polyuronic acids which are entirely composed of uronic acids. Such polyuronic acids reported so far are alginic acid, mucoric acid, pectic acid and protuberic acid (Haug *et al.*, 1967; Bartnicki-Garchia & Reyes, 1968; Fujibayashi *et al.*, 1970; Tsuchihashi *et al.*, 1981, 1983). Among them, mucoric acid and pectic acid are homouronic acids. These polyuronic acids are distributed mainly in plants, algae and fungi. Only alginic acid, which is a well-known constituent of brown algae, is also found as an extracellular polysaccharide produced by certain bacteria belonging to the genera *Pseudomonas* and *Azotobacter* (Carlson & Matthews, 1966; Linker & Jones, 1966; Gorin & Spencer, 1966; Skjak-Braek *et al.*, 1986).

My laboratory has investigated structural components of cell walls of alkalophilic *Bacillus* spp. (Aono & Horikoshi, 1983). Two acidic polymers are found in cell walls of alkalophilic *Bacillus* C-125. One is a teichuronic acid (TUA) made up of galacturonic acid, glucuronic acid and *N*-acetylfucosamine (Aono & Uramoto, 1986). The other is teichuronopeptide (TUP), composed of polyglucuronic acid and poly-γ-L-glutamic acid (Aono, 1985, 1987, 1989). These acidic polymers may contribute the ability of the organism to grow at alkaline pH (Aono, 1990).

The present paper reports a structural analysis of the polyglucuronic acid moiety from TUP of alkalophilic *Bacillus* sp. C-125. Glucuronic acid residues are bound through alternative linkages of α-1,4 and β-1,4 in the moiety.

MATERIALS AND METHODS

Organism and isolation of TUP

The alkalophilic strain of *Bacillus* sp. C-125 (FERM 7344) was grown at pH 10. Cell walls were prepared from early-stationary-phase cells by inactivation of autolytic enzymes in hot SDS, disruption of cells by sonication and differential centrifugation as described previously (Aono & Horikoshi, 1983). TUA and TUP were extracted from the cell walls with 5% (w/v) trichloroacetic acid and separated by DEAE-cellulose column chromatography (Aono, 1985). Polyglucuronic acid was isolated from TUP by hydrazinolysis (Aono, 1989).

Periodate oxidation of TUP

TUP (12 mg) was oxidized with 10 mM-$NaIO_4$ in 3 ml of 30 mM-acetic acid/NaOH buffer, pH 5.3, or 30 mM-$KHCO_3$/HCl, pH 7.6, at 15 °C in the dark. The concentration of glucuronic acid was 3.6 mM. The oxidation procedure was monitored by measuring the absorbance of periodic ion at 310 nm (Glick *et al.*, 1962). After 120 h oxidation, excess periodic ion was consumed by addition of ethylene glycol (40 μmol). Aldehyde groups produced by periodate oxidation were reduced by addition of solid $NaBH_4$. Excess $NaBH_4$ was destroyed by adjusting the reaction mixture to pH 5 with acetic acid. The samples were dialysed against distilled water (M_r cut-off of dialysis tubing 1000).

The non-diffusible product of dialysis was divided into two parts. One part was hydrolysed in 0.2 M-HCl at room temperature for 48 h. The hydrolysate was subjected directly to cellulose t.l.c. in the solvent A (see below). Aldehyde compounds were located by an alkaline $AgNO_3$ spray. The other part was hydrolysed in 0.5 M-H_2SO_4 at 100 °C for 3 h. The hydrolysate was neutralized with solid $BaCO_3$ and passed through a small column of Dowex 50-X4 (200–400 mesh; H^+ form). Effluent was concentrated with a rotary evaporator at 35 °C and heated at 50 °C for 30 min with $BaCO_3$. Acidic products were analysed on Avicel cellulose thin-layer plates in the solvent B or C (see below) and located with Bromocresol Green.

