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Gas chromatographic determination of the configuration of alanine and serine in staphylococcal cell walls

In 1966, KORMAN[1] reported the presence of small amounts of serine in the cell walls of wild-type strains of staphylococci and elevated levels of this amino acid in mutants isolated from the wild-type strains. TIPPER AND BERMAN[2] have confirmed that there are low but significant amounts of serine in *Staphylococcus aureus* Copenhagen and increased amounts in *S. epidermitis* Texas 26. They have located this amino acid within the pentapeptide cross bridges of the peptidoglycan moiety, and BROWDER *et al.*[3] have established its L-configuration in several strains by microbiological assays.

In the present communication further data are reported on the configuration of the alanine, as well as the serine, in staphylococcal walls as determined by a new gas chromatographic method[4,5]. The procedure, which is based on the use of an asymmetric stationary phase, permits one to resolve and analyze different amino acids conveniently and simultaneously.

TABLE I

MOLAR RATIOS AND CONFIGURATION OF AMINO ACIDS IN STAPHYLOCOCCAL CELL WALLS

The molar ratios were determined on a Beckman-Spinco amino acid analyzer. Configuration was determined by gas chromatography; r = relative retention volume with respect to the corresponding glycine derivative. D/L = molar ratio of the D- to the L-isomer; average of 2–3 determinations.

Strain No.	*Source of reference*	*Amino acids*							
		Lys	*Glu*	*Gly*	*Ala*	*Ser*	*Thr*	*Ile*	*Leu*
NCTC 8511	See ref. 1	1	1.14	4.93	2.91 D/L = 1.90	0.11 L*	Traces	0.10	0.13 §
HS 2123	HS 968 See ref. 1	1	1.02	4.3	3.6 D/L = 2.03	1.0 L**	Traces	Traces	Traces
HS 1159	HS 968	1	1.1	5.10	2.44 D/L = 1.60	0.03 L*	0.004	Traces	Traces
HS 1449	See ref. 1	1	1.08	4.39	2.9 D/L = 2.33	0.70 L*	Traces	Traces	Traces
HS 2136	HS 1449	1	1.1	4.6	2.7 D/L = 1.47	0.28 L*, ***	0.11	0.12	0.16 L§

* Isopropyl ester, $r = 2.16 \pm 0.04$ (standard: D-Ser, 1.98; L-Ser, 2.19).
** Determined by coinjection.
*** Methyl ester, $r = 2.60 \pm 0.03$ (standard: D-Ser, 2.44; L-Ser, 2.63).
§ Methyl ester, $r = 2.22 \pm 0.01$ (standard: D-Leu, 2.08; L-Leu, 2.21).

The staphylococcal strains chosen for analysis are listed and their reference sources given in Table I. Cell walls (2–25 mg) were prepared as previously described[1]. They were resuspended in 6 M HCl after lyophilization and subjected to hydrolysis for 6 h at 100°, dried in a rotary evaporator and redissolved in distilled water. *N*-Trifluoroacetyl methyl and/or isopropyl ester derivatives were prepared of aliquots of the redried residues, as previously described[5]. After the trifluoroacetylation step, care was taken not to chase the reagent and solvent completely. The derivatives were dissolved in chloroform and 1 μl injected without splitter into a 400 ft × 0.02 inch capillary column coated with *N*-trifluoroacetyl-L-valyl-L-valine cyclohexyl ester (Research Products Division, Miles Labs. Ltd., Elkhart, Ind.). The chromatograph was provided with a flame ionization detector; the column temperature was 108° for the isopropyl esters and 105° for the methyl esters and the He pressure 35 lb/inch^2 and 20 lb/inch^2, respectively.

The use of both methyl and isopropyl esters has the advantage of providing two sets of retention data. Where leucine was present, methyl esters had to be employed of necessity, since *N*-trifluoroacetyl isopropyl esters of L-leucine and D-serine could not be separated.

For determining the D/L ratio of alanine, conditions were optimized to obtain peak resolution down to baseline. Accuracy was found to be ± 1–5 % and was not affected by the presence of other amino acids, D-amino sugars and L-lactic acid. In some samples the area corresponding to D-alanine had to be corrected for overlap with a small unknown peak. The identity of the alanine peaks was checked by gas chromatography–mass spectrometry in one case (HS 1449).

The method can be applied directly to the cell wall hydrolysates, since experiments with a synthetic mixture of *N*-acetylmuramic acid and serine did not show any interference by the former.

It should be noted that the amino acid composition of the samples was determined on a Beckman-Spinco Analyzer (Table I). The gas chromatographic method has not yet been sufficiently refined to give a quantitative measure of the ratios of the different amino acids, in addition to that of the enantiomers. Serine, in particular, gave low yields on derivatization (about 20 % for the isopropyl esters and 60 % for

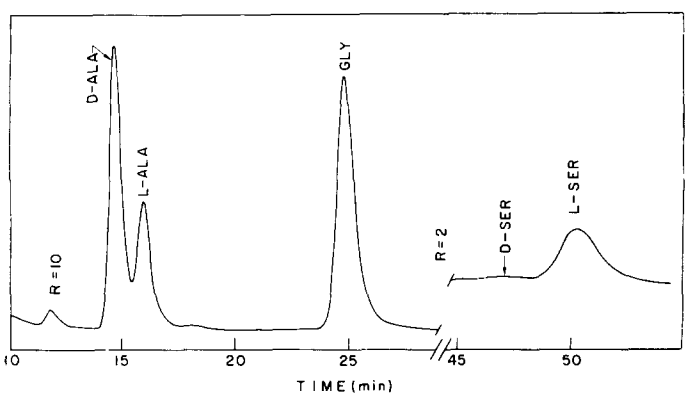

Fig. 1. Proof of the L-configuration of serine in a staphylococcal cell wall. Chromatogram of the *N*-trifluoroacetyl amino acid isopropyl esters from the cell wall hydrolysate of strain HS 2123.

the methyl esters). It was, however, shown that the loss did not affect one of the enantiomers selectively.

Configuration of serine. The increase of the serine peak on coinjection with *O,N*-di-trifluoroacetyl-L-serine isopropyl ester established the L-configuration of serine in the cell wall of HS 2123, a strain particularly rich in this amino acid and containing only traces of leucine. As can be seen in Fig. 1, the enantiomeric serine peaks are well separated. Under our conditions, the relative retention volumes were reproducible within ± 1–2 % and were used for determining the configuration of serine in all other strains studied (Table I). Irrespective of its relative amount, the serine was found to have the L-configuration in all cases, in confirmation of previous results[3].

Ratio of D- *and* L-*alanine.* The various strains differ in the molar ratio of alanine, as well as in the proportion of the D- and L-isomers. In confirmation of many other reports[6] the alanine to glutamic acid ratio is approx. 3:1. The observed variation from the value of 3 of the molar ratio of alanine in the pseudo-wild-type derivatives, HS 2136 and HS 1159, may be interpreted as representing variations in the amount of D-alanine substitution of the teichoic acid moiety. However, this explanation does not seem to be valid for the elevated serine strains. An excess over 2 moles of D- per mole of L-alanine (*e.g.* HS 1449, Table I) suggests the presence of greater quantities of di-D-alanine in the peptide moiety of these mutants. TIPPER AND BERMAN[2] have shown that indeed more di-D-alanine end groups were found in the elevated serine strain than in the wild-type strain which they studied.

It should be noted that gas chromatography also showed traces of threonine, isoleucine and leucine, as well as an unknown peak near threonine in low serine strains.

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