ELECTROPHORETIC SEPARATION OF SOME AMINO ACIDS FROM THEIR COPPER COMPLEXES*

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Chelate compounds of amino acids with copper ion have been studied for many years[1-4].

It has been stated that copper ion combines with monocarboxylic α-amino acids, giving compounds of the type $Cu(A)_2$, where A = amino acid, and with glutamic acid, aspartic acid, isoserine and polypeptides giving compounds of the type CuA[5,6].

The equilibrium constants of the reactions between heavy metal ions and the amino acids containing two ionizable groups[7] and those containing three ionizable groups[8] were also determined. MILLS[9] used paper chromatography for the separation of copper ions from organic complexes occurring in the aqueous extracts of green Australian herbage.

CRUMPLER AND DENT[10], using the same technique, found that β- and γ-amino acids were unable to react with copper.

BECK AND CSÁSZAR[11] have succeeded in separating some of the copper–amino acid complexes by paper chromatography; most of them however, had very close R_F values.

Separation of amino acids into three major groups (acidic, neutral and basic) employing low-voltage (gradient potential of 5 V/cm) paper electrophoresis was achieved as early as 1948 by WIELAND *et al.*[12].

DOBBIE *et al.*[13-15] have studied copper complexes with glycine, glycylglycine, glycyl-L-leucine, glycyl-L-tyrosine, carnosine and diglycylglycine using potentiometric titration, copper-electrode potential measurement, electrophoretic mobilities on Whatman No. 54 paper and spectrophotometric observations.

Our preliminary study on the resolution of some amino acids and their copper complexes by paper chromatography gave rather unsatisfactory results. The application of paper electrophoresis, however, proved to be more promising.

In this paper the results of the separation of a number of free amino acids from their copper complexes by the paper electrophoresis method are presented.

EXPERIMENTAL

Materials

The following reagents were used throughout this work: sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) c.p., cupric chloride ($CuCl_2 \cdot 2H_2O$) c.p., amino acids (L. Light & Co., Ltd.),

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ninhydrin (British Drug Houses Ltd.), rubeanic acid (Merck). The paper strips (Whatman No. 4) were 30 cm long and 4 cm wide.

Apparatus

The apparatus used was a conventional horizontal one made of plexiglass with tight cover-plate, operating in the potential range 0–400 V d.c. and at a current strength in the range 0–100 mA.

Methods

Amino acid–cupric complexes were prepared according to NEUBERG, LUSTIG AND MANDL[16] with the following amino acids: glycine, serine, threonine, asparagine, glutamine, aspartic acid, glutamic acid, lysine and ornithine. Amino nitrogen was determined according to POPE AND STEVENS[17]. Determinations of copper were performed colorimetrically with the use of diethyl dithiocarbamate[18]. The Cu/N_{NH_2} ratio indicated that the complexes obtained in this way have the formula mentioned above[5,6]. The following conditions were taken into account in the preliminary experiments: potential gradient, ionic strength, duration of electrophoretic process, and quality of the buffer employed. Three buffers were chosen:

(1) 0.025 *M* sodium tetraborate + 0.05 *M* NaOH, pH = 10, $\mu = 0.05$.
(2) 0.025 *M* sodium tetraborate + 0.05 *M* HCl, pH = 9.1, $\mu = 0.05$.
(3) 0.025 *M* sodium tetraborate + 0.1 *M* H_3BO_3, pH = 9.1, $\mu = 0.05$.

The most satisfactory results were obtained at the potential gradient 10 V/cm of paper strip length, 0.7 mA/cm of strip width and 1.5 h electrophoresis. Whatman

TABLE I

ELECTROPHORETIC MIGRATION RATES (R) UNCORRECTED AND CORRECTED FOR ELECTROENDOSMOTIC EFFECT

Amino acid or copper complex	*R in mm (uncorrected)*	*R in mm (corrected for electroendosmotic effect)*
Glycine	+ 15.3	+ 38.3
Cu–glycine	— 11.4	+ 11.6
Serine	+ 24.2	+ 47.2
Cu–serine	— 7.5	+ 15.5
Threonine	+ 25.5	+ 48.5
Cu–threonine	— 10.0	+ 13.0
Asparagine	+ 29.1	+ 52.1
Cu–asparagine	— 8.7	+ 14.3
Glutamine	+ 20.5	+ 43.5
Cu–glutamine	— 10.0	+ 13.0
Aspartic acid	+ 55.4	+ 78.4
Cu–aspartic acid	+ 63.9	+ 86.9
Glutamic acid	+ 50.7	+ 73.7
Cu–glutamic acid	+ 57.7	+ 80.0
Lysine	— 36.9	— 13.9
Cu–lysine	— 50.7	— 27.7
Ornithine	— 25.5	— 2.5
Cu–ornithine	— 37.7	— 14.7
Creatinine	— 23.0	