Reduction of glucuronic acid residues and preparation of oligosaccharides

TUP (2.5 g) was partially hydrolysed in 0.5 M-HCl at 100 °C for 1 h. After removal of HCl by a rotary evaporator at 37 °C, the hydrolysate was passed through a column (1.7 cm × 12.5 cm) of Dowex 50-X4 (200–400 mesh, H^+ form). Effluent and water wash were combined and freeze-dried. The freeze-dried matter was dissolved in water and reduced with 1-cyclohexyl-3-(2-morpholinoethyl)carbodi-imide metho-*p*-toluenesulphonate and $NaBH_4$ (Taylor & Conrad, 1972). The reaction product was passed through a column (2 cm × 43 cm) of Dowex 50-X4 (200–400 mesh, H^+ form). Effluent and water wash were freeze-dried. Boric acid was removed by a rotary evaporator at 30 °C with several additions of methanol.

The product was partially hydrolysed in 0.15 M-H_2SO_4 at 100 °C for 1 h. The partial hydrolysate was desalted with Dowex 50-X4 (H^+ form) and Dowex 1-X4 (HCO_3^- form) after removal of

Abbreviations used: TUA, teichuronic acid; TUP, teichuronopeptide; saccharide abbreviations are defined in Table 2.

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H_2SO_4 with $BaCO_3$, and freeze-dried. The freeze-dried matter was dissolved in 3 ml of 50 mM-acetic acid/pyridine, pH 4.1, and applied to a column of Sephadex G-15 (3.0 cm × 96 cm) which had been equilibrated with the same buffer. The column was eluted with the buffer at a flow rate of 35 ml/h. Fractions (3 ml) were assayed for reducing groups and glucose residues.

Fractions of reducing sugars (monomer to tetramer) were separately pooled and freeze-dried. Each of the fractions was loaded on a column (1 cm × 10 cm) of activated charcoal CHR-30 (60–150 mesh; Nakarai Chemicals, Tokyo, Japan). The column was eluted by a step-wise elution with water containing ethanol at a flow rate of 20 ml/h. Reducing sugars were further purified by Avicel cellulose t.l.c. in solvent A.

Enzymic hydrolysis of oligosaccharides

The saccharides (10 nmol) were incubated with 0.01 unit of α- or β-glucosidase in 20 μl of 10 mM-acetic acid/NaOH buffer (at respectively pH 6.5 or 4.5) containing 1 mM-EDTA at 30 °C overnight. The samples were treated with 0.01 unit of glucoamylase or α-amylase in 20 μl of 10 mM-acetic acid/NaOH buffer (at respectively pH 5.0 or 6.0). Authentic oligomers of α- and β-(1→4)-glucose were also treated as references. After incubation, 40 μl of ethanol was added to the reaction mixture. The mixture was centrifuged at 15000 *g* for 10 min. The supernatant was dried over NaOH *in vacuo*. The residue was dissolved in 6 μl of distilled water and one half was subjected to Avicel t.l.c. in solvent C.

Analysis

(1) Glucuronic acid was directly determined by the carbazole/H_2SO_4 reaction (Spiro, 1966). (2) Glucose was directly estimated by the anthrone/H_2SO_4 reaction (Spiro, 1966). Monomeric D-glucose was assayed with hexokinase and glucose-6-phosphate dehydrogenase (Bergmeyer *et al.* 1974). (3) D-Glucitol was determined with sorbitol dehydrogenase (Beutler, 1984). (4) Reducing groups were determined with ferricyanide, with glucose as a reference (Park & Johnson, 1949). (5) The p.m.r. spectrum in ^2H_2O was measured at room temperature with a JEOL JHN-FX 400 FT n.m.r. spectrometer (400 MHz). ^{13}C-n.m.r. in ^2H_2O was recorded at room temperature with a JEOL-FX-100 spectrometer with complete proton-decoupling. Tetramethylsilane was used as an external standard for the spectra.

Cellulose t.l.c.

Ascending chromatograms were run on Avicel cellulose thin layers at room temperature in the following solvents: (A) *n*-butanol/pyridine/water (6:4:3, by vol.); (B) *n*-butanol/formic acid/water (4:1:2, by vol.); (C) ethyl acetate/pyridine/water/acetic acid (5:5:3:1, by vol.).

