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Occurrence of Glucomuramic Acid in Gram-Positive Bacteria

R. W. WHEAT AND J. M. GHUYSEN

Department of Microbiology and Immunology and Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27706 and Service de Bacteriologie, 32 B[d] Constitution, Universite de Liege, Liege, Belgium

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Analyses of 48 gram-positive bacteria indicate only glucomuramic acid and no galactomuramic acid in cell walls.

The chemical synthesis of galactomuramic acid and the development of a rapid assay procedure by use of a modified commercial amino acid analyzer resulted in the observation that only glucomuramic acid occurs in the 16 gram-positive and gram-negative bacteria examined to date (5, 7). The present report extends this survey to include over 40 species of gram-positive bacteria from a wide variety of taxonomic groups.

Walls were prepared according to standard procedures by mechanical disruption with the use of glass beads in a Bühler disintegrator (Tubingen, Germany), or by French press disruption (*Mycobacteria* species), or by ultrasonic treatment with an MSE 500-w disintegrator (*Nocardia* species). Samples of 1 to 3 mg were heated in 1 ml of 6 N HCl for 18 to 24 hr in sealed evacuated tubes suspended in a refluxing water bath. In general, better yields of most cell wall components were obtained with the longer hydrolysis times. Samples were filtered, and HCl was removed by repeated evaporation under reduced pressure at 40 C. The residue was redissolved in water, and 1- to 0.5-mg portions were then analyzed for galactomuramic acid, glucosamine, glucomuramic acid, alanine, and glutamic acid by procedures described previously (5, 7). An example of the cochromatographic identification of glucomuramic acid in a *Streptomyces albus* G cell wall hydrolysate by retention time and both ninhydrin and reducing group functions by use of these procedures is shown in Fig. 1. (The peak appearing as a reducing compound just before diaminopimelic acid is due to a contaminant in the buffer used in this particular experiment.) No galactomuramic acid was observed in any sample examined. Only glucomuramic acid or a compound exhibiting identical mobilities, ninhydrin, and reducing group functions was observed in the amounts shown in Table 1 relative to several other cell wall components determined.

From the present and previous studies, it appears that galactomuramic acid does not occur naturally. Only glucomuramic acid has so far been identified in the bacterial species examined. Further, although galactosamine and other amino sugars may be found occasionally in some cell wall preparations, only glucosamine, identified both by chromatographic retention time and by means of the specific D-glucosamine 6-phosphate *N*-acetylase, appears to be universal among bacterial peptidoglycans. These findings strengthen the prevailing hypothesis (2) that the bacterial wall glycans are consistently chitin-like structures of β-1,4 linked pyranoside *N*-acetylglucosamine residues, except, however, that every other sugar is substituted at the C-3 hydroxyl position by a lactyl group which has the D configuration. Cellulose, chitin, and the glycan of peptidoglycans appear to play similar roles as cell-supporting structures in plants, fungi, and bacteria and are stable, linear β-1,4-linked polysaccharides. Models of bacterial glycans reveal that, as in chitin, hydrogen bonds extend between the C-3 hydroxyl group of *N*-acetylglucosamine and the C_1 to C_5 ring oxygen of the adjacent *N*-acetylglucomuramic acid, and that glycan chains which run head to tail can therefore be hydrogen-bonded (3). The consistency of the glycan structure throughout the bacterial world indicates that this structure must be essential for the functions of the polymer and suggests that any mutation which would alter its conformation is probably lethal. Strikingly, the only variations of the peptidoglycan muramic acids so far encountered consist of substitution of C-6 hydroxyl position by an acetyl group or a phosphodiester group (2) and in the replacement of *N*-acetylmuramic acid by *N*-glycolylmuramic acid (1) or by muramic lactam (6). With the possible exception that small amounts of mannomuramic acid (P. Sinay, R. W. Jeanloz, and P. H. Gross, Amer. Chem. Soc. **156**: Carbohydrate Div. Abstr. 21, 1968) may occur along with glucomuramic acid in *Micro-*

