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Quantitative Chemical Composition of Peptidoglycan of *Listeria monocytogenes*

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The qualitative and quantitative chemical composition of the peptidoglycan layer of *Listeria monocytogenes* cell walls was determined. The peptidoglycan layer was isolated from intact cells after treatment with sodium lauryl sulfate and digestion with Pronase. The isolated peptidoglycan contained carbohydrates which included hexose, rhamnose, and amino sugars. The amino sugar was identified as glucosamine which was present in very high micromole concentration, as compared with the amino acids, muramic acid, or diaminopimelic acid. In addition, protein and phosphorus were also present. However, the presence of teichoic acid was not detected in the peptidoglycan. Protein moiety contained a total of 18 amino acids with lysine as the N-terminal amino acid.

Recently, the peptidoglycan (synonyms, murein, mucopeptide, glycosaminopeptide) layer from many organisms has been studied. Slade and Slamp (15) employed the differences in peptidoglycan composition as a criterion to classify the various streptococci into different groups. Kane et al. (7) reported in detail the complete structure of mucopeptides from *Streptococcus bovis*. Kolenbrander and Ensign (9) reported a detailed study on the peptidoglycan structure and composition of *Spirillum serpens*. In our present studies, by using the peptidoglycan preparation technique of Kolenbrander and Ensign (9), we have been successful in isolating the peptidoglycan layer of *Listeria monocytogenes* cell walls. The present paper describes chemical studies and amino acid composition of a virulent strain of *L. monocytogenes* 9-125.

MATERIALS AND METHODS

Organisms and growth conditions. *L. monocytogenes* strain 9-125, serotype 4b, was employed for these studies and was obtained through the courtesy of K. F. Girard, Boston, Mass. The cells were grown and prepared by the procedures of Jenkins and Watson (6).

Preparation of peptidoglycan. *L. monocytogenes* peptidoglycans were prepared from intact cells by the sodium lauryl sulfate and Pronase treatment method of Kolenbrander and Ensign (9). A flow diagram of the procedure for isolation of peptidoglycan is presented in Fig. 1.

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Analytical methods. Quantitative analysis of amino acids and glucosamine was done by the method of Spackman et al. (16), by using an amino acid analyzer (Beckman model 120 B, Beckman Instruments, Inc., Palo Alto, Calif.). The analyzer was not equipped to detect the presence of muramic acid (James Travis, personal communication). Therefore, muramic acid was determined colorimetrically by the method of Ghuysen et al. (5) by employing the acid-hydrolyzed sample, and the calculation was done by Beer's law. Total carbohydrate was determined by the method of Dische (1), hexose was determined by the anthrone method (14) and the phenol-sulfuric method (4), hexosamine was determined by the method of Rimington (12), rhamnose was determined by the method of Dische and Shettles (2), protein was determined by the method of Lowry et al. (10), and phosphorus was determined by the method of Dryer et al. (3). Teichoic acid was determined by the acid and alkali extraction procedure of Knox and Wicken (8).

Preparation of dinitrophenyl (DNP)-amino acids. Peptidoglycan was treated with 2,4-dinitrofluorobenzene (Sigma Chemical Co., St. Louis, Mo.) by the method of Sanger (13). The DNP-peptide was then hydrolyzed with 6 N HCL for 18 h at 110 C. After hydrolysis, samples were dried in vacuo and suspended in sterile distilled water. This process was repeated five times or until the acid was removed. Chromatography of DNP-amino acid was performed on thin-layer plates of silica gel in two dimensions by employing the technique, solvents, and spray reagent as described by Randerath (11).

RESULTS

Yield of L. monocytogenes peptidoglycan. The final yield of purified *L. monocytogenes*

