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Composition of Peptidoglycans in *Bacteroidaceae*: Determination and Distribution of Lanthionine

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Peptidoglycans of organisms belonging to the strictly anaerobic family *Bacteroidaceae* were investigated for the presence of lanthionine. Different procedures for the quantitation of lanthionine were compared. Performic acid and peroxide oxidation procedures on ^{35}S-labeled peptidoglycan from *Fusobacterium nucleatum* Fev1 resulted in low yields of cysteic acid (42 and 60%, respectively) and many other additional unidentified oxidation products. Lanthionine was, however, recovered in high yield (89% or more) from acid hydrolysates of unoxidized peptidoglycans. Lanthionine was found exclusively in some species of *Fusobacterium*, in particular *F. nucleatum*, *F. necrophorum*, *F. russi*, and *F. gonidiaformans*, for which lanthionine may be ascribed a function as a taxonomic marker. Peptidoglycans of these bacteria are thus proposed to belong to a new chemotype, assigned Alδ. One strain of *Fusobacterium*, *F. mortiferum* VPI 0473 contained both lanthionine and diaminopimelic acid in about equal proportions. Species of *F. plauti* had a composition atypic of gram-negative cells. Chemotypic differences were also indicated among the species of *Bacteroides* investigated. Thus, some species contained lysine and not diaminopimelic acid as the major dibasic amino acid (e.g., *F. asaccharolyticus*). It is concluded that peptidoglycans of gram-negative organisms constitute a somewhat more heterogeneous group than hitherto assumed.

Peptidoglycans isolated from gram-negative bacteria normally contain the amino acids D-glutamic acid, *meso*-diaminopimelic acid (A_2pm), and alanine in molar ratios of about 1:1:2 and have been considered to constitute the same peptidoglycan type (7, 25). Few studies have been carried out on the cell wall of the nonspore-forming, anaerobic, gram-negative bacteria in the family *Bacteroidaceae* (1, 2, 10, 15–17, 26, 28–30). Recently, lanthionine, the monosulfur analog of A_2pm, was shown to be a natural constituent of the peptidoglycans of *Fusobacterium nucleatum* Fev1 (30) and, independently, of *F. nucleatum* ATCC 25586 (16), replacing A_2pm in these bacteria.

During the preparation of this manuscript Kato et al. (15) reported similar work on various bacteria of genus *Fusobacterium*. They found *F. mortiferum* 15 and *F. freundi* ATCC 9818 to contain both lanthionine and A_2pm.

The present paper deals with the chemical composition of peptidoglycans isolated from eight species of *Fusobacterium*, several *Bacteroides* species, and a single strain of *Leptotrichia buccalis*. The peptidoglycans were especially examined for the presence of lanthionine.

In general peptides containing sulfur amino acids are oxidized before acid hydrolysis to render these amino acids more stable to the analytical conditions employed (9). Lanthionine has been quantitated either as the sulfone after performic acid oxidation (33) or as the sulfoxide after peroxide oxidation (4, 30). The lack of convenient analytical systems for unmodified lanthionine has favored the use of these methods. The different oxidation procedures were compared with the conventional hydrolysis procedure of unoxidized samples performed with 4 N HCl at 105°C under vacuum. It was thus found that the conventional hydrolysis procedure was superior, especially when combined with analytical conditions which permitted the complete resolution of lanthionine (31).

MATERIALS AND METHODS

Chemicals. DL-Lanthionine and *meso*-lanthionine were from EGA Chemie, Steinheim, Albach, Federal Republic of Germany, and L-lanthionine was obtained through Serva Chemie, Heidelberg, Federal Republic of Germany. Radioactive materials were from the Radiochemical Centre, Amersham, England. All other reagents were reagent grade.