Materials

Yeast α-glucosidase (EC 3.2.1.20), and sweet-almond β-glucosidase (EC 3.2.1.21), glucoamylase (EC 3.2.1.3), hexokinase (EC 2.7.1.1), glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and sorbitol dehydrogenase (EC 1.1.1.14) were purchased from Boehringer Mannheim. α-Amylase was obtained from Wako Pure Chemical, Osaka, Japan. L-Glucose was purchased from Sigma Chemical Co. A dialysis tube (Spectra/Por 6; M_r cut-off 1000) was obtained from Spectrum Medical Industries, Los Angeles, CA, USA. α-(1 → 4)-D-glucose oligomers were prepared from soluble starch by hydrolysis with α-amylase. β-(1→4)-D-glucose oligomers were prepared from cellulose by acetolysis (Dickey & Wolfrom, 1949). Erythronic acid was produced by oxidation of D-erythrose with bromine water (Mandl & Neuberg, 1957). α- or β-D-Glucopyranosyl-(1→4)-D-glucitol was prepared from maltose or cellobiose by reduction with $NaBH_4$.

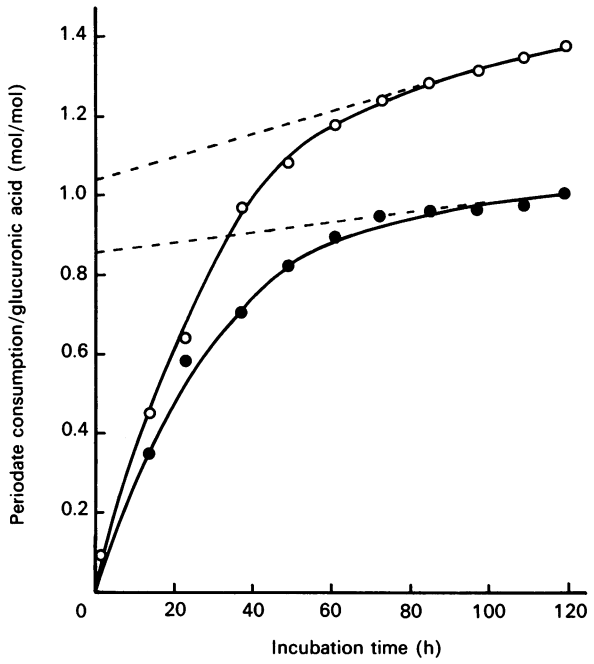

Fig. 1. Periodate oxidation of glucuronic acid present in TUP

Glucuronic acid residues (3.6 mM) were oxidized with 10 mM-$NaIO_4$ in 30 mM-acetic acid/NaOH buffer (pH 5.3; ●) or 30 mM-$KHCO_3$/HCl (pH 7.6; ○) at 15 °C. Consumption of periodic ion was monitored by measuring A_{310}. The broken line extrapolates to 0 h in order that the consumption of periodate ion by glucuronic acid residues can be calculated.

RESULTS

Consumption of periodic ion by TUP

The final consumption of periodic ion varied according to the pH value at which the TUP was oxidized with periodate (Fig. 1). The consumption of periodic ion was 1.02 mol/mol of initially present glucuronic acid when TUP was oxidized at pH 7.6. It was 0.86 mol/mol when it was oxidized at pH 5.3. It is likely that a portion of free hydroxy groups was blocked, owing to spontaneous formation of ester or lactone at acidic pH.

Smith-degradation products

An aldehyde compound derived from the C-1 anomeric carbon atom of glucuronic acid was released by oxidative fission with periodate, reduction with $NaBH_4$ and hydrolysis under weak conditions. The aldehyde product was detected without any purification or concentration and corresponded to authentic glycolaldehyde (R_F 0.72), but not to glyceraldehyde (R_F 0.58) in solvent A.

Complete Smith degradation was carried out to detect a carboxy compound derived from the C-6 carboxy group of glucuronic acid. An acid spot could be detected by weak alkaline treatment with $BaCO_3$ after partial purification and concentration. The acid was considered to form readily a stable lactone form. The acid spot corresponded to erythronic acid (R_F 0.13), which could form a stable γ-lactone, but not to glycolic acid (R_F 0.53), glyceric acid (R_F 0.29) or glucuronic acid (R_F 0.02) in solvent B.

