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Note

Simple method for the separation of amino acids, amino sugars and amino alcohols related to the peptidoglycan components on a standard amino acid analyser

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Conditions for the separation and quantitation of amino acids and amino sugars in hydrolysates of polymers of microbial origin by using the automatic amino acid analyser have been reported in a number of papers. For example, Peterson and Bernlohr[1], by using a double-column system, developed a procedure that permits the separation of 2,6-diaminopimelic acid, ornithine, muramic acid, glucosamine and galactosamine together with all of the common protein amino acids, and Guire[2] reported the separation of ninhydrin-positive peptidoglycan components by using a simple two-buffer elution programme on a single column. On the other hand, separations of hexosamines from the corresponding hexosaminitols in hydrolysate mixtures derived from borohydride-treated glycoprotein samples have also been reported[3–5]. It seems, however, that no conditions have been described for the simultaneous separation of ninhydrin-positive components that can be expected to occur in hydrolysates of borohydride-reduced peptidoglycan material.

EXPERIMENTAL AND RESULTS

We report here a simple elution scheme that enables one to measure the amino acids and amino sugars of some bacterial cell-wall peptidoglycans together with their reduction products derived from the carboxyl-free amino acid residues and the terminal hexosamine units, respectively. For example, alaninol and 5-hydroxy-4-aminopentanoic acid would be obtained from the hydrolysate of a borohydride-reduced peptidoglycan fragment if the carboxyl group of the C-terminal alanine and the γ-glutamyl residue, respectively, were originally unsubstituted. Muramicitol or glucosaminitol would be obtained from the reducing sugar unit. Parallel determinations of muramic acid, glucosamine and their glycitols will give information about the sequence and the length of the glycan chain involved. Accordingly, the separation reported in this paper deals with a mixture that includes the above compounds.

Apparatus and reagents

An LKB BC-200 amino acid analyser equipped with a column (54 × 0.9 cm) of Aminex A-6 resin was used. All chemicals were of analytical grade.

Preparations of samples

The samples, if not stated otherwise, were of commercial origin and, if necessary, were recrystallized to give compounds of analytical purity. Glucosaminitol (2-amino-2-deoxy-D-glucitol) was prepared by sodium borohydride reduction of 2-acetamido-2-deoxy-α-D-glucose, followed by deacetylation in 2 *M* hydrochloric acid for 3 h at 100° [6]. Muramicitol [2-amino-3-O-(D-1'-carboxyethyl)-2-deoxy-D-glucitol] was prepared by sodium borohydride reduction of muramic acid following essentially the procedure of Hara and Matsushima[7]. L-Isoglutamine was synthesized according to Ressler[8]. 5-Hydroxy-4-amino-L-pentanoic acid was conveniently prepared by reduction of L-glutamine methyl ester hydrochloride with lithium borohydride (10-fold excess) in dry tetrahydrofuran at 0° for 2 h and then at room temperature for 5 h. The excess of borohydride was decomposed with methanolic hydrogen chloride to pH 1, and boric acid was removed by repeated evaporation with methanol. The residue was eluted from a column of Dowex 50-X8 (H^+) resin, the material displaced by 1 *M* ammonia solution was hydrolysed in an evacuated sealed tube with 6 *M* hydrochloric acid (100°, 20 h), and the residue was eluted from a Dowex 50-X8 (H^+) column with 1 *M* ammonia solution to give the title compound, 5-Hydroxy-4-amino-L-pentanoic acid. After crystallization from methanol plus a few drops of water, the product (36% yield) had m.p. 160–161°, α_D +20.1° (1 g/100 ml water); literature m.p.[9] = 160–161.5°. Found: C, 44.86; H, 8.13; N 10.41%. $C_5H_{11}NO_3$ calculated: C, 45.10; H, 8.33; N, 10.52%.

Buffers

Samples were added in 0.2 *M* sodium citrate buffer (pH 2.2). Buffers of the following compositions were used for the elution: I, 0.2 *M* sodium citrate, pH 3.17; II, 0.35 *M* sodium citrate, pH 5.28; and III, 1.2 *M* sodium citrate, pH 6.45.

Procedure

A buffer flow-rate of 60 ml/h and a ninhydrin flow-rate of 30 ml/h were used. The column temperature was maintained at 55° during the whole analysis. The buffer changes were sequenced as follows: buffer I to II after 64 min; buffer II to III after 100 min; and buffer III to 0.2 *M* sodium hydroxide solution after 150 min of analysis. The elution was complete in 190 min.