TABLE 1. *Relative concentrations of muramic acids and certain cell wall components of some gram-positive bacteria*

Organism	Cell wall component determined (μmoles per mg)				
	Galactomuramic acid[a]	Glucosamine	Glucomuramic acid	Alanine	Glutamic acid
Micrococcus citreus R 266 (IP)[b]	0.0	0.26	0.34	1.05	0.54
M. falvus (IP)	0.0	0.21	0.40	1.53	0.87
M. freudreichii ATCC 407	0.0	1.06	1.49	3.63	3.30
M. luteus ATCC 385	0.0	0.91	1.35	2.09	3.54
M. luteus ATCC 398	0.0	0.14	0.52	0.61	0.82
M. lysodeikticus NCTC 2665	0.0	0.37	0.38	1.22	0.63
M. radiodurans R_1	0.0	0.36	0.16	1.04	0.50
M. roseus R_{27} (IP)	0.0	0.35	0.21	1.75	0.44
M. varians NCTC 7281	0.0	0.20	0.22	0.56	0.93
Staphylococcus aureus Copenhagen	0.0	0.53	0.22	1.31	0.53
Gaffkya homari ATCC 10400	0.0	0.93	1.63	1.70	1.09
Sarcina lutea R 262 (IP)	0.0	0.20	0.29	0.98	0.75
Sporosarcina ureae	0.0	1.83	1.20	1.33	1.87
Aerococcus viridans 201	0.0	1.00	1.32	1.38	1.21
A. viridans ATCC 11563	0.0	0.74	1.67	1.71	1.89
Streptococcus faecium var. *durans* (thr)[c]	0.0	0.28	0.23	0.84	0.53
S. faecium var. *durans* (val)[c]	0.0	0.30	0.26	1.05	0.62
S. pyogenes, group A, type 14	0.0	0.58	0.44	3.62	1.00
Lactobacillus acidophilus 63 AM Gasser (IP)	0.0	0.34	0.25	1.54	0.62
L. (Bifidobacterium) bifidus 305a (IP)	0.0	0.16	0.14	0.73	0.40
Propionibacterium peterssonii 5962	0.0	0.47	0.71	0.81	0.63
P. rubrum NCIB 8901	0.0	0.35	0.62	0.63	0.37
P. technicum NCIB 5965	0.0	0.39	0.22	0.87	0.49
Butyribacterium rettgeri ATCC 10825	0.0	0.23	0.22	0.44	0.52
Corynebacterium anaerobicum Prevot 3103 (IP)	0.0	0.02	0.17	0.72	0.53
C. anaerobicum Prevot 3471 (IP)	0.0	0.07	0.26	0.82	0.44
C. fermentans 5211 (IP)	0.0	0.09	0.16	0.46	0.25
C. insidiosum ATCC 10253	0.0	1.28	0.69	0.41	0.62
C. poinsettiae 9682	0.0	0.10	0.10	0.04	0.05
C. tritici	0.0	0.10	0.22	0.24	0.30
Microbacterium lacticum ATCC 8081	0.0	0.47	0.12	0.21	0.06
Bacillus cereus	0.0	0.83	0.10	0.57	0.54
B. megaterium KM	0.0	0.50	0.46	1.52	0.88
B. pasteurii	0.0	1.65	0.74	1.14	0.49
B. stearothermophilus	0.0	1.55	1.71	3.46	1.52
Clostridium histolyticum 973 (IP) (collagenase, +; toxin, −)	0.0	0.12	0.13	0.61	0.55
C. perfringens type A, BP6K (toxin +) (IP)	0.0	0.11	0.08	0.14	0.10
Mycobacterium avium 802	0.0	0.08	0.10	0.19	0.11
M. kansasii P21 (defatted walls)	0.0	0.09	0.18	0.32	0.26
M. smegmatis (defatted walls)	0.0	0.18	0.24	0.52	0.25
M. segmatis (enriched peptidoglycan)	0.0	0.32	0.49	1.24	0.76
Nocardia kirovani (crude walls, defatted, saponified)	0.0	0.28	0.34	0.74	0.46
Streptomyces albus G	0.0	0.38	0.38	0.72	0.38
S. coelicolor A_3 (2) N_2	0.0	1.48	0.85	0.58	0.53
Streptomyces sp. K27	0.0	0.49	0.94	1.13	1.32
Streptomyces sp. R61	0.0	0.59	0.87	0.73	0.53
Planococcus sp.	0.0	0.79	0.74	0.90	1.26
Blue-green algae cells	0.0	0.12	0.11	0.79	0.90

