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Primary Structure of the Peptidoglycan from the Unicellular Cyanobacterium *Synechocystis* sp. Strain PCC 6714

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A peptidoglycan fraction free of non-peptidoglycan components was isolated from the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6714. Hydrofluoric acid treatment (48%, 0°C, 48 h) cleaved off from the peptidoglycan non-peptidoglycan glucosamine, mannosamine, and mannose. The purified peptidoglycan consists of *N*-acetyl muramic acid, *N*-acetyl glucosamine, L-alanine, D-alanine, D-glutamic acid, and *meso*-diaminopimelic acid in approximately equimolar amounts. At least partial amidation of carboxy groups in the peptide subunits is indicated. Peptide analyses and 2,4-dinitrophenyl studies of partial acid hydrolysates revealed the structure of the *Synechocystis* sp. strain PCC 6714 peptidoglycan to belong to the A1γ type (direct cross-linkage) of peptidoglycan classification. The degree of cross-linkage is about 56% and thus is in the range of that found in gram-positive bacteria. Some of the peptide units are present as tripeptides lacking the carboxy-terminal D-alanine.

The presence of peptidoglycan in cyanobacteria is well documented by different observations such as sensitivity of cyanobacteria cells towards penicillin or lysozyme, by fine-structural studies, and by chemical analyses of peptidoglycan constituents (for a review, see references 3 and 4). The typical constituents of the A1-type of peptidoglycan have been found in unicellular and filamentous strains as well. Molar ratios of GlcN-MurN-Ala-Glu-A_2pm = 1:1:2:1:1 found with peptidoglycan of *Phormidium foveolarum* and *Tolypothrix tenuis* (14) have confirmed that they belong to this type. (Abbreviations are defined below.) However, molar ratios of GlcN:MurN up to 2:1 were found in peptidoglycan fractions of *Anacystis nidulans* (synonym *Synechococcus*) (14), *Synechococcus lividus* (26), *Aphanothece halophytica* (15), or *Phormidium uncinatum* (9), and GalN, ManN, or Man was found in the peptidoglycan fraction from *Synechococcus* (12).

It will be shown that the peptidoglycan fractions of *Synechocystis* sp. strain PCC 6714, used for this study, contain ManN, GalN, GlcN, and Man in addition to the constituents of the A1-type peptidoglycan. Non-peptidoglycan components can be separated from peptidoglycan by hydrofluoric acid treatment. Identification of the amino acid isomers and peptide analyses allowed the elucidation of the primary structure of the purified peptidoglycan of *Synechocystis* sp. strain PCC 6714.

MATERIALS AND METHODS

Abbreviations. Ala, alanine; A_2pm, diaminopimelic acid; DNP, dinitrophenyl; FDNB, 1-fluoro-2,4-dinitrobenzene; GalN, galactosamine; GlcN, glucosamine; Glc, glucose; Glu, glutamic acid; ManN, mannosamine; Man, mannose; MurN, muramic acid.

Cultivation of cyanobacteria. *Synechocystis* sp. strain PCC 6714 was obtained from the Pasteur Culture Collection (PCC), Institut Pasteur, Paris (France). The strain was cultivated photoautotrophically in BG-11 medium (pH 7.5) at 25°C (21). Mass cultures were prepared in a 12-liter fermentor (Jungkeit, Göttingen, Germany) and were gassed continuously by a stream of air (250 liters/h) and CO_2 (2.5 liters/h). Cells were harvested from the stationary phase of growth, washed once with 20 mM *N*-Tris-hydrochloride, pH 9.0 (Tris buffer, used throughout the isolation procedure) and stored at −20°C until use.

Isolation of peptidoglycan. Cells were suspended in buffer at 4°C, mixed with glass beads (0.17 to 0.18 mm in diameter; cell-to-glass bead ratio was 1:2 [vol/vol]), and shaken at full speed for 10 min in a Vibrogen shaker (E. Bühler, Tübingen, Germany). After removal of glass beads by filtration, crude cell envelopes were separated by differential centrifugation (12,000 × *g*, 4°C, 30 min) and washed 10 times with Tris buffer. Discontinuous sucrose gradients (10 ml of 60, 55, 50, 45, and 40% sucrose in Tris buffer) were loaded with 10 ml of the crude cell envelope suspension (2 to 3 mg of protein per ml) and run in an SW25.2 rotor (Beckman Instruments, Fullerton, Calif.) at 20,000 rpm for 4 h. Cell walls were recovered from both the pellet and the band at 60% sucrose and were further purified by using the same gradient (three times). Sucrose was removed by washing the cell walls with ice-cold Tris

buffer at least five times. The purified cell walls were suspended in 4% sodium dodecyl sulfate (SDS) and extracted five times at 100°C for 15 min (2).

