Column Chromatography of 2-Aminoethylphosphonic Acid

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Abstract

Column chromatography of 2-Aminoethylphosphonic acid on a Beckman 116 Amino Acid Analyzer under routine operating conditions showed that this amino acid is eluted after 27 to 28 min with a buffer pH 3.28 (.20 N). This agrees with the acidic nature of this amino acid.

Introduction

2-Aminoethylphosphonic acid (AEP) has been suggested as a marker of protozoa in the rumen since it is absent from the bacteria and feed (1). Ibrahim et al. (6) reported a method for separating and identifying AEP in protozoa and contents from the rumen. This method was applied to the contribution of protozoa to microbial protein synthesis in the rumen by Ibrahim and Ingalls (5), who reported that AEP was eluted after 185 min when an additional elution buffer of pH 6.25 (.40 N) was used. In my experience, however, AEP is eluted within 30 min with the same initial buffer of pH 3.28 (.20 N) with AEP from the same source as Ibrahim et al. (6).

Results and Discussion

A chromatogram run with AEP alone under the conditions described showed that this amino acid had an elution time of 27 to 28 min (Fig. 1). When a calibration mixture together with AEP was run, AEP was eluted with the same peak time of 27 to 28 min. This is in accord with the acidic nature of this amino acid and results of other workers. Alhadeff and Daves (2) using the same conditions as the present study obtained a similar elution time of 28 min for AEP on a Beckman 120 C Analyzer. AEP eluted with a peak time of 43 to 44 min on a Beckman 120 C Analyzer with a buffer flow of 70 and ninhydrin flow of 35 ml/h (9). The longer elution time probably was due to the fact that flow rates were slower than those in the present study. But even in this case elution time was much shorter than that (185 min) reported by Ibrahim et al. (6). AEP and phosphorylethanolamine are acidic compounds with almost identical chromatographic properties and structures. Separation of these two compounds has been achieved with an Amberlite CG 120 resin and eluting buffer of pH 2.50 (3).

In the present study a peak was found in the

Materials and Methods

Standards were prepared so that .2 ml of buffer pH 2.2 contained .3 μmol of AEP (Grade A, Calbiochem, Los Angeles, California), alone or together with .1 μmol of each amino acid in a calibration mixture (Type 1, Beckman, California). Analysis was according to the Beckman manual for protein hydrolysates with buffers of pH 3.28 (.20 N) and pH 4.25 (.20 N). Ninhydrin was pumped in from zero time, and the recorder started after 15 min. Flow rates of 80 and 40 ml/h for buffer and ninhydrin, respectively, were checked before the sample was applied to the column. Sample volumes (.2 ml) were applied to the acid-neutral column (55 × .9 cm) of a Beckman 116 Amino Acid Analyzer containing Beckman resin type PA 28.

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FIG. 2. Amino acid chromatogram of a calibration mixture without 2-Aminoethylphosphonic acid. Portion of the chromatogram eluted with buffer pH 6.25 (.40 N) containing an Ammonia peak in the region reported for 2-Aminoethylphosphonic acid by Ibrahim et al. (6).

region reported for AEP by Ibrahim et al. (6) when a calibration mixture containing no AEP was run with their system of elution buffers (Fig. 2). This peak is due to ammonia (8) and appears to be the peak attributed to AEP by Ibrahim et al. (6) since they ran their AEP standard together with a full calibration mixture and never alone.

A second error in the work of Ibrahim et al. (6) concerns their finding of phosphoserine in hydrolysates of ruminal protozoa and contents. The C-O-P bond of this amino acid is destroyed on hydrolysis with 6N-HCl for 24 h at 100 C (7). Thus, the peak attributed to phosphoserine by these workers (6) was due to other ninhydrin positive compound(s) in their hydrolysates.

From my results and work of others, it appears that AEP has an elution time of 27 to 28 min under normal operating conditions on a Beckman 116 Analyzer. Thus, AEP would be a convenient marker of ruminal protozoa providing it can be well separated from the numerous other amino acids and ninhydrin-positive compounds which elute before aspartic acid (4).

References