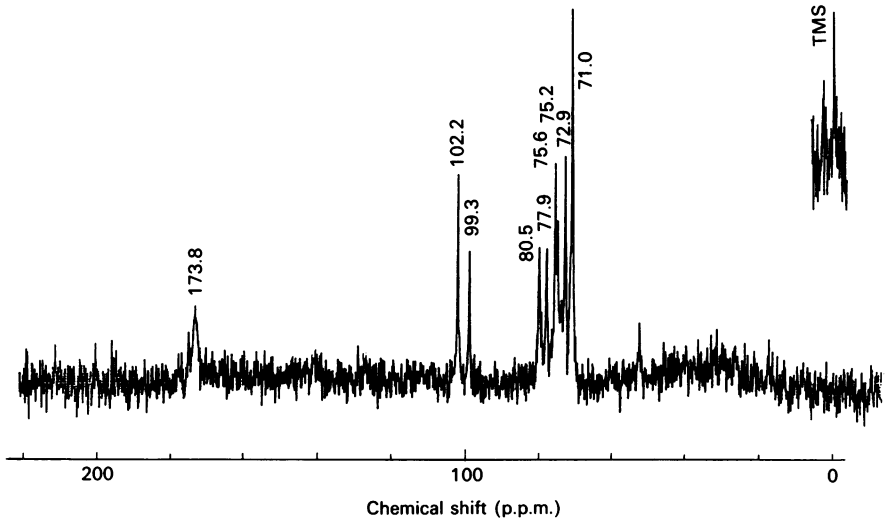

Fig. 2. ^{13}C-n.m.r. spectrum of polyglucuronic acid prepared from TUP

Chemical-shift values are relative to trimethylsilane (TMS).

Table 1. Assignment of chemical-shift values of polyglucuronic acid prepared from TUP

Each signal was assigned on the comparison with values reported previously. Chemical-shift values were measured using tetramethylsilane as an external standard.

	Chemical shift (p.p.m.)						
Residue	C-1	C-2	C-3	C-4	C-5	C-6	H-1
α-Glucuronic acid	99.3	71.0	71.0	77.9	71.0	173.8	5.40
β-Glucuronic acid	102.2	72.9	75.2	80.5	75.6	173.8	4.58

N.m.r. spectra of the polyglucuronic acid moiety

The ^{13}C-n.m.r. spectrum of the polyglucuronic acid showed several signals, suggesting that the glucuronic acid residue was not bound through a sole linkage (Fig. 2). The ^{13}C-n.m.r. spectra of β-(1 → 4)-linked glucuronic acid were reported by Tsuchihashi *et al.* (1981, 1983). The signals were assigned on the assumption that the polyglucuronic acid is composed of glucopyranosyluronic acid (Table 1). The p.m.r. spectrum of the polyglucuronic acid showed two signals for H-1 at 4.58 and 5.40 p.p.m. that corresponded to H-1 in β- and α-anomers of glucuronic acid respectively (Table 1). The integral of the H-1 signals showed that a molar ratio of α- and β-anomers was 1:1.07.

Reduction of the polyglucuronic acid moiety and preparation of oligosaccharides

Glucuronic acid residues were reduced to glucose residues for structural analysis of the polyglucuronic acid moiety. TUP (2.5 g dry wt.; glucuronic acid, 2.3 mmol; L-glutamic acid, 11 mmol) was partially hydrolysed. Most of glutamic acid was removed with Dowex 50 (H^+), since large amounts of glutamic acid interfered with the reduction of glucuronic acid with the carbodi-imide. The hydrolysate (dry wt. 0.69 g; glucuronic acid, 1.9 mmol; L-glutamic acid, 0.12 mmol) was reduced to oligoglucan (glucose, 1.1 mmol; glucitol, 0.4 mmol). Glucitol was produced from glucuronic acid at the reducing ends of oligoglucuronic acids produced during the partial hydrolysis. The oligoglucan was partially hydrolysed to produce lower oligosaccharides. Recovery of the oligosaccharides was 0.75 mmol of glucose after desalting with the ion-exchange resins. Some of the glucose was adsorbed to Dowex 1, probably because glucuronic acid residues or boric acid partially remained. The oligosaccharides were fractionated by gel chromatography on Sephadex G-15 (Fig. 3). Peaks of reducing sugars were different from those of glucose residues, because a portion of the oligosaccharide was non-reducing sugar (glucitol). Monosaccharides (fractions 146–156), disaccharides (fractions