Rigid layers (SDS-insoluble fractions of the cell wall) were collected by ultracentrifugation (175,000 × g, 2 h) and washed with distilled water until they were free of SDS (13). The rigid layers were suspended in 20 mM Tris buffer (pH 7.4) and treated at 37°C with pronase (from *Streptomyces griseus*, 6 U per mg of solid, Boehringer Mannheim Corp.) and trypsin (from bovine pancreas, EC 3.4.21.4, 10,000 to 13,000 units of *N*-α-benzoyl-L-arginine ethyl ester per mg of protein, Sigma Chemical Co., St. Louis, Mo.), respectively, to remove non-peptidoglycan amino acids. The peptidoglycan fraction obtained was extracted twice with boiling 4% SDS, and the detergent was removed as described above.

A sample (30 to 50 mg) of the lyophilized peptidoglycan was suspended in 2 ml of ice-cold 48% hydrofluoric acid and kept at 0°C for 48 h. Hydrofluoric acid was removed by evaporation in vacuo. The lyophilized residue was suspended in ice-cold distilled water and neutralized with 0.1 M LiOH. After several washes with distilled water, the hydrofluoric acid-treated peptidoglycan was finally collected by centrifugation (175,000 × *g*, 4°C, 2 h).

Electron microscopy. Peptidoglycan fractions were fixed in 1% osmiumtetroxide for 6 h and were embedded in Epon. Thin sections were stained with uranyl acetate and then by lead citrate. The specimens were examined in a Philips EM 400 microscope at 80 kV. For negative staining, the broken sacculus preparation was treated with 1% phosphotungstic acid. Methods for preparation of ultrathin sections and negative staining were as described previously (12).

Isolation of peptides from peptidoglycan. Peptidoglycan was hydrolyzed in 4 N HCl at 100°C for 30 min. The peptides formed were separated by combined low voltage–thin-layer electrophoresis (first dimension, pyridine-acetic acid-water = 1:2:250 [vol/vol/vol], pH 4.4; 20 V/cm, 3 h, 4°C) and thin-layer chromatography (second dimension, ethylacetate-pyridine-acetic acid-water, 5:5:1:3 [vol/vol/vol/vol] [16]) on cellulose plates (20 by 20 cm, Merck, Darmstadt). Peptides were detected by ninhydrin reaction or were isolated from fluorescamine-stained fluorograms (28) for preparative purposes (5).

Chemical analysis of peptidoglycan and peptides. Amino acids and amino sugars were liberated by 4 N HCl (105°C, 18 h) and were identified and quantified by an automatic amino acid analyzer (Durrum, model D-500). Mannosamine and GalN, which could not be separated from each other by the elution program used, were identified by gas-liquid chromatography, on a Poly A-103 glass column (2 m length, 2 mm inside diameter, packed with 3% polyamide on 100/120 mesh Gas-Chrom Q, temperature program 200 to 260°C, 4°C/min [20]). Neutral sugars were liberated by 0.1 N HCl (100°C, 48 h) and determined as alditol acetate derivatives as described previously (23). The configuration of amino acids was identified by separation of the *N*-heptafluoro-butyryl-isobutylester derivatives (18) of the isomers on a Chirasil Val glass capillary column (25 m long, 0.2 mm inside diameter; temperature program: 90°C, 4 min isothermal; 90 to 170°C, 2°C/min; 170°C isothermal; Applied Science, Europe).

Total acetyl (amide- and ester-bound) residues, removed with 0.2 N HCl (100°C, 12 h), were determined by gas-liquid chromatography on a Porapak Q column (2 m length, 2 mm inside diameter; column temperature 200°C, internal standard propionic acid).

O-Acetyl (ester-bound) residues were split off with 0.05 N NaOH (23°C, 4 h). *N*-Acetyl (amide-bound) residues were calculated from the difference of total acetyl and *O*-acetyl (8). Fatty acids were analyzed as methylester derivatives by gas-liquid chromatography (11) on EGSS-X and Castorwax columns, respectively. Organic phosphorus was determined by the Lowry method (19).

