

Amino Acid Composition of Certain Bacterial Cell-Wall Proteins¹

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ABSTRACT

HOWE, JEAN M. (Purdue University, Lafayette, Ind.), W. R. FEATHERSTON, W. J. STADELMAN, AND G. J. BANWART. Amino acid composition of certain bacterial cell-wall proteins. *Appl. Microbiol.* **13**:650-652. 1965.—Analyses were made to determine the amino acid composition of the cell-wall proteins of *Salmonella pullorum*, *S. senftenberg* 775W, *S. derby*, and *Escherichia coli*. These proteins consist of the usual 18 amino acids found in most proteins with diaminopimelic acid in addition. Quantitative determination of these amino acids showed that their amounts were similar.

The development by Salton and Horne (1951a, b) of a mechanical method for the isolation of a relatively homogeneous cell wall has fostered an increase of information concerning the chemical constitution of walls of bacteria (Cummins and Harris, 1956a, b; Ikawa and Snell, 1960; Perkins and Rogers, 1959). The picture with gram-negative organisms is less complete than that of gram-positive organisms, however, and most analyses of amino acid composition are qualitative in nature. The present study was initiated to quantitatively compare the amino acid composition of cell-wall protein of three *Salmonella* species and that of *Escherichia coli*. These organisms exhibit different agglutination reactions (Kauffman, 1941).

MATERIALS AND METHODS

Organisms. *S. pullorum* and *S. senftenberg* 775W were obtained from the Western Regional Research Laboratory. *S. derby* was supplied by M. J. Woodburn (Department of Foods and Nutrition, Home Economics, Purdue University), and was identified by the National Animal Disease Laboratory, Ames, Iowa. *E. coli* was obtained from the collection of the Biological Sciences Department, Purdue University.

Medium and cultural conditions. Brain Heart Infusion (Difco) broth was used as the growth medium. Growth conditions used were those described by Ikawa and Snell (1960). Cells were harvested at 1 C in a Servall refrigerated centrifuge.

Preparation of cell walls. The washed cells were

suspended in distilled water and placed in a polyethylene bottle, together with acid-washed Superbrite type 100-5005 glass beads (Minnesota Mining and Manufacturing Co., St. Paul, Minn.). The suspension, containing approximately equal portions of bacteria, glass beads, and distilled water, was shaken for two 25-min periods by use of a paint shaker [ca. 620 strokes (3.8-cm) per min, Miracle Paint Rejuvenator Co., St. Paul, Minn.] The mixture was kept cool by refrigeration at 2 to 7 C intermittently during shaking.

Staining of samples of the cell wall with methylene blue indicated almost complete breakage of cells. The slurry was diluted with distilled water and centrifuged for 10 min at $480 \times g$ at 1 C to settle glass beads and intact bacteria. The $480 \times g$ supernatant fraction was centrifuged at $5,000 \times g$ for 25 min. The sediment from this centrifugation was dialyzed against distilled water overnight in casing dialysis tubing in the cold, and was then centrifuged at $25,000 \times g$ for 25 min. This $25,000 \times g$ residue was suspended in distilled water and centrifuged at $600 \times g$ for 10 min. The sediment was discarded. The supernatant suspension was then centrifuged at $7,500 \times g$ for 20 min to sediment the cell-wall fraction, which was washed and was then resuspended in water and lyophilized.

Purity of the preparation was checked by comparing the composition of the cell-wall fraction with that of the same material which was further washed with 1 M NaCl (three times) and with distilled water (six times). Amino acid composition of the latter material was comparable to that of the cell-wall fraction.

Extraction of lipid. The cell-wall fraction was extracted with approximately 10 times its weight of petroleum ether-diethyl ether (24:1) to remove the lipid-containing material. The solvent was removed from the proteinaceous residue by drying in air with mild heat for approximately 3 hr.

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Hydrolysis. Tared amounts of cell walls weighing from 60 to 150 mg were hydrolyzed with 5 ml of 6 N HCl per 100 mg in sealed tubes in an autoclave at 121 C for 16 hr. The hydrolysate was filtered through a medium-porosity sintered-glass funnel, and was then dried in a vacuum oven to evaporate HCl, and diluted with distilled water to approximately 1 mg/ml.