Strains and growth conditions. The 39 test strains examined are listed in Table 1. Cultivation was performed in 0.5-liter screw-cap bottles filled to the top

TABLE 1. Strains used in the study

Species	Designation	Received from[a]
F. nucleatum	Fev1	1
F. nucleatum	ATCC 10953	2
F. nucleatum	F1	Own isolate
F. nucleatum	F9	Own isolate
F. nucleatum	F18	Own isolate
F. necrophorum	SPH1	3
F. necrophorum	VPI 6161	4
F. necrophorum	N167	5
F. russi	VPI 0307	4
F. gonidiaformans	VPI 4381	4
F. gonidiaformans	VPI 0482A	4
F. gonidiaformans	VPI 11360	4
F. mortiferum	VPI 5696	4
F. mortiferum	VPI 0473	4
F. varium	VPI 0499A	4
F. naviforme	VPI 4877	4
F. naviforme	VPI 11936C	4
F. plauti	VPI 4145	4
L. buccalis	L11	Own isolate
B. fragilis	NCTC 9343	6
B. distasonis	ATCC 8503	2
B. thetaiotaomicron	VPI 5333	4
B. oralis	VPI 9958	4
B. oralis	VPI 8906	4
B. oralis	NP333	7
B. oralis	5540	8
B. malaninogenicus subsp. *melaninogenicus*	30	5
B. melaninogenicus subsp. *intermedius*	NCTC 9338	6
B. disiens	VPI 7852	4
B. bivius	VPI 6318	4
B. ruminicola subsp. *ruminicola*	C12	9
B. ruminicola subsp. *ruminicola*	D46	9
B. asaccharolyticus	VPI 4199	4
B. praeacutus	VPI 0217-1	4
B. hypermegas	VPI 2366	4
B. serpens	VPI 0-950	4
B. putredinis	VPI 4998-1	4
B. multiacidus	A405	10
B. multiacidus	GE-374-14	10

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with the following medium (in grams per liter): tryptone (Oxoid Ltd., London), 15: NaCl, 5; KH_2PO_4, 1.5; $Na_2HPO_4 \cdot 2H_2O$, 3.5; $NaHCO_3$, 0.5; yeast extract (Oxoid), 3.0; ascorbic acid or L-cysteine-hydrochloride, 1.0; vitamin B12, 0.001; haemin, 0.005; menadione, 0.001; and glucose, 2.5 (*Fusobacterium* spp.) or 5.0 (*Bacteroides* spp. and *L. buccalis*); pH 7.0. For cultivation of *Bacteroides oralis*, *B. melaninogenicus*, *B. asaccharolyticus*, and *L. buccalis*, the medium was supplemented with 2% human plasma. Cultures were grown for 48 h. The cells were harvested by centrifugation, washed twice with phosphate-buffered saline (pH 7.2), and stored as a paste at −21°C until used.

Preparation of peptidoglycan. The peptidoglycans were obtained by the use of boiling 4% sodium dodecyl sulfate followed by pronase digestion (30). The dry weight of the preparations accounted for 0.5 to 1.5% of the wet weight of the cells.

Radioactive labeling of cells. *F. nucleatum* Fev1 was incubated for 24 h in 1 liter of medium containing 1 mCi of L-[^{35}S]cysteine-hydrochloride (82 mCi/mmol). The cells were harvested, and the peptidoglycan was purified as described above. The specific radioactivity was about 800,000 cpm/mg of peptidoglycan.

Analytical methods. (i) Thin-layer chromatography (TLC). Ascending chromatography of samples of acid hydrolysates (4 N HCl, 105°C, 16 h under vacuum) was performed on cellulose thin-layer plates (Merck, Darmstadt, Federal Republic of Germany) in solvent 1 (*n*-butanol–pyridine–water–acetic acid, 60:40:30:3 by volume) and solvent 2 (methanol–pyridine–water–12 N HCl, 80:10:18:2 by volume).

(ii) Quantitative amino acid and amino sugar analysis. Routine quantitative analysis of acid hydrolysates (4 N HCl, 105°C, 16 h under vacuum) was performed in a microbore single-column system which allowed the complete separation of specific peptidoglycan constituents from other naturally occurring compounds (31).

