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A Rapid Procedure for the Quantitative Separation of α,ε-Diaminopimelic Acid (DAP)

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NOTE

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Abstract

A simple and rapid procedure for the quantitative separation of α,ε-diaminopimelic acid (DAP) from mixture of amino acids, using anion-exchange resin, is presented. The recovery of DAP from the column ranges between 96 and 98%.

INTRODUCTION

α,ε-Diaminopimelic acid (DAP) has been shown to be present as an important cell-wall constituent in most gram-negative bacteria and blue-green algae (*1–3*). Rhuland (*4*) has aptly reviewed the distribution, chemical properties, metabolism, and biosynthesis of DAP. The methods reported for the quantitative separation of DAP from the mixture of amino acids present in cell hydrolysate of bacteria are by paper chromatography (*3*, *5–7*), electrophoresis (*3*), and ion-exchange chromatography (*6*, *8–10*). Sen and co-workers (*11*) reported methods for the determination of DAP both by thin-layer (TLC) and gas–liquid (GLC) chromatography; the TLC method is rapid and sensitive, whereas GLC method is extremely sensitive but time consuming.

A perusal of literature reveals that the methods for the separation of

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DAP by ion-exchange chromatography are essentially based on using cation-exchange resins. The present report, however, pertains to a simple, rapid, and quantitative procedure for the isolation of DAP from the mixture of amino acids using anion-exchange resin.

EXPERIMENTAL

Dowex 2X-8 (20–50-mesh) from J. T. Baker Chemical Company, Phillipsburg, New Jersey, available in chloride form, was converted to the free base by suspending and shaking in 1 *N* NaOH (until free from Cl ions), and subsequently into the acetate form with glacial acetic acid. The resin was washed well with deionized water and the fines removed by decantation. After suspending the resin two to three times in 1 *N* acetic acid, it was loaded, under gravity, on a 0.9-cm diameter water-jacketed column, and a resin height of 30 cm was attained. The column was maintained at 45 °C. Prior to loading of the sample, the column was washed for an hour with 0.1 *N* acetic acid. 1 ml sample containing 100 μg DAP in a mixture of standard amino acids was carefully added on the top of the resin. It may be pointed out that amino acids up to a concentration of 0.4 μmole of each of 18 amino acids is well retained while DAP is eluted from the column, under the present condition. Elution with 0.1 *N* acetic acid was now effected, under gravity, maintaining a flow rate of 30 ml/hr, and 1 ml fractions were collected. Suitable aliquots of each fraction were taken and DAP determined colorimetrically by the procedure of el-Shazly and Hungate (*6*). The findings were also confirmed by using radioactive DAP(^{3}H) as well. DAP (1 μmCi) in the amino acids mixture was added on the column and the elution carried out as described above. An aliquot (0.1 ml) of each fraction was placed in a vial to which 9.9 ml of Bray's scintillation fluid (*12*) was added. The radioactivity was read on a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 3320. The findings are presented graphically in Fig. 1.

Working Procedure

After rejecting the first 6 ml eluent from the 30 cm column of Dowex 2X-8 in acetate form, maintained at 45 °C, 15 ml of the subsequent eluent is collected, the flow rate being 30 ml/hr. The eluent (1 ml) may be taken directly for color development. For a concentration of DAP lower than 10 μg/ml, 15 ml of eluent from the column may be carefully

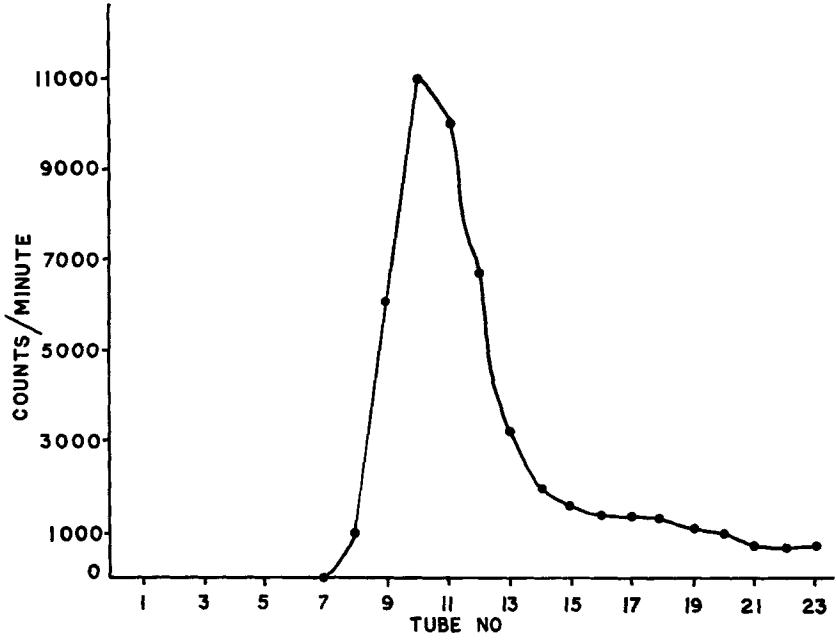

FIGURE 1

evaporated to dryness under vacuum at 40°C, made to suitable volume with 0.1 *N* acetic acid, and an aliquot taken for colorimetry.

The recovery of DAP from the column ranges between 96 and 98%.

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