C- and N-terminal group estimation. C-terminal amino acids were detected by treatment of peptidoglycan with anhydrous hydrazine (100°C, 10 h) and separated from their hydrazides (24). *N*-Norleucine was used as an internal standard. Peptidoglycan from *Escherichia coli* K-12 was used as a control preparation. N-terminal groups of peptidoglycan or of isolated peptides were reacted with 1-fluoro-2,4-dinitrobenzene (FDNB) at 60°C for 30 min in the dark (17). After removal of excess of FDNB by extraction with ether (5 to 10 times), the lyophilisate from the water phase was hydrolyzed (4 N HCl, 105°C, 18 h) and subjected to amino acid analysis to detect the nonderivatized amino acids. The DNP derivatives from the hydrolysate were chromatographed twice on silica gel plates (solvent-trichloromethane-methanol-acetic acid = 65:25:3:3 [vol/vol/vol/vol]) in parallel to authentic DNP derivatives. The yellow-colored bands were scraped off the plates and eluted with methanol and, after evaporation, the compounds were heated in concentrated ammonia (100°C, 6 h) to cleave off the DNP residue and to detect the DNP-derivatized amino acids by the amino acid analyzer.

RESULTS

Morphology of peptidoglycan fractions. The peptidoglycan fraction obtained after pronase digestion was examined by electron microscopy. In negatively stained preparations, it could be shown that the peptidoglycan had retained the rigidity and shape of broken sacculi (Fig. 1a). In ultrathin sections, the peptidoglycan appeared as an electron-dense layer with a thickness of 12 nm (Fig. 1b). There was no difference observed in the electron microscope between the peptidoglycans after trypsin and pronase digestion, respectively. The sacculus-like structure was also observed after hydrofluoric acid treatment (not shown).

Chemical composition of peptidoglycan from *synechocystis* sp. strain PCC 6714. Peptidoglycan was obtained from *Synechocystis* sp. strain PCC 6714 after SDS treatment of cell walls in a yield of 4% cell dry weight. It contained MurN, GlcN, Ala, A_2pm, and Glu in a molar ratio of 1:1:2:1:1 (Table 1). The rigid layer (i.e., the SDS-insoluble fraction of the cell wall) contained 3.4% (dry weight) of the non-peptidoglycan amino acids. These amino acids, including lysine and arginine, were removed by digestion with pronase and trypsin, respectively. Hydrofluoric acid

