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LIGAND-EXCHANGE CHROMATOGRAPHY OF AMINO ACIDS ON COPPER–, COBALT– AND ZINC–CHELEX 100

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SUMMARY

Procedures for the ligand-exchange chromatography of amino acids on copper–, cobalt– and zinc–Chelex 100 have been examined. Ligand exchange on the copper complex affords a simple and rapid method for the removal of amino acids (except for aspartic and glutamic acids) from dilute solutions. The influence of the pH on the binding of amino acids to the metal complex was also studied. The bound amino acids could be eluted with ammonium hydroxide which also causes a slight metal leakage. Chromatography on cobalt– and zinc–Chelex 100 showed that only the basic amino acids were quantitatively attached to these complexes at pH 8.3–9.5, whereas the others were predominantly excluded. This procedure can be used for the selective concentration and removal of basic amino acids in the presence of other amino acids.

INTRODUCTION

Ligand-exchange chromatography[1] has been shown to have great potentialities for the separation and removal of such ligands as amines, amino acids and others from liquid media by the formation of complexes with a transition metal attached to a solid support. This principle has been utilized to separate amino acids from peptides[2–4] and similarly to concentrate and remove amino acids from salt solutions such as sea water[5]. Mixtures of amines, hydrazines and purine and pyrimidine bases[6], as well as nucleic components[7], have also been separated by this technique. A solid support that has frequently been used is Chelex 100, which is a chelating ion-exchange resin containing iminodiacetate groups, and copper has mostly been the metal of choice. In addition, the ligand-exchange resolution of racemic amino acids can be accomplished when asymmetric resins are used[8,9].

Although chromatography on copper–Chelex 100 has been used by several groups of investigators[2–5], the results obtained by different groups are not in complete agreement. Therefore, in an attempt to clarify divergent reports in the literature, we deemed it of interest to re-examine the procedure and to introduce certain modifications.

Recently, we reported on a selective ligand-exchange chromatographic pro-

cedure in which nickel–Chelex 100 was used for the separation of the basic amino acids[10]. Here, we describe our experience with copper–Chelex 100. In order to study the behavior of each amino acid during ligand-exchange chromatography, we tested the method by using pure amino acid samples under different experimental conditions (*e.g.*, choice of buffer and pH). This paper also describes the extension of the method to include chromatography on cobalt– and zinc–Chelex 100.

MATERIALS AND METHODS

Sodium–Chelex 100 (100–200 mesh; Bio-Rad Labs., Richmond, Calif., U.S.A.) was treated as described previously[10] and was allowed to react with saturated solutions of $CuSO_4 \cdot 5\,H_2O$, $CoCl_2$ and $ZnCl_2$; these and the other reagents were of analytical grade (Merck, Darmstadt, G.F.R.). For the preparation of copper–Chelex 100, the hydrogen form of the resin was also used.

Chromatography on copper–Chelex 100

Suspensions of the copper-resin were used in water; in ammoniacal solution of pH 8–10.2; in 0.1 *M* carbonate buffer of pH 9.2–10.8 (ref. 11); and in 0.1 *M* sodium borate–sodium hydroxide buffer of pH 11 (ref. 12).

The treatment of the copper–Chelex 100 with ammonia solution was essentially as outlined earlier[10], but with two exceptions. First, since the use of 3 *M* ammonium hydroxide did not seem to be necessary, in the later experiments it was replaced with 0.02 *M* solution. Secondly, owing to the very slow decrease in pH of the washing effluent and in order to obtain wash-water with pH values lower than 9.5, the copper complex was washed with dilute acetic acid (0.5 m*M*). For the preparation of copper-resin in carbonate or borate buffer, after the complex had been washed free from copper ions (tested with Merckoquant Cu^{2+} test paper (Merck) and with 3.5% methanolic diethyldithiocarbamate), it was suspended in the desired buffer and the final pH was adjusted by adding 1 *M* sodium hydroxide.