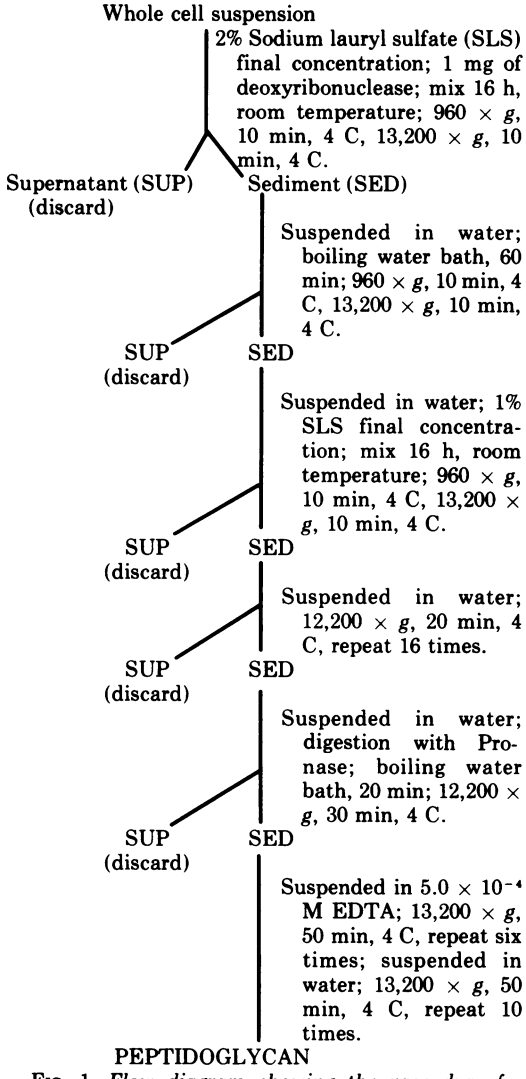

FIG. 1. *Flow diagram showing the procedure for isolation of L. monocytogenes peptidoglycan. EDTA, ethylenediaminetetraacetic acid.*

peptidoglycan was 0.4% of the dry weight of the intact vegetative cells. Electron micrographs of the peptidoglycan revealed a thin, delicate, and almost transparent material (Fig. 2), which retained the characteristic cell shape.

Quantitative chemical composition of L. monocytogenes peptidoglycan. Presented in Table 1 are the results of the quantitative chemical analysis of *L. monocytogenes* strain 9-125 peptidoglycan. It is of interest to note the reproducibility of the peptidoglycan chemical analyses. Triplicate determinations on three separate lots of peptidoglycan preparation did not differ more than 4% for any of the constituents. Protein was present in higher concentration than the carbohydrates. The presence of teichoic acid (8) was not detected in the peptidoglycan of *L. monocytogenes*. Therefore, it is believed that the phosphorus may have come from the residual cell wall membrane which has been reported to contain phosphorus (9).

The amino acid composition of the peptido-

TABLE 1. *Quantitative chemical composition of peptidoglycan of L. monocytogenes*

Components tested	Avg % per dry wt of peptidoglycan		
	lot no. 1	lot no. 2	lot no. 3
Total carbohydrate	27.27 (2)[a]	28.08 (2)	26.78 (2)
Hexose[b]	12.00 (2)	14.67 (2)	12.19 (2)
Hexose[c]	10.94 (2)	11.16 (2)	10.94 (2)
Hexosamine	4.28 (2)	5.41 (2)	5.07 (2)
Rhamnose	8.03 (3)	10.45 (2)	7.60 (2)
Protein	67.50 (2)	64.00 (2)	66.00 (2)
Phosphorus	2.89 (2)	2.23 (2)	2.66 (2)

[a] Number in parenthesis represents number of replicates.

[b] Hexose was determined by the anthrone method (14).

[c] Hexose was determined by the phenol-sulfuric acid method (4).

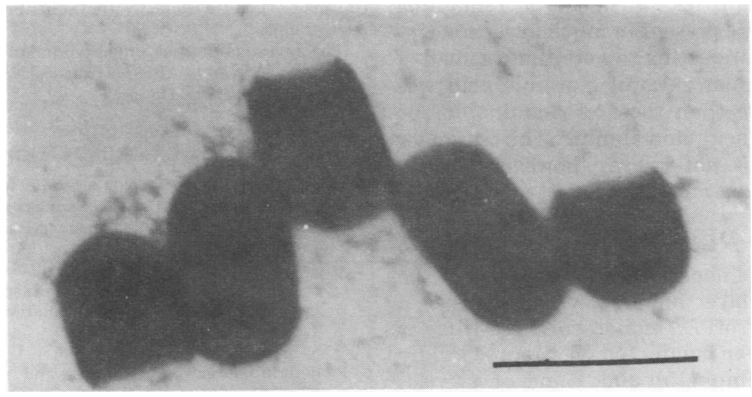

FIG. 2. *Electron micrograph of peptidoglycan of L. monocytogenes. Sample was put on Formvar-coated grid and stained with 3% phosphotungstic acid for 3 min. Magnification is 27,500; marker represents 1.0 μm.*

