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Optical Configuration of Staphylococcal Cell Wall Serine

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Studies on the amino acid content of the cell wall of gram-positive bacteria have shown them to be composed of relatively few amino acids. One of the principal amino acids found in the cell wall of certain staphylococci is serine (1, 2, 5, 6). Although the optical configuration of staphylococcal cell wall serine has not been determined, Whitney and Grula (8) found that *Micrococcus lysodeikticus* grown in the presence of radioactive D-serine incorporated labeled serine in the peptidoglycan. Harney et al. (4), in studies on the cell wall of strains of *Leuconostoc mesenteroides*, have reported the presence of L-serine.

Our interest in the nature of the configuration of the serine in staphylococcal cell wall developed as a result of the marked effect the serine content of the wall has on the lysis of strains of *Staphylococcus epidermidis* by lysostaphin (H. P. Browder et al., Bacteriol Proc. p. 14, 1966). Increasing amounts of cell wall serine resulted in a decrease in susceptibility to this lytic agent.

The strains of *S. epidermidis* and the *S. aureus* 209P used in these studies were obtained from V. T. Schuhardt of the University of Texas. The Smith, K6, Weldwood, and Adams strains of *S. aureus* were from the collection of D. E. Rogers of Vanderbilt University, and the *S. albus* 12228 is an American Type Culture Collection strain.

Staphylococcal cell walls were prepared from late log-phase cultures as previously described (2). Trypticase Soy Broth (Baltimore Biological Laboratory) was used as the source of nutrient for all of the staphylococci with the exception of *S. epidermidis* 115. Poor initial growth on Trypticase Soy Broth (TSB) prompted us to grow this organism on Micro Inoculum Broth (Difco). Following adaptation of the culture to TSB, cell wall from strain 115 was also prepared by using the TSB medium.

Hydrolysates of the lyophilized cell walls were prepared by suspending the wall (ca. 10 mg/ml) in 6 N HCl, sealing the tubes evacuated to <100 mm Hg, and heating for 20 hr in an oil bath at 110 C. Tube contents were filtered and taken to dryness over KOH in vacuo. The hydrolysates were dissolved in distilled water and assayed for serine microbiologically and on the Beckman Spinco amino acid analyzer.

To assess the loss of serine under the conditions of hydrolysis, seryl-glycine and glycyl-serine were hydrolyzed with *S. aureus* Copenhagen cell wall (contains no serine) under the described conditions. The resulting hydrolysate was assayed for serine on the amino acid analyzer with a serine recovery of 86.2%. The reported results have been corrected for the destruction of serine during hydrolysis.

TABLE 1. *Cell wall serine[a] in selected strains of staphylococci*

Organism	L-Serine[b]	Total serine[c]
S. epidermidis 27992	0.317	0.341
S. epidermidis 115 MIB[d]	0.310	0.300
S. epidermidis 115 TSB[e]	0.718	0.772
S. epidermidis 26	0.651	0.680
S. albus 12228	0.419	0.444
S. aureus 209P	0.190	0.182
S. aureus Smith	0.135	0.130
S. aureus K_6	0.080	0.082
S. aureus Weldwood	0.049	0.045
S. aureus Adams	0.217	0.205

[a] Corrected for destruction of serine during hydrolysis.
[b] By microbiological assay. Results expressed as micromole of serine per milligram of cell wall.
[c] By amino acid analyzer. Results expressed as micromole of serine per milligram of cell wall.
[d] Cells grown on Micro Inoculum Broth.
[e] Cells grown on Trypticase Soy Broth.

The microbiological assay for L-serine (7) employed *Streptococcus* sp. ATCC 8042 and Serine Assay Medium (Difco). The response of the test organism to low levels of serine was increased by successive transfer on media containing decreasing amounts of serine (*Microbiological Assay of Vitamins and Amino Acids*, p. 73, Difco Laboratories, Detroit, 1963). Using D-serine and L-serine separately and in combinations, it was clearly shown that the assay was

TABLE 2. *Effect of medium composition on the serine and glycine content of staphylococcal cell wall*

Medium	Amino acid	Noninoculated broth[a]	Spent medium[a]	Cell wall (μmoles/mg)
Trypticase Soy Broth	Glycine	0.160	0.147	1.753
	Serine	0.271	0.247	0.772
Micro Inoculum Broth	Glycine	0.323	0.356	2.329
	Serine	0.173	0.104	0.300

[a] Results expressed as micromoles of amino acid per milligram of lyophilized medium.

specific for the L-isomer and not influenced by the presence of D-serine.

Results from the microbiological assay and the amino acid analyzer are presented in Table 1. The results from the two assay methods agree quite well and suggest that the serine found in staphylococcal cell wall is of the L-configuration.

The growth medium has a pronounced effect on the serine content of the cell wall of *S. epidermidis* 115. Substituting TSB for Micro Inoculum Broth results in a 150% increase in serine content, although there is only a 56% increase in the serine content of the medium (Table 2). This is accompanied by a concomitant decrease in the amount of glycine such that the sum of serine and glycine remain constant (H. P. Browder et al., Bacteriol. Proc. p. 14, 1966). Young (9) observed a similar effect on the cell wall composition of *Bacillus subtilis* grown in different media, although the changes in the amino acid composition of the principal amino acids were not as pronounced as those reported here. Cummins and Harris (3) have suggested use of the cell wall amino acid composition as an aid to bacterial taxonomy.

Taxonomic inference may properly be drawn from the presence or absence of a specific amino acid in the cell wall. However, in light of our findings, no taxonomic significance should be attached to the amount of such amino acid.

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