Glass columns (20 × 1 cm) were packed with 10 ml of the copper-resin. A solution of a mixture of the common amino acids (1.0 μmole of each, except for Asn, Gln and Trp*) in 200–1000 ml of water (or carbonate or borate buffer solution, depending on which suspension of the copper-resin was being used), and the solution was allowed to pass through each column at a flow-rate of 2–3 ml per min. Each column was then washed with 10 ml of twice-distilled water, and the amino acids were eluted and determined[13] as described previously[10].

Chromatography on cobalt– and zinc–Chelex 100

These experiments were performed in a manner similar to that used for ligand-exchange chromatography on the nickel–Chelex 100 at low pH. After the cobalt and zinc complexes had been washed free from the corresponding metal ions (detected, respectively, with Merckoquant Co^{2+} and Zn^{2+} test papers), each metal resin was stirred in 3 *M* ammonium hydroxide and filtered off; the complexes were then washed

* The symbols used for amino acids are those recommended by the IUPAC–IUB Commision on Biochemical Nomenclature, *J. Biol. Chem.*, 247 (1971) 977. All the asymmetric amino acids used in these experiments are of the L configuration.

with twice-distilled water until the washings showed a pH of 9.1–9.5. The bound amino acids were eluted with 3 *M* or 6 *M* ammonium hydroxide, and working up of the samples and analyses of amino acids[13] were carried out as before[10].

RESULTS AND DISCUSSION

The initial purpose of performing ligand-exchange chromatography on copper–Chelex 100 at pH 8–9 was to concentrate amino acids and separate them from inorganic ions and other constituents of wastewater (as has been accomplished with sea water[5]). Since there were discrepancies among results reported by different investigators who used this method[2–5], the practicality and reliability of the procedure for the quantitative removal of all the amino acids from solutions had to be established by using samples of pure amino acids.

The experiment was first performed on the ammonia-equilibrated copper–Chelex, which had been washed with water until the effluent showed a pH of 9.5. After the elution of the amino acids from the complex, and their determination, however, it became evident that aspartic acid and glutamic acid were present only in minute amounts. This result is at variance with those of Siegel and Degens[5]. Theoretically, there are two explanations for this anomaly. First, the two amino acids are not bound to the copper–Chelex under the experimental conditions, and secondly, these amino acids are so strongly attached to the complex that they are not eluted with the ammonium hydroxide solution. This was clarified by performing amino acid analyses both on the original solutions after passage through the columns (after lyophilization of the samples) and on the ammonia-eluted fractions. The results showed that the two dicarboxylic amino acids passed through the columns without significant sorption. When the loading of the amino acids on the columns was decreased, there was no demonstrable change in the results, indicating that the low sorption of these amino acids was not due to the high loading of the columns and

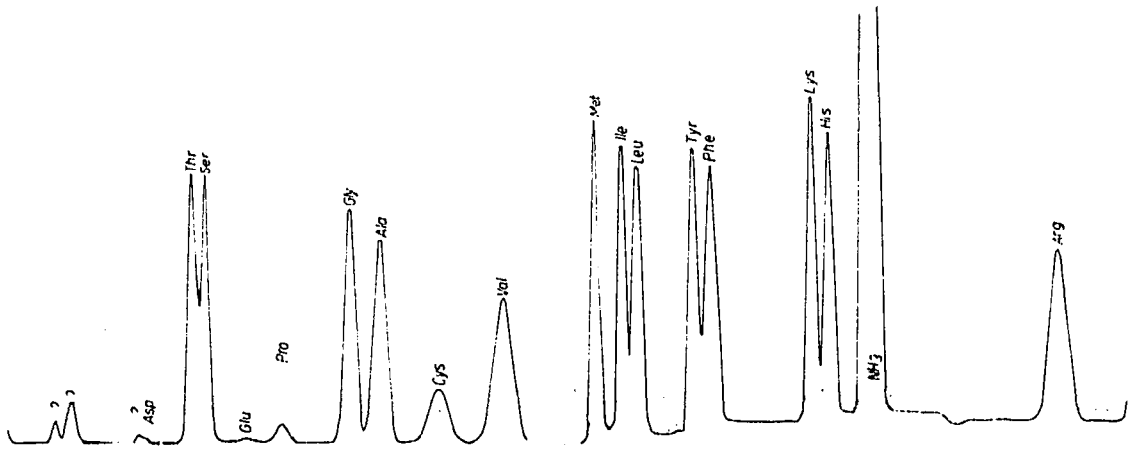

Fig. 1. Ion-exchange chromatogram of amino acids bound to ammonia-equilibrated copper–Chelex 100 at pH 10.1 and eluted with 6 *M* ammonium hydroxide. The unknown peaks at the beginning of the run also appear on the chromatogram of the blank test. ———, Absorbance at 570 nm, ·····, absorbance at 440 nm.

