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SINGLE COLUMN ION EXCHANGE ANALYSIS OF ASPARAGINE AND CLUTAMINE IN THE PRESENCE OF OTHER AMINO ACIDS

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During the acid hydrolysis of proteins preparatory to analysis for amino acids, the amino acids asparagine and glutamine are converted to aspartic acid and glutamic acid respectively. Therefore, during calculation the quantity of each is included within the quantity of its respective acid. However, when physiological fluids are examined for amino acid content, hydrolytic conditions are not necessarily used. In this case separate analysis for each acid and amide pair is practical.

At the present time the most common method for analysis of amino acids is the use of ion exchange resins as developed by Moore and Stein(2) and modified by Spackman, Stein and Moore (4). By the use of improved resins and two columns, resolution of asparagine and glutamine from other naturally occurring amino acids is possible. However, when using the single column method of Pies and Morris(3), some difficulties arise. Asparagine and glutamine appear together, and appear at the approximate Rf as two other amino acids, threonine and serine. Those workers limited by equipment to using the method of Pies and Morris(3) had difficulties in measuring these amides.

The purpose of this study was to develop a method of single column amino acid analysis which would allow a mixture of amino acids which includes asparagine and glutamine to be quantitatively analyzed.

MATERIAL AND METHODS

A solution 0.25 micro-moles/ml in asparagine, glutamine, aspartic acid, glutamic acid, and alanine was prepared in 0.01 N HCL from analytical grade standards (Nutritional Biochemical Corporation). Two ml of this preparation was placed in each of five 20 ml glass vials. Into four of these vials was added 0.5 ml of pH 2.90 citrate buffer and 0.5 ml of redistilled 6 N HCl. Into the fifth was placed 0.5 ml of 2.90 citrate buffer and 0.5 ml distilled water. The vials were capped with polyethylene snap cap. The three vials containing the HCl were placed on a hot plate to reflux at 52ºC. for the following times: 10, 20, 30 hours.

Immediately upon completing its designated reflux time, each vial was cooled, 0.5 ml of pH 2.90 buffer and 0.2 ml 12.6 N NaOH were added to the solution. The pH of the solution was adjusted to approximately 2.0 with a mixture of equal volumes of 2 N NaOH and pH 2.90 citrate buffer. Final volume was

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approximately 4.0 ml. To the control vial (no acid treatment 2.0 ml of pH 2.2 citrate buffer was added to adjust volume to 4.0 ml.

Ion Exchange Chromatography

For quantitative analysis of the amino acids, the 6 hour procedure of Pies and Morris(3) and Technicon Auto Analyzer (Technicon Corporation) with a 130cm column were used. However, only the first 5 chambers of Autograde were used with each analysis. Two ml of buffered sample was applied manually each time to the column and compressed nitrogen used to force the solution into the resin. Column temperature was maintained at 60ºC. After the emergence of the alanine peak of each run, the chromatography was stopped, and the column regenerated for 15 minutes with 2.0 N NaOH followed by 3.10 citrate buffer for 1/2 hour.

Calculation of the amounts of each amide originally present was made by using alanine as an internal standard for calculation of corrected peak areas, using the following formula:

$$\frac{(An - Ao)\ S1}{So}$$

where

An=Area under acid peak after hydrolysis

Ao=Area under acid peak before hydrolysis

S1=Area under alanine peak after hydrolysis

So=Area under alanine peak before hydrolysis.

After peak area correction, the amount of each amino acid present was calculated using a correction factor, Qa, derived from a standard aminogram of 0.25 micro-moles of each amino acid.

RESULTS AND DISCUSSION

Preliminary experiments confirmed the difficulties encountered by Boulter (1) in separation of the four acids by using various buffer gradients. A separation of asparagine from glutamine in the absence of serine and threonine was achieved, but only when the two amides were present in less than 0.1 micro-molar quantities.

Therefore, for complete quantitative analysis, it was necessary to run two chromatograms, one complete run for calculation of concentrations of all acids except the four mentioned above, and a second partial run after mild acid hydrolysis. By subtraction of the original amounts of aspartic and glutamic acids from that present after hydrolysis, the original quantities of asparagine and glutamine, respectively, were determined. In addition, after hydrolysis, the analysis of serine and threonine was completed in the normal manner by using the correction factor computed for alanine.

Results of hydrolysis for different times is shown in Fig. 1. Reflux of the mixture in 1N HCl on a hot plate at 52ºC gave values which showed a linnear decrease of amide and increase of acid for thirty hours after which hydrolysis was completed. On the other hand, when a water bath at 52ºC was used, the mixture reached equilibrium at about 20 hours with significant amounts of amide remaining un-hydrolized. Further heating at this temperature did not promote additional hydrolysis. The difference between the two methods of hydrolysis is likely due to the fact that during refluxing a part of the solution is in contact momentarily with greater temperatures.

Examination of the percentage of recovery of aspartic acid and glutamic acid after hydrolysis of their respective amides by 1 N HCl for 30 hours shows that recovery of both was greater than 98%. This recovery is significantly greater than the 90% found by Thurman and Boulter(5) who used a shorter time but a more rigorous treatment (6N HCl, 100ºC). It is felt that the mild conditions of hydrolysis described in the method presented above will avoid the acid hydrolysis of any soluble peptides which may be present in a physiological fluid and will give a precise analysis of the free amino acids present.

SUMMARY

A method of single column amino acid analysis is described in order to

express quantitatively the content of asparagine and glutamine present in a mixture of amino acids. In addition, the conditions used in this method allow the analysis of any other amino acid present in physiological fluids.

REFERENCES

BOULTER, D., 1966 — An Introduction to Automatic Amino Acid Analysis with Plant Extracts. "In Techniques in Amino Acid Analysis". p. 99. Technicon International Division S.A. Geneva, Switzerland.

MOORE, S., AND STEIN, W.H., 1954 — Chromatography of Amino Acids on Sulfonated Polystyrene Resins. "J. Biol. Chem. 211-895.

PIES, K.A. AND MORRIS, L., 1960 — A Modified Procedure for the Automatic Analysis of Amino Acids, "Anal. Biochem". 1(3): 187.

SPACKMAN, D.H., STEIN, W.H., and MOORE, S., 1958 — Automatic Recording Apparatus for use in the Chramatography of Amino Acids. "Anal. Chem". 30. 1190.

THURMAN, D.A. and BOUèLTER, D., 1966 — The Determination of Proteins. *In* Techniques in Amino Acid Analysis", p. 101. Technicon International Division S. A., Geneva, Switzerland.