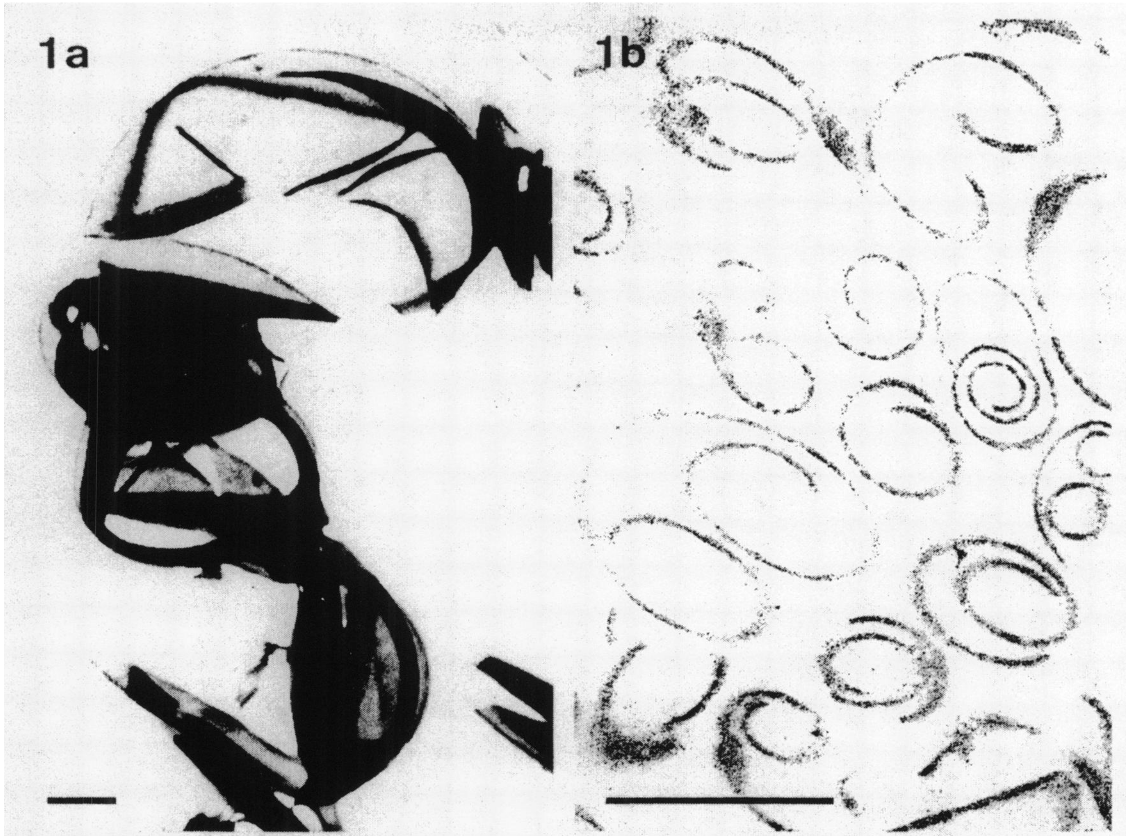

FIG. 1. Isolated SDS-extracted broken peptidoglycan sacculi after digestion with pronase. (a) Preparation by negative staining. (b) Ultrathin section of the peptidoglycan layer. Bar represents 0.5 μm.

treatment in the cold solubilized 41% (dry weight basis) of the protease-digested peptidoglycans. The isolated peptidoglycan was free of ManN, GalN, and Man; GlcN was reduced to an amount equimolar to MurN. The data of acetyl determination and dinitrophenylation show that the amino sugars were completely *N*-acetylated. Only small amounts of *O*-acetyl were detected.

Configuration of the constituting amino acids. The D- and L-isomers in the different amino acid monomer and peptide fractions of peptidoglycan were separated and quantitatively determined, by gas-liquid chromatography, as to their *N*-heptafluoro-butyryl-isobutylester derivatives by the use of a Chirasil-Val glass capillary column. Glutamic acid was found to be present as D-Glu exclusively (Table 1), and the Ala fraction consisted of equimolar amounts of D-Ala and L-Ala. It was further possible to identify the total of A_2pm as *meso*-A_2pm by using the same column (Fig. 2): the A_2pm from *Synechocystis* sp. strain PCC 6714 eluted identically to authentic *meso*-A_2pm but differently from D,D-A_2pm or L,L-A_2pm (standards: mixture of L,L-, D,D-, and *meso*-isomers of A_2pm [Sigma]).

Amidated carboxyl groups. Liberation of ammonia during the first hour of hydrolysis (4 N HCl, 105°C) without amino acid degradation indicates amidation of carboxyl groups in peptidoglycan (10). The amount of ammonia liberated from the *Synechocystis* sp. strain PCC 6714 peptidoglycan was compared with respective amounts obtained for *E. coli* K-12 or *Streptomyces albus* G peptidoglycan. *E. coli* K-12 peptidoglycan which lacked amidation of carboxyl groups of both D-Glu and *meso*-A_2pm showed a molar ratio of D-Glu–*meso*-A_2pm–ammonia = 1:0.7:0.5. In *S. albus* G peptidoglycan, known to possess amidated α-carboxyl group of D-Glu (20), a respective value of 3.7 mol for ammonia was found. *Synechocystis* sp. strain PCC 6714 peptidoglycan showed a molar ratio of D-Glu–*meso*-A_2pm–ammonia = 1:0.7:1.5. This indicates amidation of some carboxyl groups in the *Synechocystis* peptidoglycan. It is not known whether D-Glu or *meso*-A_2pm is amidated.

Identification of peptides. Eight peptides were detected in partial acid hydrolysates of the isolated peptidoglycan after low-voltage thin-layer electrophoresis and thin-layer chromatography

TABLE 1. Chemical composition of peptidoglycan fractions of *Synechocystis* sp. strain PCC 6714

Component	Amt in peptidoglycan fraction (nmol per mg of fraction [dry wt])		
	Pronase-digested	Trypsin-digested	Pronase-digested and hydrofluoric acid (48%, 0°C, 48 h)-treated
Amino sugars			
MurN	520	514	837
GlcN	890	876	953
ManN	222	225	—[a]
GalN	15	16	—
Amino acids			
D-Ala	504	503	812
L-Ala	669	667	1,077
D-Glu	604	598	959
meso-A_2pm	642	632	933
Neutral sugars			
Man	313	289	—
Glc	33	32	—
Acetyl groups			
N-Acetyl	1,402	1,372	1,346
O-Acetyl	12	10	15
Phosphorus	150	139	—

[a] —, None.