The ion-exchange analysis was performed on a single column with three sodium citrate buffers followed by 0.2 *M* sodium hydroxide solution (Fig. 1). The

TABLE I

ELUTION TIMES OF SOME AMINO ACIDS, AMINO ALCOHOLS, HEXOSAMINES AND HEXOSAMINITOLS FROM THE START OF THE CHROMATOGRAM

Compound	*Time (min)*	*Compound*	*Time (min)*
Muramicitol	40	Isoglutamine	110
Muramic acid	62	5-Hydroxy-4-aminopentanoic acid	115
Glutamic acid	65	Glucosamine	125
Glycine	90	Glucosaminitol	135
Alanine	95	Ammonia	167
2,6-Diaminopimelic acid	100	Alaninol	170

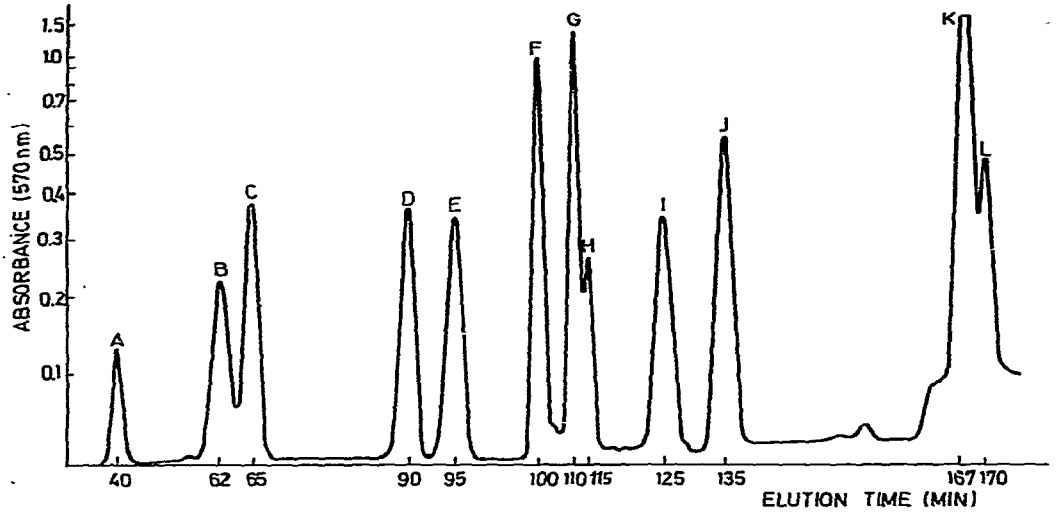

Fig. 1. Elution pattern of a mixture of amino acids, amino sugars and amino alcohols related to the peptidoglycan components on a single column (54 × 0.9 cm) of LKB Aminex A-6 resin with a three-buffer elution programme. Peaks: A = muramicitol; B = muramic acid; C = glutamic acid; D = glycine; E = alanine; F = 2,6-diaminopimelic acid; G = isoglutamine; H = 5-hydroxy-4-aminopentanoic acid; I = glucosamine; J = glucosaminitol; K = ammonia; L = alaninol.

whole procedure was accomplished in about 3 h under conditions of standard amino acid analysis; Table I lists the mean elution times of the compounds involved. The amounts of the components submitted to analysis ranged from 0.2 to 1.0 μmole, depending on the colour intensity of the relevant ninhydrin complex (*e.g.*, 0.5 μmole for 5-hydroxy-4-aminopentanoic acid and 1 μmole for muramicitol and alaninol). For a good separation, the maintenance of the pH of the first buffer (I) is of particular importance: at pH below 3.16 the peaks of 2,6-diaminopimelic acid and alanine overlap, whereas at pH above 3.18, the muramic acid and glutamic acid peaks overlap.

REFERENCES

1 D. E. Peterson and R. W. Bernlohr, *Anal. Biochem.*, 33 (1970) 238.
2 P. Guire, *Anal. Biochem.*, 42 (1971) 1.
3 P. Weber and R. J. Winzler, *Arch. Biochem. Biophys.*, 129 (1969) 534.
4 A. M. Bella, Jr. and Y. S. Kim, *J. Chromatogr.*, 51 (1970) 314.
5 P. J. Durda and M. A. Cynkin, *Anal. Biochem.*, 59 (1974) 407.
6 P. D. Bragg and L. Hough, *J. Chem. Soc.*, (1957) 4347.
7 S. Hara and Y. Matsushima, *Bull. Chem. Soc. Jap.*, 39 (1966) 1826.
8 C. Ressler, *J. Amer. Chem. Soc.*, 82 (1960) 1641.
9 A. C. Chibnall, C. Haselbach, J. L. Mangan and M. W. Rees, *Biochem. J.*, 68 (1958) 122.