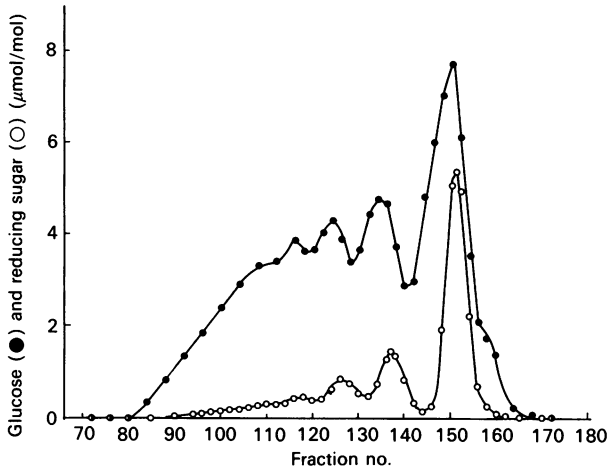

Fig. 3. Sephadex G-15 gel chromatography of the oligosaccharides prepared from reduced TUP

TUP (2.5 g) was partially hydrolysed with 0.5 M-HCl at 100 °C for 1 h. Most of glutamic acid was removed by passing through a Dowex 50-X4 (H^+ form) column. Glucuronic acid residues were reduced with carbodi-imide and $NaBH_4$. The reduced product was partially hydrolysed in 0.15 M-H_2SO_4 at 100 °C for 1 h. After removal of H_2SO_4 with $BaCO_3$, the partial hydrolysate was desalted, then fractionated on a column of Sephadex G-15 (3.0 cm × 96 cm) which had been equilibrated with 50 mM-acetic acid/pyridine, pH 4.1. The column was eluted with buffer at a flow rate of 35 ml/h. Fractions (3 ml) were assayed for reducing groups (○) and glucose residues (●).

Table 2. Characterization of oligosaccharides

Saccharides α-2–α-4 represent maltose, maltotriose and maltotetraose. Saccharides β-2–β-4 represent cellobiose, cellotriose and cellotetraose. Saccharides OS are described in the text. [EtOH] represents the percentage concentration of ethanol at which the oligosaccharide was eluted from a column of activated charcoal. These saccharides were subjected to Avicel cellulose t.l.c. in solvent A. R_{Glc} represents mobility of each saccharide relative to glucose. The saccharides were hydrolysed with HCl, α- or β-glucosidases or glucoamylase. Products were analysed by t.l.c. Products in parentheses indicate that the original saccharides were not completely hydrolysed under the conditions described in the Materials and methods section. — Means that the saccharide was not hydrolysed. A blank space means 'not tested'. An identification in parentheses is tentative.

Saccharide	[EtOH]	R_{Glc}	Hexoses: Reducing end	Products obtained by hydrolysis with: HCl	α-Glucosidase	β-Glucosidase	Glucoamylase	Identification
OS-2-1	7	0.85	2.3	Glc	Glc	—	(Glc)	α-Glc*p*-(1→4)-Glc*p*
OS-2-2	10	0.80	1.8	Glc	—	Glc	—	β-Glc*p*-(1→4)-Glc*p*
OS-2-3	5	0.72		Glc, glucitol	Glc	—	(Glc)	α-Glc*p*-(1→4)-Glucitol
OS-2-4	5	0.62		Glc, glucitol	—	(Glc)	—	β-Glc*p*-(1→4)-Glucitol
OS-3-1	15–20	0.68	3.1	Glc	—	(Glc, OS-2-1)	—	β-Glc*p*-(1→4)-α-Glc*p*-(1→4)-Glc*p*
OS-3-2	20–25	0.61	3.1	Glc	Glc, OS-2-2	—	Glc, OS-2-2	α-Glc*p*-(1→4)-β-Glc*p*-(1→4)-Glc*p*
OS-4-1	20	0.34	4.2	Glc	—	—	(Glc, OS-3-1)	α-Glc*p*-(1→4)-β-Glc*p*-(1→4)-α-Glc*p*-(1→4)-Glc*p*
OS-4-2	30	0.32	4.4	Glc	—	(Glc)	—	(β-Glc*p*-(1→4)-α-Glc*p*-(1→4)-β-Glc*p*-(1→4)-Glc*p*)
α-2		0.83			Glc	—	(Glc)	
α-3		0.72			Glc, α-2	—	Glc	
α-4		0.56			(Glc)	—	Glc	
β-2		0.80			—	Glc	—	
β-3		0.47			—	Glc, β-2	—	
β-4		0.20			—	(Glc, β-2, β-3)	—	

133–145), trisaccharides (fractions 123–132) and tetrasaccharides (fractions 115–122) were separately pooled. Di-, tri- or tetrasaccharide fractions were rechromatographed on the same Sephadex G-15 column. Recoveries of the reducing saccharides were 95 μmol of monosaccharide, 30 μmol of disaccharides, 21 μmol of trisaccharides and 15 μmol of tetrasaccharides.