The disaccharide tetrapeptide GlcNAc-MurNAc-L-Ala-D-Glu(A_2pm-D-Ala) was isolated from hen egg white lysozyme digest of *Escherichia coli* peptidoglycan (20) and used for calculating the color factor for specific amino sugars and A_2pm. A few samples were analyzed as the acetylated butyl esters (22) on a Perkin-Elmer gas chromatograph (model 900) with a glass column (0.175 by 185 cm) packed with Tabsorb (Regis C Chemical Co., Morton Grove, Ill.).

(iii) Detection of radioactivity. Hydrolyzed samples of ^{35}S-labeled Fev1 peptidoglycan were also monitored by passing the effluent from the long column of the Beckman 120 B amino acid analyzer through a flow scintillation spectrometer (Packard Tri-Carb 3200) before the effluent reacted with ninhydrin (12). The conditions for the analysis in this case are essentially those described by Inglis et al. (13), i.e., the column temperature was 52°C throughout, and the starting buffer was adjusted to pH 3.05. The buffer change followed at 90 min. The elution profile of the acidic and neutral amino acids under these conditions is the same as that reported for the microbore single-column system (31). Radioactivity was also assayed on glass filters by using Scint Hei 1 solution (Koch-Light). Radioactive material subjected to TLC was detected by exposure to X-ray film (X-Omat; Eastman Kodak Co., Rochester, N.Y.).

(iv) Oxidative procedures. *F. nucleatum* Fev1 peptidoglycan labeled with ^{35}S (500,000 cpm equivalents) was suspended in 0.2 ml of formic acid at 0°C, and then 1 ml of performic acid was added. The mixture was incubated for 20 h at 0°C (9). Excess reagent was removed by lyophilization, and hydrolysis was performed as described above. Another sample (1,000,000 cpm) was hydrolyzed, and half of it was treated with peroxide at room temperature (30). The samples were assayed for radioactivity after separation on TLC or in the Beckman 120 B amino acid analyzer.

(v) Other techniques. Acid hydrolysates of peptidoglycans containing A_2pm were subjected to TLC in solvent 2. In this system *meso*- and DD-A_2pm cochromatographed and were separated from LL-A_2pm, which has a higher R_f value (21). The chromatograms were sprayed with 0.2% ninhydrin in acetone. Densitometric tracings of the chromatograms were performed with a Zeineh soft laser scanning densitometer (Biomed Instruments, Chicago, Ill.), and the tracings were analyzed by using a graphical measuring instrument (MOP-AH/03, Kontron Meßgeräte, Munich, Federal Republic of Germany). Autoradiographs of acid hydrolysates of ^{35}S-labeled Fev1 peptidoglycan run on TLC in solvent 1 were analyzed by using the same technique.

RESULTS

Quantitative estimation of lanthionine. The effect of performic acid oxidation on lanthionine before acid hydrolysis and peroxide oxidation after hydrolysis was studied by using *F. nucleatum* Fev1 ^{35}S peptidoglycan. The products were separated on TLC in solvent 1 and detected both by autoradiography (Fig. 1, lanes A through C) and by ninhydrin (Fig. 1, lanes a through c). As revealed by autoradiography, the major product formed by performic acid and by peroxide oxidation had the mobility of cysteic acid, both in solvent 1 (Fig. 1) and in solvent 2 (data not shown). Densitometric tracing of the chromatogram gave 42 and 66% cysteic acid for performic and peroxide oxidation, respectively (Fig. 1, lanes A and B). In the control 89% of the applied radioactivity was found in the position of lanthionine (Fig. 1, lane C). In addition to cysteic acid, both oxidation procedures generated several products (Fig. 1, lanes A and B), most of which were not ninhydrin positive (Fig. 1, lanes a and b). No attempts were made to identify these products. The relative proportions of these products varied to some extent from one experiment to another.

The complexity of the mixtures of lanthionine oxidation products was also demonstrated when samples of products obtained as described above were run on the amino acid analyzer (Fig. 2). Material not retained on the column and thus eluting as cysteic acid (Fig. 2, peak I) accounted for 55 and 94% of the applied radioactivity in the case of performic acid and peroxide oxidized material, respectively. The identity of peak II (Fig. 2) eluting at 40 min is unknown.

