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Note

Improved method for the separation of lysine from N-ε-monomethyllysine in plasma using cation-exchange chromatography

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During studies involving the metabolism of lysine in sheep it became necessary to determine the specific activity of lysine-4,5-H3(n) in plasma. This required the use of a chromatographic system capable of giving a baseline separation of lysine from other ninhydrin-positive components present in sheep plasma. When a pH 4.19 sodium citrate buffer (0.4 *M* Na^+) and a 29 × 0.9-cm column of Aminex A5 resin (Bio-Rad Labs., Richmond, Calif., U.S.A.) at a temperature of 29° were used, as suggested by Atkin and Ferdinand[1] for the separation of basic amino acids present in physiological fluids, a peak appeared in the position expected for lysine during chromatography of deproteinised sheep plasma. On occasions, however, there were indications that there were two compounds running in this position. Chromatography of authentic standards indicated that the other component of the peak was probably N-ε-monomethyllysine. It had been reported that sheep plasma contains significant quantities of this compound[2]. Previously reported methods for separating lysine completely from N-ε-monomethyllysine would appear to cause ornithine to overlap with lysine (see for example ref. 3). We therefore investigated the possibility of obtaining a system to give baseline separation of lysine from other ninhydrin-positive compounds present in plasma, notably ornithine and N-ε-monomethyllysine.

The system adopted was based on the Technicon NC1 amino acid analyser (Technicon, Basingstoke, Great Britain). A column (48 × 0.9 cm) of Aminex A5 resin, maintained at 22°, in order to resolve ornithine and lysine, was eluted with pH 5.65 sodium citrate buffer (0.35 Na^+; 34.31 g trisodium citrate·$2H_2O$, 2 ml Brij 35 (32% w/v), 0.1 ml octanoic acid, approximately 3.5 ml concentrated HCl all dissolved in 1 l water). With a flow-rate of 1.15 ml/min a back pressure of 320–400 p.s.i. was obtained. Each run took 216 min excluding regeneration and equilibration. A typical chromatogram of deproteinised sheep plasma is presented in Fig. 1.

To determine the specific activity of the lysine the effluent from the column was divided, 0.23 ml/min being mixed with ninhydrin[4] (0.32 ml/min) and segmented with nitrogen (0.60 ml/min) before being passed through the reaction bath for colour development. The remainder was collected in a fraction collector prior to counting

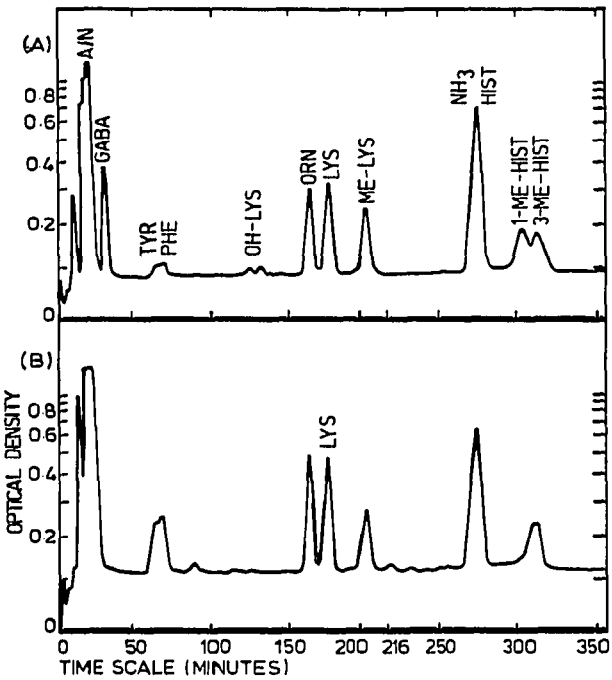

Fig. 1. The elution patterns given by 0.5 ml sheep plasma extract with the addition of 1.0 ml standard amino acid mixture (A) and by 2.0 ml sheep plasma extract alone (B). No evidence was obtained for the presence of ninhydrin compounds in plasma running close to lysine other than those marked. N-ε-dimethyllysine and N-ε-trimethyllysine would be expected to run slower than N-ε-monomethyllysine[3]. The plasma extract was prepared by mixing 2.0 ml 10% (w/v) sulphosalicylic acid with 3.0 ml sheep plasma. After maintaining the mixture at 4° for 30 min the protein precipitate was removed by centrifugation (5,000 *g* for 10 min). 1.0 ml of the standard amino acid mixture contained 0.19 μmole γ-aminobutyric acid (GABA), 0.06 μmole hydroxylysine, 0.27 μmole lysine, 0.25 μmole N-ε-methyllysine, 0.45 μmole $(NH_4)_2SO_4$, 0.26 μmole histidine, 0.24 μmole 1-methylhistidine, and 0.18 μmole 3-methylhistidine. Peaks at beginning of trace are acidic and neutral amino acids (A/N).

in a scintillation counter. The 2.0-ml fractions were mixed with 10 ml of a 2:1 mixture of toluene and Triton X100 containing 4.0 g 2,5-diphenyloxazole and 0.1 g 1,4-di-[2-(5-phenyloxazolyl)]-benzene per litre of mixture.

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