No. 4 strips 30 × 4 cm were placed on the supporting part of the electrophoresis chamber and the solutions to be separated (10–15 μg) were applied to the centre line across the paper strip. After the electrophoretic process was finished the paper strips were taken out, dried horizontally in an oven at 105° on a glass supporting frame for 10 minutes. The dried paper strips were cut along the long axis; one part

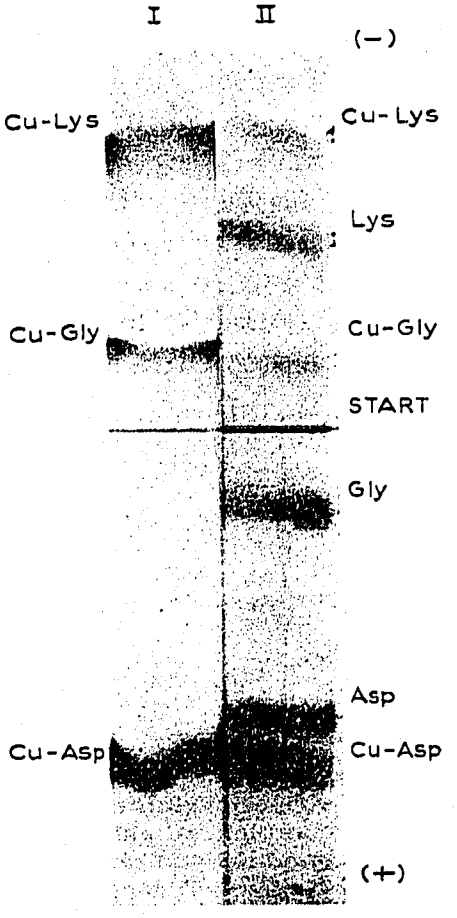

Fig. 1. Separation of glycine, lysine and aspartic acid from their copper complexes. Experimental conditions: borate–NaOH buffer, pH 10, μ 0.05, duration of electrophoresis 1.5 h, potential gradient 10 V/cm. I, paper strip developed with rubeanic acid; II, paper strip developed with ninhydrin.

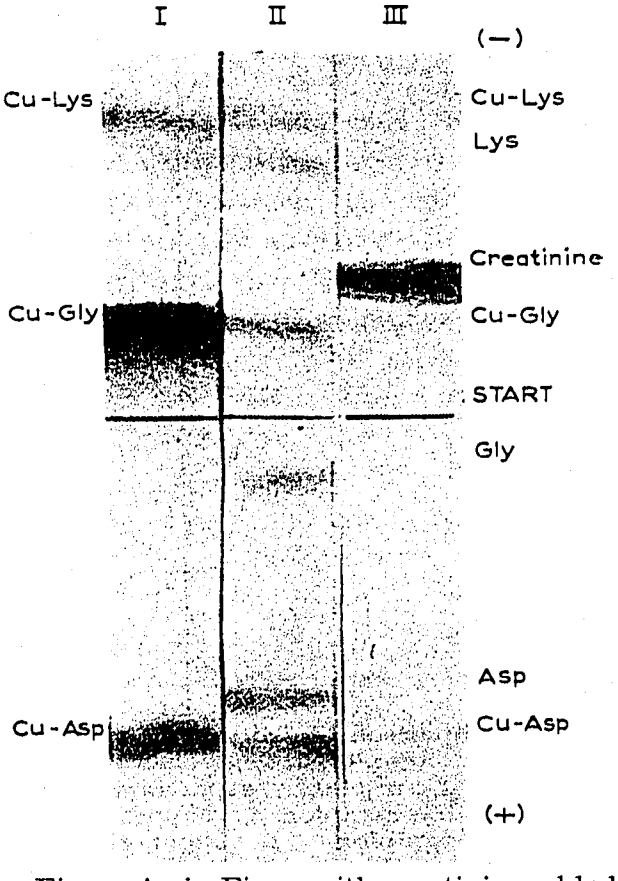

Fig. 2. As in Fig. 1 with creatinine added (serving as a control for electroendosmotic effect). Experimental conditions as in Fig. 1. I and II as in Fig. 1; III, paper strip developed with alkaline picrate for creatinine.

was sprayed with 10–15 % solution of acetic acid in acetone, dried at room temperature for approx. 15 minutes and developed with 0.1 % ninhydrin (300 ml of methyl alcohol + 185 ml of *n*-butanol + 15 ml of 2 *N* acetic acid) at 100–105°; the other part was sprayed with 0.1 % solution of rubeanic acid in methanol (the positive reaction for copper appears almost immediately).