In parallel experiments various amounts of

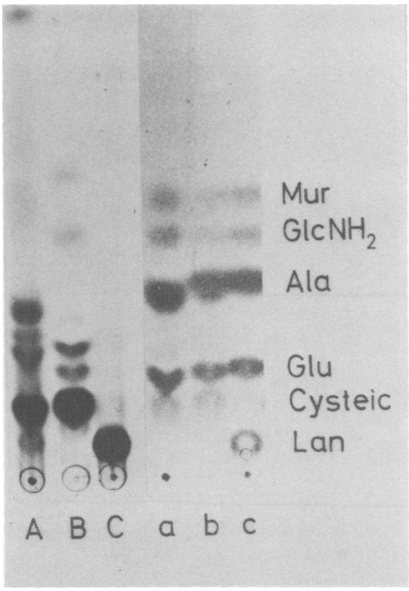

FIG. 1. TLC of acid hydrolysates of ^{35}S-peptidoglycan from *F. nucleatum* Fev1. Each sample contained 25,000 cpm. A and a, Sample treated with performic acid before acid hydrolysis; B and b, sample treated with peroxide after acid hydrolysis; C and c, control sample; A, B, and C, Positive print of the autoradiogram exposed for four days; a, b, and c, Same TLC plate developed with ninhydrin; Cysteic, cysteic acid; Lan, lanthionine.

compounds eluted at the retention times expected for lanthionine sulfoxide (as a split peak at 48 and 53 min) and lanthionine sulfone (at 61 min). The identification of these peaks was based on the work of Lipton et al. (18).

After performic acid oxidation, 36% of the radioactivity eluted as cystine (Fig. 2A, peak V). When the analyzer was run under the conditions of Spackman et al. (27), i.e., with pH 3.25 buffer, peak V eluted before valine, again as cystine. This material, therefore, is most likely cystine.

In the control sample about 85% of the radioactivity was eluted as lanthionine (Fig. 2C, peaks III and IV), of which 84% eluted as *meso*-lanthionine (peak IV) and 16% eluted as DL-lanthionine (peak III). The positions of the lanthionine isomers were established by using standards of commercially available L-, DL-, and *meso*-lanthionine. Advantage was also taken of the original report on the behavior of the lanthionine isomers upon ion-exchange chromatography (3). The recovery of lanthionine from unoxidized peptidoglycan under these conditions was found to be 90% or more, determined as combined radioactivity in lanthionine and cysteic acid. The latter accounted usually for 5% of this, or less.

With a few exceptions (6, 19), the same ninhydrin color factor has been used in the estimation of the isomers of lanthionine. The ratio of *meso*-lanthionine to DL-lanthionine in the ^{35}S-peptidoglycan of *F. nucleatum* Fev1 was thus found to be 85:15 (Table 2). Since the same ratio (actually 86:14) was calculated based on radioactivity in the isomers (Fig. 2C), we take this as strong evidence that the ninhydrin color factor is the same for the various lanthionine isomers.

It is still a question whether both isomers of lanthionine are present in the peptidoglycan or whether one isomer arise due to the treatment before analysis. Some racemication is actually expected to take place during acid hydrolysis. Thus, when the peptide nisin, which contains only *meso*-lanthionine, was analyzed after standard acid hydrolysis, both *meso*- and DL-lanthionine were found in the ratio of 60:40 (8). Essentially the same effect of acid hydrolysis upon lanthionine has been reported by others (32). We observed that our conditions of hydrolysis (4 N HCl, 16 h, 105°C) yielded 11% *meso*-lanthionine from pure L-lanthionine (data not shown). It is likely, therefore, that the isomer present in a peptide will be found in the highest yield after acid hydrolysis, and it seems reasonable to suggest that *meso*-lanthionine is the only isomer in those peptidoglycans that contains lanthionine (Table 2).

Composition of the peptidoglycans. The chemical composition of the peptidoglycans of the different *Fusobacterium* and *Bacteroides* strains and of *L. buccalis* L11, is summarized in Table 2.