Chromatography. Quantitative determination of most of the amino acids of the cell wall were obtained by ion-exchange chromatography of 1-ml samples of the hydrolysate by use of a Technicon amino acid analyzer. The conditions used in the ion-exchange chromatography resulted in the masking of diaminopimelic acid on the chromatogram by ammonia. Single-dimensional paper chromatograms with a solvent system of methanol-water-pyridine-10 N HCl (30:17.5:10:2.5) were used to detect diaminopimelic acid as described by Rhuland et al. (1955).

Tryptophan was determined by spectrophotometric analysis of samples of the cell wall dissolved in 0.1 N NaOH. This method was based on the selective absorption of tyrosine and tryptophan and the application of the solution of simultaneous linear equations (Goodwin and Morton, 1946).

RESULTS AND DISCUSSION

Average values of triplicate determinations of the amino acid composition of the four species of organisms are presented in Table 1. The values are expressed as moles relative to alanine. Under the conditions of hydrolysis, recovery was almost complete for the various amino acids, with the exception of methionine, tyrosine, and cystine. The recoveries of methionine and tyrosine were 70 and 91%, respectively. Most of the cystine was oxidized to cysteic acid.

Cummins and Harris (1956a, b), in their extensive study of cell walls, reported that the cell-wall protein of gram-positive organisms consists mainly of alanine, glutamic acid, and lysine. Diaminopimelic acid, aspartic acid, and glycine occurred in some, but not all gram-positive cell walls. The distribution of amino acids varies widely from one species to another; yet, in some cases very similar ratios have been reported (Salton and Pavlik, 1960; Rogers and Perkins, 1959; Hancock, 1960; Strominger, Park, and Thompson, 1959). In this study, the amino acid compositions of the gram-negative organisms were much more complex than those reported for the gram-positive organisms. These results follow the pattern of results from comparative studies of cell-wall components by Salton (1953). They are also in agreement with reports which indicate that a more complicated physical structure of multilayered walls is encountered more frequently in gram-negative organisms than in gram-positive

TABLE 1. Amino acid composition* (moles relative to alanine)

Amino acid	<i>Escherichia coli</i>	<i>Salmonella senftenberg</i>	<i>S. derby</i>	<i>S. pul-lorum</i>
Aspartic acid.....	1.01	1.00	0.93	1.00
Threonine.....	0.48	0.48	0.54	0.50
Serine.....	0.41	0.43	0.44	0.48
Glutamic acid.....	1.14	1.14	1.11	1.11
Proline.....	0.25	0.28	0.28	0.26
Glycine.....	0.93	0.96	0.98	1.02
Alanine.....	1.00	1.00	1.00	1.00
Valine.....	0.73	0.75	0.72	0.66
Methionine†.....	0.31	0.23	0.30	0.37
Isoleucine.....	0.55	0.55	0.51	0.51
Leucine.....	0.83	0.78	0.77	0.78
Tyrosine†.....	0.12	0.08	0.10	0.15
Phenylalanine.....	0.34	0.33	0.33	0.33
Lysine.....	0.56	0.56	0.55	0.59
Histidine.....	0.18	0.19	0.18	0.21
Arginine.....	0.51	0.52	0.49	0.48
Tryptophan‡.....	0.05	0.04	0.06	0.05
Diaminopimelic acid (<i>meso</i> form).	+	+	+	+

* Cysteic acid was not determined because of lack of material.

† Observed values corrected for recovery.

‡ Spectrophotometric determination relative to tyrosine concentration.

organisms (Kellenberger and Ryter, 1958; Glauert, 1962).

The relative ratios of amino acids from cell-wall proteins among the species analyzed in this study were very similar. The results obtained with *E. coli* agreed quite well with those reported by Roberts et al. (1957), except that the ratios of aspartic acid, glutamic acid, glycine, valine, and leucine were somewhat higher in this study. This discrepancy could be due to strain difference or to different methods of analysis.

The ion-exchange determination revealed appreciable amounts of ammonia in the hydrolysates. Possible sources of this ammonia include: (i) the destruction of cell-wall compounds such as the amino sugars; (ii) deamination of glutamine and asparagine; (iii) decomposition of amino acids; and (iv) the possibility that, since diaminopimelic acid eluted at the same position as ammonia, part of the calculated ammonia was diaminopimelic acid.

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