(Fig. 3). They were found to be composed of equimolar amounts of the constituting amino acids (Table 2) and were identified as dipeptides (MurN-L-Ala, L-Ala-D-Glu, D-Glu-*meso*-A_2pm, *meso*-A_2pm-D-Ala), tripeptides (L-Ala-D-Glu-*meso*-A_2pm, MurN-L-Ala-D-Glu), and a tetrapeptide (L-Ala-D-Glu-*meso*-A_2pm-D-Ala), respectively. The structure of the peptides was deduced from the molar ratios of amino sugars and amino acid isomers in the different peptides and from N-terminus determination of each of the eight peptides (Table 2). The dipeptides were further characterized by their chromatographic behavior on the amino acid analyzer and were also identified in the whole peptidoglycan partial hydrolysate (Fig. 4). No contamination of peptides by non-peptidoglycan components was observed.

The ninhydrin-positive spot no. 8 (Fig. 3) contained two peptides with the same amino acid composition, namely, *meso*-A_2pm and D-Ala in a 1:1 molar ratio, but with different structures. This became obvious after dinitrophenylation of the peptides of spot no. 8. The DNP derivatives formed were DNP-D-Ala, mono-DNP-*meso*-A_2pm, and di-DNP-*meso*-A_2pm. In addition, nonderivatized D-Ala was detected. These findings are in agreement with the occurrence of two different peptides: one involved in direct cross-linkage of the tetrapeptides, and the other containing the C-terminus of the tetrapeptide. The two peptides were separated on the amino acid analyzer (peaks 8a and 8b in Fig. 4) but were not separately isolated for preparative purposes. The peak area ratio of peptides 8a and 8b was found to be 2:1 and 5:1 for *Synechocystis* sp. strain PCC 6714 and *E. coli* K-12, respectively.

γ-Carboxyl group of glutamic acid in the link to NH_2(-L)-*meso*-A_2pm. The dipeptide D-Glu-*meso*-A_2pm was isolated from partial acid hydrolysates of both *E. coli* K-12 and *Synechocystis* sp. strain PCC 6714 peptidoglycans. They were chromatographically and electrophoretically (Fig. 3) indistinguishable. In addition, both peptides had identical retention times on the amino acid analyzer (Fig. 3). It is concluded that the γ-

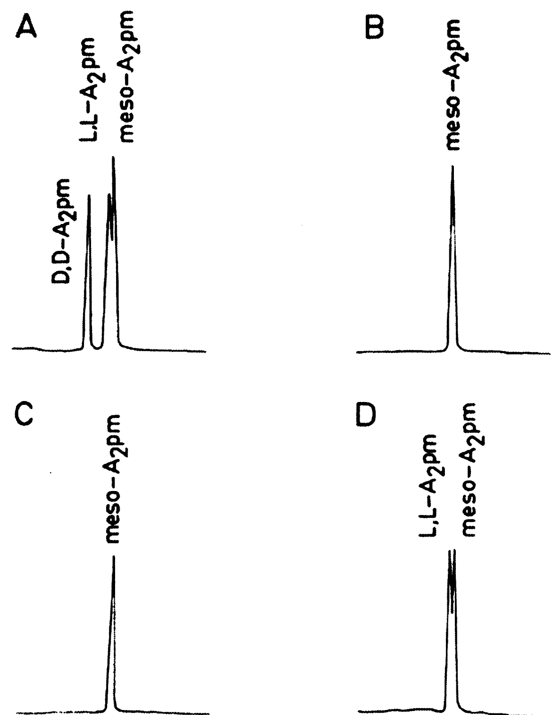

FIG. 2. Separation of the isomers of A_2pm as *N*-heptafluoro-butyryl-isobutylester derivatives by gas-liquid chromatography on a Chirasil-Val glass capillary column (for technical details, see the text). (A) Mixture of authentic isomers of A_2pm. (B) *meso*-A_2pm from peptidoglycan of *Synechocystis* sp. strain PCC 6714. (C) Mixture of *meso*-A_2pm from the peptidoglycans of *Synechocystis* sp. strain PCC 6714 and *E. coli* K-12, respectively. (D) Mixture of *meso*-A_2pm and L,L-A_2pm from the peptidoglycans of *Synechocystis* sp. strain PCC 6714 and *S. albus* G, respectively.