In most cases the isolation procedure for the peptidoglycan moiety was satisfactory, i.e., the dry weight of the complex contributed to about 0.5 to 1.5% of the wet weight of the cells, and the peptidoglycan-specific components accounted for the main part of the complex (Table 2).

In some preparations polysaccharides might have contributed to a considerable part of the dry weight, since no particular precautions were taken to remove such contaminants. In *B. fragilis* NCTC 9343 about 30% of the dry weight was polysaccharide as estimated from the anthrone test (data not shown). In the present study, however, we have only dealt with the ninhydrin-positive components of the peptidoglycans. In a few cases (e.g., with *F. gonidiaformans* VPI 0482A and VPI 11360) the isolation procedure apparently failed, since the recovery of the peptidoglycan was very low, and since several amino acids not commonly associated with the peptidoglycan were detected in considerable amounts. Such peptidoglycans have not been included in Table 2.

Genus *Fusobacterium*. The peptidoglycans isolated from the strains of *Fusobacterium* con-

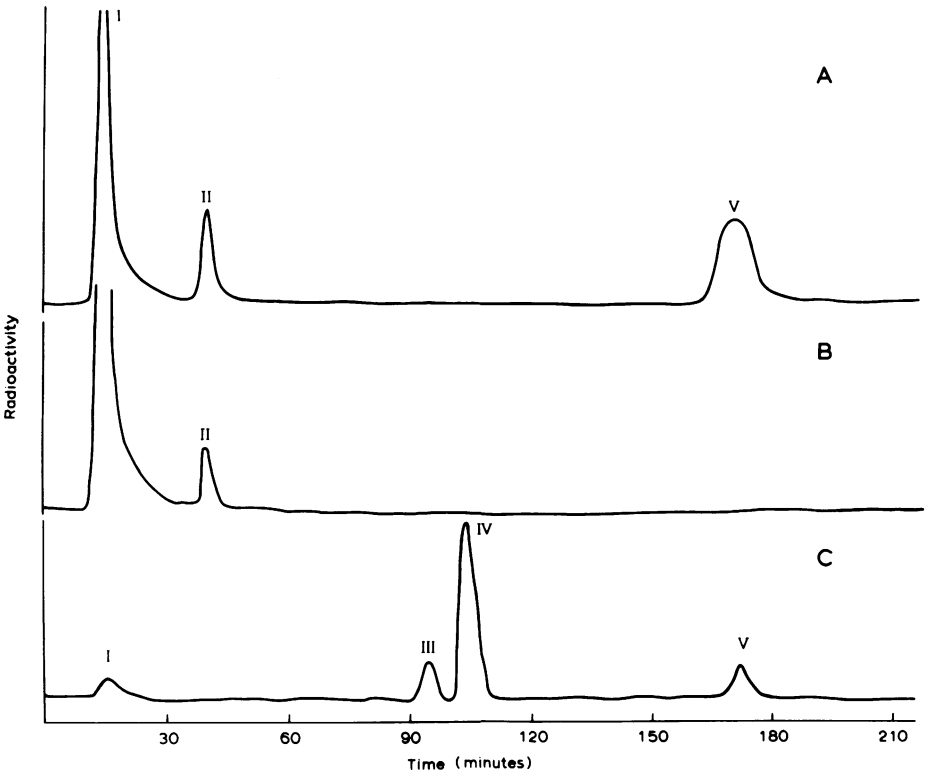

FIG. 2. Elution profiles of radioactive material in acid hydrolysates of ^{35}S-peptidoglycan from *F. nucleatum* Fev1. The samples were analyzed in a Beckman 120 B amino acid analyzer (see text). A, Sample (250,000 cpm) treated with performic acid before acid hydrolysis; B, sample (250,000 cpm) treated with peroxide after acid hydrolysis; C, control sample (150,000 cpm). Peaks: I, cysteic acid; II, unknown oxidation product(s); III, DL-lanthionine; IV, *meso*-lanthionine; V, cystine.