The experiments concerning the applicability of the alkaline borate buffers were performed using three amino acids (glycine, lysine, aspartic acid) and their copper complexes. The most useful buffer appeared to be the borate–NaOH buffer (pH 10, μ 0.05), as indicated by the calculated electrophoretic migration rates of the substances tested. The electrophoretic mobilities were calculated in millimetres and recorded as

(+) or (—) with respect to localization of a detected compound at the anode or cathode zone respectively of the paper strip (Table I).

The results of the separation of some of the compounds studied are shown in Figs. 1, 2 and 3.

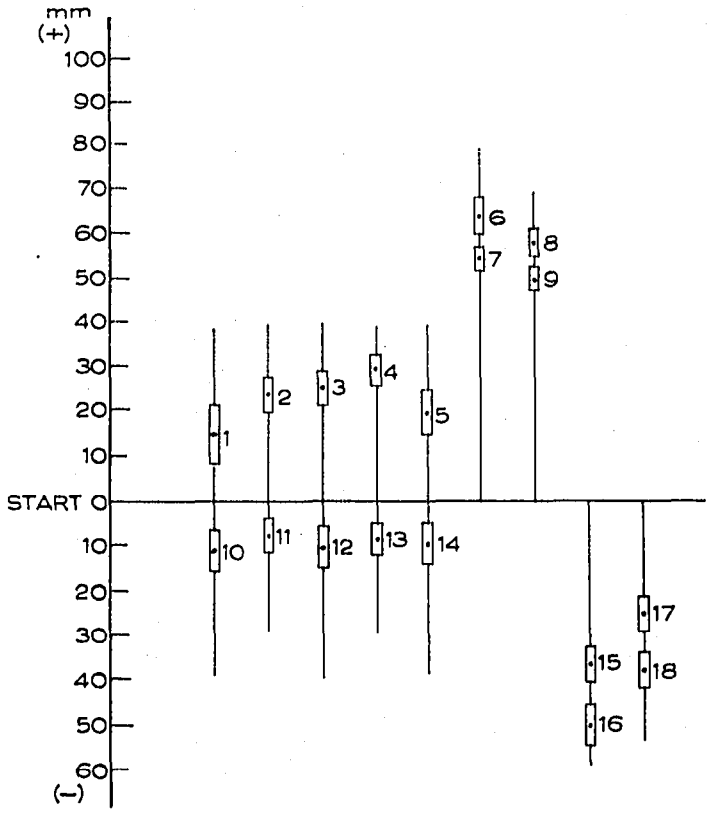

Fig. 3. Comparison of the migration values and the band widths of developed amino acids and their copper complexes. 1. glycine. 2. serine. 3. threonine. 4. asparagine. 5. glutamine. 6. copper–aspartic acid complex. 7. aspartic acid. 8. copper–glutamic acid complex. 9. glutamic acid. 10. copper–glycine complex. 11. copper–serine complex. 12. copper–threonine complex. 13. copper–asparagine complex. 14. copper–glutamine complex. 15. lysine. 16. copper–lysine complex. 17. ornithine. 18. copper–ornithine complex. Experimental conditions as in Fig. 1.

DISCUSSION

There is little information in the literature concerning the separation of free amino acids from their copper complexes. The resolution of amino acids and of amino acid–copper complexes on separate paper electropherograms was reported by WIELAND *et al.*[12].

DOBBIE *et al.*[13-15] reported electrophoretic mobilities of copper complexes with glycine and some dipeptides, without attempting to separate a larger number of amino acid–copper complexes.

In this report the results of the application of low-voltage paper electrophoresis for the purpose of separating some free amino acids from their copper complexes are described.

BORSOOK[19] has demonstrated that the complexes of the type $Cu(A)_n$ can be formed not only in the alkaline but also in the acid range of pH. We are able to state that at pH 5–6 in acetate buffer all the complexes tested are liable to considerable

decomposition, while in phosphate buffer the stability of basic amino acid complexes is lost almost completely.

Below pH 7, there was no resolution between free amino acids and their copper complexes since both had the same migration rate (R) values. The optimal separation is reached in the pH range of 9–11.

In WIELAND's work cited above[12] only 0.1 M acetate buffer at pH range of 3.7–7.5 was employed.

At higher pH values the detection of amino acids by the ninhydrin test becomes increasingly difficult.

Of the borate buffers at pH 9–11 the borate–HCl and borate–H_3BO_3 buffers gave better results as far as the resolution of basic amino acids and their copper complexes were concerned; in the borate–NaOH buffer, however, the separation of neutral amino acids from their complexes was more clearly demonstrable.