[a] Limit of detection of galactomuramic acid was 0.01 μmoles/mg.

[b] IP, Institut Pasteur, Paris.

[c] Formerly *Streptococcus faecalis* ATCC 9790 but identified as *S. faecium* var. *durans* by O. Kandler (*private communication*). Thr and val: stationery-phase cells grown in media containing a limited supply of threonine or valine (4).

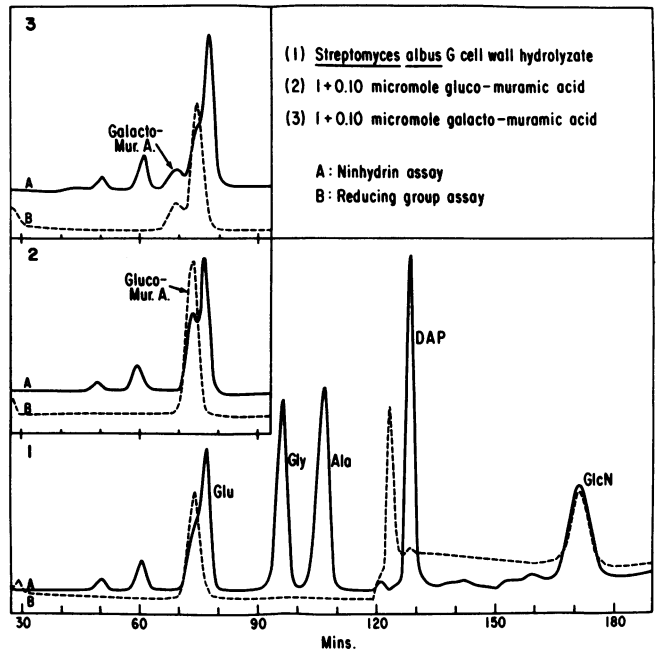

FIG. 1. *Chromatographic identification of glucomuramic acid and galactomuramic acid by addition of known amounts of the respective synthetic standards (7) to a cell wall preparation before hydrolysis as compared to the cell wall hydrolyzed alone. Recovery of the known muramic acids ranged from 70 to 80% or better. The separation was carried out on 55-cm columns of Aminex A-4 resin (Bio-Rad Laboratories, Richmond, Calif.), eluted at 68 ml/hr at 50 C with 0.2 N Na^+ sodium citrate buffers at pH 3.10, followed at 70 min (the buffer change appears after alanine) by pH 4.25 buffer containing 1.5% benzyl alcohol and 3% n-propanol, on a Beckman-Spinco 120 C amino acid analyzer modified as previously described (5), to assay both ninhydrin and reducing group reactivities.*

coccus lysodeikticus, the variations appear never to affect the D-gluco configuration of muramic acid.

The walls analyzed in the present report were prepared for more complete structural studies (1, 2, and *manuscript in preparation*). We are indebted for the preparation of some of the walls to Regina Tinelli (Paris), Micheline Guinand (Lyon), Melina Leyh-Bouville (Liege), O. Kandler and K. Schleifer (Munich), and J. F. Petit (Paris). We express our appreciation to Winfred Clingenpeel, Richard Smith, and Mrs. Hsu Chung for technical assistance.

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