tained muramic acid, glutamic acid, alanine, and glucosamine in fairly similar ratios. However, they differed with respect to their content of dibasic amino acid; the strains of *F. nucleatum*, *F. necrophorum*, *F. russi*, and *F. gonidiaformans* contained lanthionine, whereas those of *F. mortiferum* VPI 5596, *F. varium*, and *F. naviforme* contained A_2pm. In one case, *F. mortiferum* VPI 0473, lanthionine and A_2pm were present in about equal proportions. This result was confirmed with different batches of pure stock solutions of the strain. *F. russi* VPI 0307 was unique by having two glycine residues per glutamic acid. The composition of *F. plauti* was clearly atypical; it contained an excess of glucosamine as well as galactosamine and several amino acids.

L. buccalis. The finding of glutamic acid, alanine, and A_2pm in *L. buccalis* confirms results of previous studies (10).

Genus *Bacteroides*. The peptidoglycans from the *Bacteroides* species all contained A_2pm and, in a few instances, considerable amounts of lysine. The distribution of the isomers of A_2pm was examined by TLC in solvent 2. All strains but two contained exclusively *meso*-/DD-A_2pm. In *B. oralis* VPI 9958 and NP333, LL-A_2pm constituted 78 and 86%, respectively, of the A_2pm present.

Both A_2pm and lysine were apparently a prominent dibasic amino acid in *B. melaninogenicus* 30 and *B. bivius* VPI 0318. In *B. asaccharolyticus* VPI 4199 only small amounts of A_2pm were found, whereas lysine was the major dibasic amino acid.

Galactosamine was present in the preparations from *B. multiacidus* A405 and GE-374-14 and *B. praecutus* VPI 0217-1. Another unexpected finding was the presence of glycine, e.g., in the preparations of peptidoglycans from *B. oralis* VPI 9958 and NP333, *B. melaninogenicus* 30 and in small amounts in *B. hypermegas* VPI 2366.

An unknown ninhydrin-positive component (*x*, Table 2) was found in some preparations. No attempts were made to identify this component, the amount of which was calculated by using the color factor of histidine.

TABLE 2. Composition of peptidoglycans[a]

Strain	Asp	Ser	Mur	Glu		Lanthionine			Gly	Ala	$GlcNH_2$	$GalNH_2$	A_2pm	x[b]	His	Lys
				Relative amt	nmol/mg of peptidoglycan	Total	% Isomers									
							DL	*meso*								
F. nucleatum																
Fev1			1.1	1.0	647	1.1	15	85		1.7	1.2					
ATCC 10953			1.1	1.0	634	0.9	12	88		1.9	1.3					
F1			0.8	1.0	608	0.8	10	90		1.9	0.9					
F9			0.9	1.0	265	1.2	17	83		1.9	1.3					
F18			0.9	1.0	387	1.2	17	83		2.1	1.4					
F. necrophorum																
SPH1			0.9	1.0	643	1.1	13	87		1.5	1.2					
VPI 6161			1.1	1.0	314	1.1	7	93		1.4	1.3					
N167			1.0	1.0	295	0.8	6	94		1.2	1.0					
F. russi VPI 0307			1.5	1.0	672	0.9	11	89	2.1	2.1	1.5					
F. gonidiaformans VPI 4381			1.3	1.0	544	1.1	17	83		2.4	1.1					
F. mortiferum																
VPI 5696			1.2	1.0	120					2.0	1.1		0.9			
VPI 0473			1.0	1.0	640	0.6	17	83		1.6	1.0		0.5			
F. varium VPI 0499A			1.2	1.0	430					2.7	1.8		1.2			
F. naviforme																
VPI 4877			0.7	1.0	—[c]					2.2	1.4		1.1			
VPI 11936C			0.6	1.0	298					1.6	1.2		1.0			
F. plauti VPI 4145	0.5	1.5	1.1	1.0	306					2.8	4.0	1.5	0.3		0.9	1.3
L. buccalis L11			0.5	1.0	461					1.8	1.0		1.2	1.5		
B. fragilis NCTC 9343			1.0	1.0	231					1.5	1.1		1.0			
B. distasonis ATCC 8503			1.2	1.0	—					1.7	1.3		0.9			
B. thetaiotaomicron VPI 5333[d]				1.0	—					1.5			1.1			