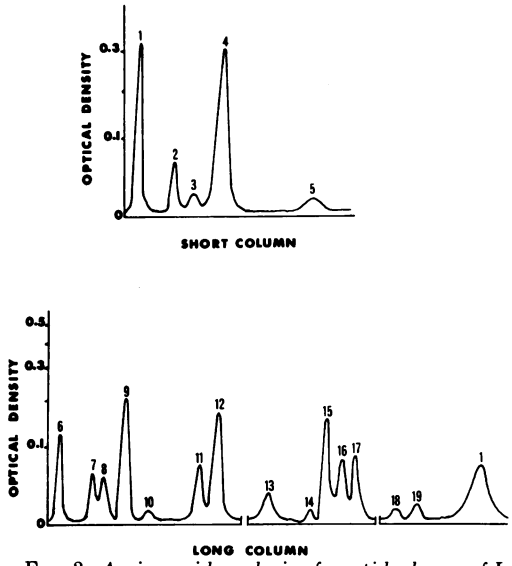

FIG. 3. *Amino acid analysis of peptidoglycan of L. monocytogenes employing a Beckman model 120 B amino acid analyzer. Analysis was carried out on a 60-cm long column for the acidic or neutral amino acids and on a 9-cm short column for the basic amino acids. For total analysis, 1.0-ml portions of the samples were pipetted onto the columns. Column packing was polystyrene, and chart speed was 0.1 inch/min. Identification was made by a comparison with known amino acids. Short column: 1, glucosamine; 2, lysine; 3, histidine; 4, ammonia; 5, arginine. Long column: 6, aspartic acid; 7, threonine; 8, serine; 9, glutamic acid; 10, proline; 11, glycine; 12, alanine; 13, valine; 14, methionine; 15, diaminopimelic acid; 16, isoleucine; 17, leucine; 18, tyrosine; 19, phenylalanine; and 1, glucosamine.*

glycan of *L. monocytogenes* is presented in Fig. 3, and quantitative yield is presented in Table 2. The ratio of diaminopimelic acid to muramic acid, glutamic acid, alanine, and glucosamine was 1:1:2:1:44. Aspartic acid, leucine, and isoleucine were the only amino acids found in significant quantities, whereas the remaining amino acids were present in much lower concentrations. It is interesting to note that instead of alanine, the molar ratio of glutamic acid was two times more than those of diaminopimelic acid, muramic acid, and alanine. The reason for such a result is not known; however, it may be unique with *L. monocytogenes*.

TABLE 2. *Amino acids and amino sugar analyses of peptidoglycan of L. monocytogenes*

Components	Peptidoglycan (0.75 mg hydrolyzed)		
	μM values	μg yield	Ratio to diaminopimelic acid
Diaminopimelic acid	0.068	12.93	1
Muramic acid[a]	0.042	12.32	1.05
Glucosamine[b]	3.155	564.75	43.67
Glutamic acid	0.154	22.66	1.75
Alanine	0.148	13.19	1.02
Aspartic acid	0.068	9.05	0.67
Leucine	0.060	7.87	0.61
Isoleucine	0.050	6.56	0.51
Lysine	0.040	5.85	0.45
Histidine	0.010	1.55	0.12
Arginine	0.008	1.39	0.11
Threonine	0.040	4.76	0.37
Serine	0.036	3.78	0.29
Proline	0.034	3.91	0.30
Glycine	0.060	4.50	0.35
Valine	0.046	5.39	0.42
Methionine	0.012	1.79	0.14
Tyrosine	0.026	4.71	0.36
Phenylalanine	0.020	3.30	0.26

[a] Muramic acid was estimated by the method of Ghuysen et al. (8).

[b] Glucosamine which was eluted from both columns was calculated as one component.

DISCUSSION

This study demonstrated the presence of the peptidoglycan layer among the cell walls of a virulent strain of *L. monocytogenes*. Similar studies have been conducted on streptococci by Slade and Slamp (15) and Kane et al. (7). Preliminary studies indicate that a complete, detailed chemical analysis of peptidoglycan would aid greatly in defining the immunology or toxicity of the organism in terms of exact chemical or structural features. Whether the mechanism of pathogenesis of *L. monocytogenes* in man or animals is related to the peptidoglycan will require further analysis of this strain as well as other virulent listerial strains.

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