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A Method for the Separation of Peptides and α-Amino Acids

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In dealing with extracts from natural sources a method was needed for the separation of peptides from amino acids. Fazakerley & Best (1965) achieved separation of amino acids from proteins and peptides by filtration over Sephadex loaded with copper hydroxide. The Cu^{2+} chelates of proteins and peptides are eluted with borate buffer pH 11·0, the amino acids are retained. The latter are obtained together with the Cu^{2+} ions by elution with 0·2N-hydrochloric acid.

We developed a new method because for several peptides pH 11·0 is harmful, the column capacity is small and the fractions are contaminated with salts. The underlying principle is the difference in electrical charge between the cupric chelates of amino acids and peptides. At pH 8·0 the Cu^{2+} chelates (Cu^{2+}:amino acid = 1:2) of the basic and neutral amino acids are positively charged or neutral. The Cu^{2+} complexes of the peptides and of the acidic amino acids are (partly) negatively charged. A mixture of the Cu^{2+} chelates of peptides and amino acids can be separated by anion exchange chromatography, using the following procedure. A mixture up to 75mg. of amino acids and peptides, dissolved in 0·01M-collidine acetate buffer of pH 8·0, was converted into the corresponding Cu^{2+} complexes by shaking with an excess of cupric carbonate at 45° for 15min. or by filtration over a column consisting of cellulose:cupric carbonate (2:1) (the reaction time on the column must be 1hr. at room temp.). The mixture, concentrated to a volume of 2 to 3ml., is applied to a column (30cm. × 1·4cm.) of DEAE-cellulose in the acetate form, which has been equilibrated with 0·01M-collidine–acetate buffer, pH 8·0. The basic and neutral amino acids are eluted by this buffer. Subsequently, the peptides are obtained by stepwise elution with 0·01M-collidine–acetate buffer, pH 4·5, 1% acetic acid and 0·1N-hydrochloric acid. The acidic amino acids are present in the last fraction. Extinction of the collected fractions can be measured at 620mμ. Cu^{2+} is removed from the fractions by extraction with 8-hydroxyquinoline in chloroform at pH 4 to 9. Measurement of the extinction of the chloroform layer at 396mμ is a very sensitive procedure to estimate the Cu^{2+} concentration.

This method has been checked on several artificial and natural mixtures.

Fazakerley, S. & Best, D. R. (1965). *Anal. Biochem.* **12**, 290.