TABLE 2—*Continued*

Strain	Asp	Ser	Mur	Glu		Lanthionine			Gly	Ala	$GlcNH_2$	$GalNH_2$	A_2pm	x^b	His	Lys
				Relative amt	nmol/ml of peptidoglycan	Total	% Isomers									
							DL	*meso*								
B. oralis																
VPI 9958			0.6	1.0	278				0.9	1.6	0.6		0.7	0.6	0.9	
VPI 8906			0.8	1.0	256					2.2	1.4		1.3			
NP333			0.6	1.0	320				1.9	1.9	1.1		1.0	0.5	0.4	
5540			1.5	1.0	514					2.5	1.4		1.4			
B. melaninogenicus																
30		0.8	0.9	1.0	327				2.3	1.8	1.1		0.6			0.7
NCTC 9336[d]				1.0	—					3.4			0.7			
B. disiens VPI 7852			1.5	1.0	341					1.5	1.5		1.0			
B. bivius VPI 6318			0.5	1.0	—					1.8	1.0		0.6			0.3
B. ruminicola																
C12			0.6	1.0	—					2.2	0.8		0.9			
D46			1.0	1.0	401					2.0	1.1		1.1			
B. asaccharolyticus VPI 4199			0.7	1.0	810					1.9	0.7		0.2			1.1
B. praeacutus VPI 0217-1			0.8	1.0	674					2.3	2.3	1.1	1.5	0.4	0.5	
B. hypermegas VPI 2366			1.15	1.0	142				0.2	1.6	1.2		1.0			
B. serpens VPI 0950			1.0	1.0	—					2.2	1.0		0.9			
B. putredinis VPI 4998-1			0.9	1.0	—					2.0	0.9		1.0			
B. multiacidus																
A405			1.2	1.0	411					1.6	1.3	0.3	1.2		0.3	
GE-374-14			0.6	1.0	327					1.9	2.7	0.5	1.1		0.4	

[a] The values presented are the means of two or more analyses.
[b] Color factor for histidine.
[c] —, Not determined.
[d] Determined on GLC.

DISCUSSION

Recently we reported the presence of lanthionine in the peptidoglycan of *F. nucleatum* Fev1 (30). The composition of the peptidoglycan was calculated from acid hydrolysates treated with peroxide (4% final concentration) before ion-exchange chromatography. The rationale for this procedure was the finding that lanthionine coeluted with muramic acid and glutamic acid during chromatography, whereas the oxidation products of lanthionine all eluted before threonine (30). One obvious drawback of the oxidation procedure was that the two amino sugars present in the peptidoglycan were partially destroyed. Moreover, the recovery of lanthionine sulfoxide varied considerably, and in addition many other products were formed. It is quite possible that the presence of the amino sugars caused some of the reactions observed with lanthionine (5). Figures 1 and 2 show that the oxidation had proceeded further than lanthionine sulfoxide, and that the major product was cysteic acid. There were many other oxidation products (Fig. 1), most of which are unknown. The formation of unknown oxidation products by peroxide treatment of lanthionine has been reported by others (18).

Low amounts of cystine were found in different hydrolysates of unoxidized *F. nucleatum* Fev1 peptidoglycans, ranging from 0 to 10% of the amount of lanthionine. Most likely this cystine arises as an artifact from lanthionine during the treatment. This hypothesis is supported by the variable amounts found in hydrolysates of identical (unoxidized) samples (see above) and by the increase in cystine after performic acid treatment (Fig. 2A compared with Fig. 2C).

Besides this occasional byproduct the recovery of lanthionine was always very satisfactory when the samples were kept away from oxidizing agents (e.g., air) during hydrolysis.