Changes of potential gradient between 3.3 V/cm and 10.2 V/cm are of minor importance in the process of zone separation, providing the necessary time adjust-

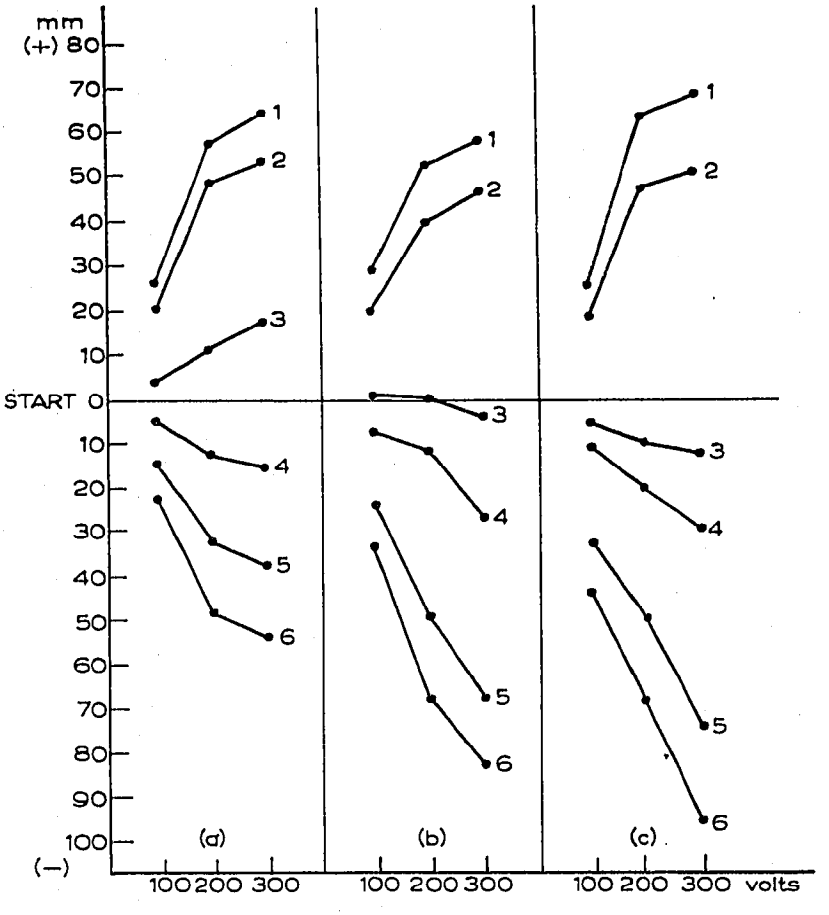

Fig. 4. Dependence of migration values of amino acids and their copper complexes on the potential applied. 1. copper–aspartic acid complex. 2. aspartic acid. 3. glycine. 4. copper–glycine complex. 5. lysine. 6. copper–lysine complex. Experimental conditions: (a) borate–NaOH buffer, pH 10; (b) borate–HCl buffer, pH 9.1; (c) borate–H_3BO_3 buffer, pH 9.1. μ 0.05, duration of electrophoresis 1.5 h, Whatman No. 4 paper strips 30 × 4 cm. Ordinates: migration rates in millimetres. Abscissae: potential applied in volts (100, 200 and 300 V correspond to gradient potentials: 3.3 V/cm, 6.6 V/cm and 10 V/cm).

ments are made. Fig. 4 would suggest that the potential gradients ranging between 6.6 and 10.2 V/cm are the most appropriate.

When one places a solution of amino acid on the starting line of a paper strip, followed by a solution of copper salt, apparently the same complexes are formed as those prepared by the classical methods.

On applying to the paper a mixture of an amino acid–copper complex with another amino acid, a partial displacement of the complexed amino acid occurs and a new complex is formed. The competitive interaction is observed throughout the whole range of pH studied, *viz.* 5–11.

Similar observations have been made by WIELAND *et al.*[12] in the case of (histidine)$_2$–Cu complex capable of exchanging one histidine molecule with another amino acid molecule and forming in this way a mixed amino acid–Cu complex.

Under our conditions it was possible to separate amino acid–copper complexes into three main groups: acidic, neutral and alkaline, and in each group to separate a given complex from its amino acid.

If necessary, the composition of an amino acid–copper complex can be determined following copper precipitation with H_2S and subsequent paper chromatography of the liberated amino acid.

SUMMARY

Mixtures of free amino acids and their copper complexes were fractionated by paper electrophoresis in borate buffer (pH 10, μ 0.05), potential gradient 6.6–10.2 V/cm on Whatman No. 4 paper strips into three major zones: acidic, neutral and alkaline. Within these zones free amino acids were separated from their copper complexes. Below pH 5–6 the complexes decomposed to a considerable extent.

The amino acid composition of the common zone of complexes may be determined by the removal of copper with H_2S followed by paper chromatography of liberated amino acids.

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