These saccharides were further purified by activated-charcoal column chromatography and Avicel cellulose t.l.c. (Table 2). Monosaccharide was eluted from the activated charcoal with 2% (v/v) ethanol and contained glucose and glucitol in a molar ratio of 1:0.3. The disaccharide fraction contained two reducing sugars (OS-2-1 and OS-2-2; for definition, see Table 2) and two non-reducing sugars (OS-2-3 and OS-2-4). These saccharides were eluted from the charcoal column in the following order: OS-2-3 and OS-2-4 with 5% (v/v) ethanol; OS-2-1 with 7% (v/v) ethanol; and finally OS-2-2 with 10% (v/v) ethanol. The non-reducing sugar was detected on the thin layer with alkaline $AgNO_3$ when colour development was prolonged. Two reducing trisaccharides (OS-3-1 and OS-3-2) were purified. Non-reducing sugars were found also in the trisaccharide fraction, but these were not purified. Two reducing sugars (OS-4-1 and OS-4-2) were purified from the charcoal-column eluate.

Stereoisomerism of glucuronic acid

Monomeric glucose was purified by cellulose t.l.c. in the solvent A and assayed by three basically different methods; anthrone/H_2SO_4 reaction for total hexoses, reducing-group determination with ferricyanide for total reducing sugars, and enzymic analysis with hexokinase and D-glucose-6-phosphate dehydrogenase for D-glucose. Authentic L-glucose was not catabolized at all in the enzymic analysis. A ratio of the determinations by these methods was 1.06 (total hexoses): 0.94 (total reducing sugars): 1.00 (D-glucose).

D-Glucose was converted into D-gluconolactone 6-phosphate and L-glucose was not altered during determination of D-glucose. The reaction mixture used for the enzymic analysis was desalted with Dowex 1 (HCO_3^- form) plus Dowex 50 (H^+ form) and then subjected for Avicel cellulose t.l.c. in the solvent C. A reducing spot was not found, suggesting that L-glucose was absent from the fraction.

Characterization of oligosaccharides

The saccharides were analysed by Avicel t.l.c. in solvent A and C, before and after complete hydrolysis with 1 or 4 M-HCl at 100 °C for 1 h. A major reducing spot corresponded to glucose. A faint spot appeared on prolonged colour development and corresponded to glucitol (R_{Glc} 0.89). These results suggested that all of the saccharides were oligomers of glucose and glucitol. Further characterization of the saccharides was carried out by enzymic hydrolysis and Avicel t.l.c. The results are summarized in Table 2.

Maltohexaose and higher α-(1 → 4)-glucans were digested with α-amylase to produce glucose, maltose, maltotriose and so on. A fraction containing higher (above pentasaccharide) oligosaccharides (Fig. 2) was treated with α-amylase. No glucose was released from the fraction. A slight amount of glucose was released from the fraction with β-glucosidase or glucoamylase (results not shown).

DISCUSSION

Homopolyglucuronic acid is rarely found in bacterial cells. Stereoisomerism of glucuronic acid, which is a constituent of TUP, was examined after the reduction. As a result, it was concluded that the polyglucuronic acid moiety of TUP was composed of essentially D-isomer.

Consumption of periodic ion by TUP suggests that, on average, 1 mol of glucuronic acid residue was oxidized with 1 mol of periodate and that one free α-glycol structure would be present in a residue (Fig. 1). Analyses by controlled or complete Smith

degradation of TUP indicated that hydroxy groups 2 and 3 of the glucuronic acid residue were free, and that hydroxy groups 1, 4 and 5 were blocked. The C-6 carboxy group must also be free, because the polyglucuronic acid moiety is highly acidic (Aono, 1989). The C-1 anomeric hydroxy group must blocked, because reducing ability is enhanced by hydrolysis of the polyglucuronic acid (results not shown). Therefore the polyglucuronic acid moiety in TUP is suggested to be composed of (1 → 4)-linked glucopyranosyluronic acid and/or (1 → 5)-linked glucofuranosyluronic acid.