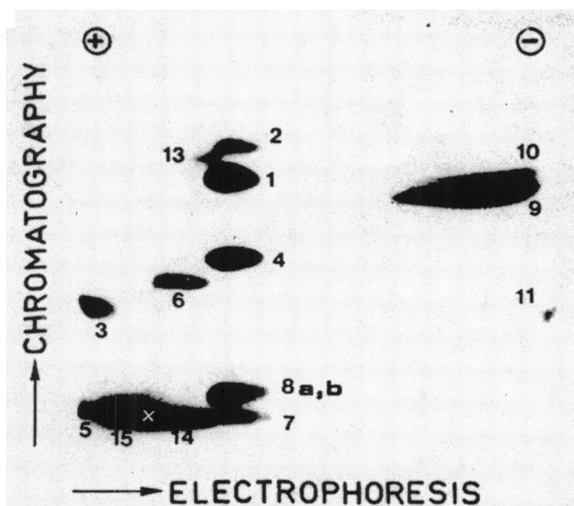

FIG. 3. Separation of peptides and monomers, released by partial acid hydrolysis (4 N HCl, 100°C, 30 min) from peptidoglycan of *Synechocystis* sp. strain PCC 6714, by combined low-voltage thin-layer electrophoresis (first dimension: pyridine-acetic acid-water, 1:2:250 [vol/vol/vol], pH 4.4, 20 V/cm) and thin-layer chromatography (second dimension: ethylacetate-pyridine-acetic acid-water, 5:5:1:3 [vol/vol/vol/vol]). Ninhydrin staining was used. Identity of peaks: for 2, 5, 6, 8a, 8b, 13, 14, and 15, see Table 2. Other peaks: 1, MurN; 3, D-Glu; 4, D-Ala + L-Ala; 7, *meso*-A_2pm; 9, GlcN. Peaks 10 (ManN) and 11 (GlcN-ManN) were observed when peptidoglycan, not treated with hydrofluoric acid, was used. ×, Origin.

carboxyl group of Glu forms a peptide linkage with NH_2(-L-)-*meso*-A_2pm.

Carboxy-terminal and free amino groups. D-Alanine and *meso*-A_2pm were the only C-terminal amino acids found on hydrazinolysis of *Synechocystis* peptidoglycan. This suggests that this peptidoglycan contains tripeptides L-Ala-D-Glu-*meso*-A_2pm and tetrapeptides L-Ala-D-Glu-*meso*-A_2pm-D-Ala bound to muramic acid. The content of C-terminal D-Ala was significantly lower than that observed with peptidoglycans from *E. coli* K-12 or *S. albus* (molar ratios of cleaved D-Ala–*meso*-A_2pm = 1:5.3 in *Synechocystis* sp. strain PCC 6714, 1:1.2 in *E. coli* K-12, and 1:0.7 in *S. albus*). Mono-DNP-diaminopimelate was the only DNP derivative formed on FDNB treatment of the peptidoglycan. It was determined as *meso*-A_2pm after cleavage of the DNP residue from mono-DNP-A_2pm by concentrated ammonia (100°C, 6 h).

Degree of cross-linkage. About 44% of the total *meso*-A_2pm present in the *Synechocystis* sp. strain PCC 6714 peptidoglycan was accessible to the FDNB reaction (27). This shows that in 56% of the peptide side chains, the free amino groups of *meso*-A_2pm were blocked as a result of cross-linkage (Fig. 5).

DISCUSSION

The presence of non-peptidoglycan components in peptidoglycan fractions from cyanobacteria has been hampering structural studies so far. They could not be removed by applying drastic methods such as SDS (14, 26) or phenol-water (14) extraction. In this study, hydrofluoric acid treatment of the *Synechocystis* sp. strain PCC 6714 peptidoglycan was shown to remove all of the ManN, Man, GalN, and non-peptidoglycan GlcN. Their complete elimination by hydrofluoric acid in the cold suggests the presence of a polysaccharide which might be covalently bound to the *Synechocystis* peptidoglycan via phosphodiester bonds. It should be noted that, in fact, organically bound phosphorus is found in this peptidoglycan fraction (Fig. 5).

The peptidoglycan of *Synechocystis* sp. strain

TABLE 2. Composition and sequence of peptides from partial acid hydrolysates (see Fig. 2) of the peptidoglycan of *Synechocystis* sp. strain PCC 6714

Peptide no.	Constituents (molar ratios of peptides)[a]					DNP derivatives,[b] formed on dinitrophenylation of the peptides	Sequence of peptides
	MurN	L-Ala	D-Glu	*meso*-A_2pm	D-Ala		
2	0.8	1.0	—	—	—	DNP-MurN	MurN-L-Ala
13	1.0	1.0	0.9	—	—	DNP-MurN	MurN-L-Ala-D-Glu
6	—	1.0	1.1	—	—	DNP-L-Ala	L-Ala-D-Glu
14	—	1.0	0.9	1.0	—	DNP-L-Ala + mono-DNP-A_2pm	L-Ala-D-Glu-*meso*-A_2pm
15	—	1.0	1.0	1.1	0.9	DNP-L-Ala + mono-DNP-A_2pm	L-Ala-D-Glu-*meso*-A_2pm-D-Ala
5	—	—	1.0	0.9	—	DNP-D-Glu + mono-DNP-A_2pm	D-Glu-*meso*-A_2pm
8a,b	—	—	—	1.0	1.0	DNP-D-Ala + mono-DNP-A_2pm	*meso*-A_2pm-D-Ala[c]
						+ di-DNP-Ap_2m	*meso*-A_2pm-D-Ala[c]