The gram-negative bacteria are considered to belong to the same peptidoglycan type, namely, the direct cross-linked type A1γ, containing A_2pm (25). The composition of the gram-negative anaerobes studied in this work, however, indicates chemotypic differences. The compositions of the peptidoglycan of the different *Fusobacterium* species all seem to fit the A1 type with, in some strains, A_2pm replaced by lanthionine in the L-R3 position. These lanthionine-containing peptidoglycans might therefore be assigned to a new A1δ type. Strong evidence for this *meso*-lanthionine directly cross-linked type has been presented for *F. nucleatum* Fev1, which so far is the only strain for which the primary structure of the peptidoglycan has been examined in some detail (29). In addition to *F. nucleatum*, *F. necrophorum*, *F. russi*, and *F. gonidiaformans* seem to belong to the A1δ type.

Structurally it is not surprising that we find the *meso* isomer of lanthionine in these peptidoglycans since the *meso* form is the dominant isomer of A_2pm in gram-negative bacteria (7, 25). Some strains of *F. mortiferum* apparently have the ability to produce and to incorporate into the peptidoglycan both A_2pm and lanthionine. This finding is consistent with a recent report from Kato et al. (15) that strains *F. mortiferum* 15 and *F. freundii* ATCC 9817 contained both A_2pm and lanthionine, whereas *F. nucleatum*, *F. necrophorum*, and *F. russi* all contained lanthionine only.

The composition of *F. plauti* VPI 4145 was clearly atypical; it contained an excess of glucosamine as well as galactosamine and several amino acids not commonly associated with the peptidoglycan of a gram-negative cell. The presence of lysine in an amount approximately equimolar to that of glutamic acid and muramic acid suggests the presence of another peptidoglycan type in this bacterial species. It is interesting to note that the fatty acid pattern of *F. plauti* is quite different from those of other *Fusobacterium* species (14), and it has been suggested that strains classified as *F. plauti* are phylogenetically unrelated to other *Fusobacterium* species (14). Electron microscopic examination of *F. plauti* VPI 4145 revealed a cell wall consistent with a gram-positive organism (11). To us this example emphasizes the validity and potential of peptidoglycan typing in the classification of bacteria in general.

The composition of the peptidoglycan from the majority of the *Bacteroides* strains examined seems to fit the A1γ type of peptidoglycan. Among probable exceptions are *B. melaninogenicus* subsp., *melaninogenicus* 30, *B. bivius* VPI 6318, and *B. asaccharolyticus* VPI 4199, all containing lysine.

As reported by Shah et al. (26), lysine is the major diamino acid in *B. asaccharolyticus* VPI 4199. In contrast to these authors, we found in addition small amounts of A_2pm in strain VPI 4199. Shah et al. prepared the cell walls by the rapid screening method of Schleifer and Kandler (25) with trichloroacetic acid. In our hands this procedure results in crude peptidoglycan preparations from gram-negative organisms. Since Shah et al. analyzed their preparations by paper chromatography only, it is possible that small amounts of A_2pm may have been overlooked.

Interestingly, in *B. oralis* VPI 9958 and NP333 the major isomeric form of A_2pm is LL-A_2pm. The presence of glycine, which was also found in these two *B. oralis* strains, and LL-A_2pm is characteristic of the peptidoglycan type A3γ (25).

Biochemically, and with respect to DNA base

composition, the genus *Bacteroides* encompasses a heterogenous group of anaerobic organisms. Different peptidoglycan types may exist within this group (Table 2). Further studies on the composition and structure of the peptidoglycans may therefore be a valuable asset in a future classification of these organisms.

The peptidoglycan has been considered to be phenotypically quite stable (23). However, modifications have been observed caused by quantitative differences in the composition of the medium, in general only in the presence of quite unbalanced growth medium and caused by transitions in growth phase (24). These factors may play a role for the observed variations in composition, in particular where two different diamino acids are found in the same peptidoglycan. The biosynthesis of lanthionine and A_2pm should be studied in *F. nucleatum* Fev1 and in *F. mortiferum* VPI 0473 as a function of growth phase and under various growth conditions to evaluate the effect of these exogenous factors on the primary structure of these peptidoglycans.

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