N.m.r. spectra revealed two types of equimolar glycosyl linkages in the polyglucuronic acid preparation (Fig. 2). That the two linkages were originally present in TUP and not artefacts is suggested by the observation that the same signals for carbon atoms of glucuronic acid were also found in the TUP preparation (results not shown). The linkages can be considered as α- and β-(1 → 4)-pyranosyl linkages (Table 1). Therefore the polyglucuronic acid is suggested to be either an equimolar mixture of poly-α- and β-(1 → 4)-glucopyranosyluronic acids or a polymer composed of α- and β-(1 → 4)-glucopyranosyluronic acids.

Structural analysis of oligomers from the polyglucuronic acid was necessary before a final conclusion could be made about structure of the polyglucuronic acid moiety. Glucuronic acid was reduced to glucoses, because several types of enzymes acting on D-glucan are commercially obtained. The reducing disaccharides prepared from reduced TUP were maltose [α-D-glucopyranosyl-(1 → 4)-D-glucopyranose] and cellobiose [β-D-glucopyranosyl-(1 → 4)-D-glucopyranose] (Table 2). These results support the notion that the polyglucuronic acid is linked through α- and β-(1 → 4)-linkages and rule out a (1 → 5)-linkage. Maltotriose or cellotriose was not obtained. The reducing tri- or tetrasaccharides were composed of D-glucose binding alternatively through α- and β-(1 → 4)-linkages. α-Amylase did not act on the pentasaccharide, suggesting that α-(1 → 4)-pentasaccharide was not present.

These results lead one to conclude that the polyglucuronic acid moiety of TUP is composed mainly of a repeating unit that is → 4)-α-D-glucopyranosyluronic acid-(1 → 4)-β-D-glucopyranosyluronic acid-(1→. Little, if any, of the structure contains branched chains.

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REFERENCES

Aono, R. (1985) J. Gen. Microbiol. **131**, 105–111
Aono, R. (1987) Biochem. J. **245**, 467–472
Aono, R. (1989) J. Gen. Microbiol. **135**, 265–271
Aono, R. (1990) Biochem. J. **266**, 933–936
Aono, R. & Horikoshi, K. (1983) J. Gen. Microbiol. **129**, 1083–1087
Aono, R. & Uramoto, M. (1986) Biochem. J. **233**, 291–294
Bartnicki-Garcia, S. & Reyes, E. (1968) Biochim. Biophys. Acta **170**, 54–62
Bergmeyer, H. U., Bernet, E., Schmidt, F. & Stork, H. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 2nd edn., vol. 3, pp. 1196–1201, Verlag Chemie, Weinheim
Beutler, H. O. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd edn., vol. 6, pp. 356–362, Verlag Chemie, Weinheim
Carlson, D. M. & Matthews, L. W. (1966) Biochemistry **5**, 2817–2822
Dickey, E. E. & Wolfrom, M. L. (1949) J. Am. Chem. Soc. **71**, 825–828
Fujibayashi, S., Abe, H. & Nisizawa, K. (1970) J. Biochem. (Tokyo) **67**, 37–45
Glick, M. C., Chen, I. W. & Zilliken, F. (1962) J. Biol. Chem. **237**, 981–987
Gorin, P. A. J. & Spencer, J. F. T. (1966) Can. J. Chem. **44**, 993–1001
Haug, A., Larsen, B. & Smidsrød, O. (1967) Acta Chem. Scand. **21**, 691–704
Linker, A. & Jones, R. S. (1966) J. Biol. Chem. **241**, 3845–3851
Mandl, I. & Neuberg, C. (1957) Methods Enzymol. **3**, 162–172
Park, J. T. & Johnson, M. J. (1949) J. Biol. Chem. **181**, 149–151
Skjak-Braek, G., Grasdalen, H. & Larsen, B. (1986) Carbohydr. Res. **154**, 239–250
Spiro, R. G. (1966) Methods Enzymol. **8**, 311–320
Taylor, R. L. & Conrad, H. E. (1972) Biochemistry **11**, 1383–1388
Tsuchihashi, T., Yadomae, T. & Miyazaki, T. (1981) Carbohydr. Res. **98**, 65–74
Tsuchihashi, T., Yadomae, T. & Miyazaki, T. (1983) Carbohydr. Res. **111**, 330–335