[a] —, None.
[b] Recognized by their lack on separation of hydrolysates (4 N HCl, 105°C, 18 h) of the FDNB-treated peptides on the automatic amino acid analyzer.
[c] With different structures (see the text).

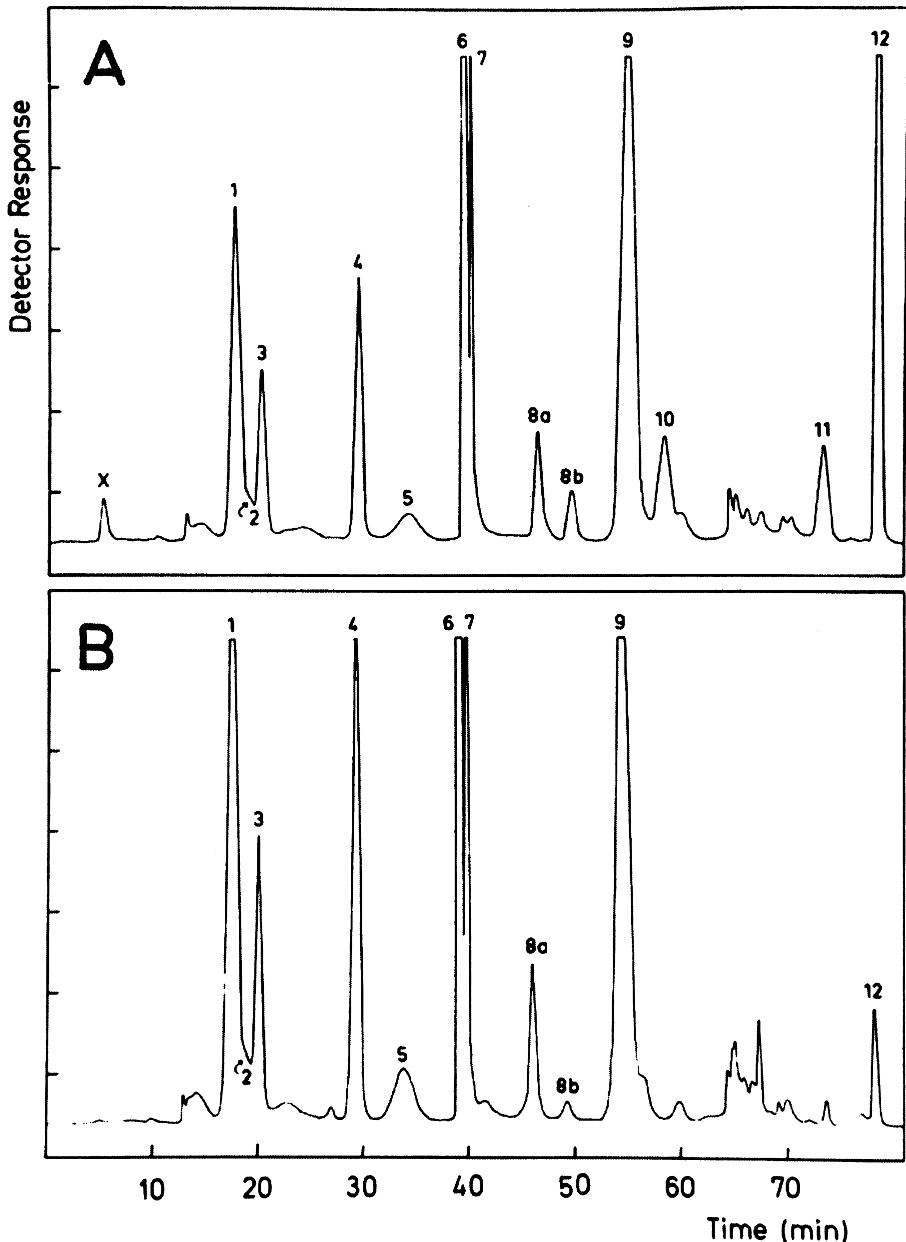

FIG. 4. Elution profiles of partial acid hydrolysates (see Fig. 2) of (A) *Synechocystis* sp. strain PCC 6714 and (B) *E. coli* K-12 peptidoglycan on the automatic amino acid analyzer. Identity of peaks: X, unknown (phosphorylated amino sugar?); 1, MurN; 2, MurN-L-Ala; 3, D-Glu; 4, D-Ala + L-Ala; 5, D-Glu-*meso*-A_2pm 6, L-Ala-D-Glu; 7, *meso*-A_2pm; 8a, *meso*-A_2pm-D-Ala; 8b, *meso*-A_2pm-D-Ala; 9, GlcN; 10, ManN; 11, GlcN-ManN; 12, ammonia. Note: the dipeptides of peaks 8a and 8b are different in structure (see the text). Peaks 10 and 11 were observed when peptidoglycan, not treated with hydrofluoric acid, was used.

PCC 6714 belongs to the A1 γ type (25). This was revealed by application of known methods for structural investigations on peptidoglycan (24) and was confirmed by a parallel investigation of *E. coli* K-12 peptidoglycan. Direct cross-linkage is strongly suggested by the finding of two different *meso*-A_2pm-D-Ala dipeptides, one of them very likely representing the cross-linking dipeptide and the other representing the C-terminal dipeptide of the tetrapeptide subunit (Fig. 5). Both of these peptides are also formed on partial acid hydrolysis of *E. coli* K-12 pepti-

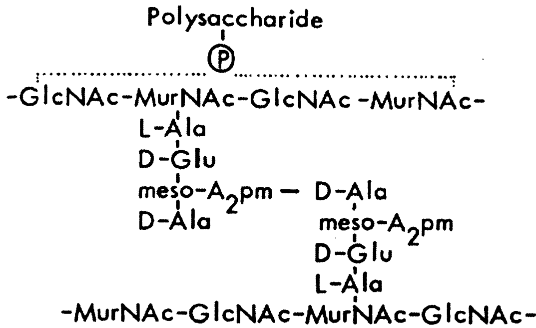

FIG. 5. Proposed structure of the peptidoglycan of *Synechocystis* sp. strain PCC 6714. It is of the A1 γ-type; the degree of cross-linkage is about 56%. Covalent binding of a polysaccharide to peptidoglycan via phosphodiester bonds is suggested.

doglycan. Within the tetrapeptide, the γ-carboxyl group of Glu seems to represent the link to NH_2(-L-)-*meso*-A_2pm, as indicated by comparison with respective dipeptides from *E. coli* K-12 peptidoglycan. As expected, the hydrazinolysis studies confirmed D-Ala to be the carboxy-terminal amino acid of the tetrapeptide. Part of the *meso*-A_2pm was detected to be carboxy-terminal, indicating that not all peptides are terminated by D-Ala. Thus, tripeptides are suggested to be present as a structural component of the peptidoglycan of *Synechocystis* sp. strain PCC 6714, or this might be due to the action of carboxypeptidase B during assembly of peptidoglycan (9). About 56% of the tetrapeptides of the *Synechocystis* peptidoglycan are involved in direct cross-linkage. This degree of cross-linkage is in the range of that found with gram-positive but not gram-negative bacteria (22).

Detailed analyses on the sugar strands of the *Synechocystis* peptidoglycan were not carried out. The acetyl values and the complete liberation of the amino sugar monomers (1) under the hydrolytic conditions used show a complete acetylation of the amino groups of both GlcN and MurN.

The cell wall of cyanobacteria is considered to correspond to the gram-negative type, mainly due to the presence of an outer membrane. On the other hand, the thickness of peptidoglycan layers in some cyanobacteria—e.g., a width up to 200 nm is observed in *Oscillatoria princeps* (3)—and the degree of cross-linkage found in the *Synechocystis* peptidoglycan correspond to typical gram-positive peptidoglycan. A polysaccharide, possibly bound to the peptidoglycan of *Synechocystis*, as indicated by the hydrofluoric acid extraction, is also known for gram-positive bacteria only. The finding of non-peptidoglycan sugars in peptidoglycan preparations of other cyanobacteria (4) might indicate binding of polysaccharide to peptidoglycan also in cyanobacteria other than *Synechocystis*. It is concluded that cyanobacteria have developed a particular cell wall organization which is different from a typically gram-negative or gram-positive cell wall structure. According to the data of molecular taxonomy, cyanobacteria have developed from ancestral eubacteria as a separate line (6), although a common source with gram-positive bacteria has also been proposed (30).

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