TABLE I

RATES OF RECOVERY OF AMINO ACIDS AFTER CHROMATOGRAPHY ON AMMONIA-EQUILIBRATED COPPER–CHELEX 100 AT pH 10.1

Amino acid	*Recovery (%)*	
	3 M ammonium hydroxide	*6 M ammonium hydroxide*
Aspartic acid	Small	Small
Threonine	91	88
Serine	80	80
Glutamic acid	Small	Small
Proline	99	101
Glycine	104	102
Alanine	90	93
Cysteine	95	83
Valine	98	101
Methionine	102	104
Isoleucine	105	104
Leucine	98	104
Tyrosine	103	102
Phenylalanine	107	106
Lysine	104	104
Histidine	106	104
Arginine	90	104

the lack of availibility of attachment sites. The experiments were repeated using ammonia-treated copper–Chelex 100 at pH 8–10.5, but again recovery of the two dicarboxylic amino acids remained insignificant. An amino acid chromatogram of a typical test performed at pH 10.1 is shown in Fig. 1. The rates of recovery of amino acids from two identical experiments (pH 10.1) after elution with 3 and 6 *M* ammonium hydroxide are shown in Table I. Slightly low recovery values for serine and threonine are also apparent, although not very significant. Since these two amino acids are acid-labile, their lower values might be due to preliminary treatment of the samples with hydrochloric acid before the amino acid analyses.

When ligand-exchange chromatography was accomplished on copper–Chelex in water (without the ammonia treatment), only lysine, histidine, arginine and cysteine were bound to the complex, the aromatic amino acids tyrosine and phenylalanine were also partially retained, but the rest of the amino acids were predominantly excluded and were found in the original solution. This confirmed the influence of pH on ligand-exchange chromatography[10].

Chromatography on copper–Chelex 100 in carbonate and borate buffers

Since aqueous ammonia is not a complete buffer system, and because sorption of amino acids on the complex appeared to depend on the pH of the medium, it seemed necessary to repeat the experiment with use of the copper complex in solutions with complete buffering capacity. A series of experiments was therefore performed using carbonate buffers at pH 9.2, 9.6, 10.0, 10.2 and 10.8. The results showed that, as the pH of the copper complex was increased, there was a corresponding increase in sorption of aspartic and glutamic acids; however, there was never quantitative

TABLE II

RATES OF BINDING OF ASPARTIC AND GLUTAMIC ACIDS TO COPPER–CHELEX 100 AT DIFFERENT pH VALUES

Buffer	*pH*	*Recovery (%)*	
		Aspartic acid	*Glutamic acid*
Carbonate	9.2	23	17
Carbonate	9.6	27	18
Carbonate	10.0	35	25
Carbonate	10.2	36	27
Carbonate	10.8	52	34
Borate	11.0	80	54

binding. A higher degree of attachment was observed when borate buffer at pH 11 was used (with a loading of 0.2 μmole of each amino acid). The rates of sorption and recovery of the two acidic amino acids at different pH values are shown in Table II.

In all the experiments, the elution with ammonia caused slight leakage of copper, this leakage being higher when inorganic buffers were used. The fractions were freed from traces of metal by passing them through columns of sodium–Chelex 100. Although 50 ml of 3 *M* ammonium hydroxide appeared to be sufficient to elute all the bound amino acids when the chromatography was performed on the ammonia-treated copper complex (see Table I), removal of histidine (and, to a lesser extent, that of arginine) was incomplete when the inorganic buffers at higher pH values were used. Complete elution of these amino acids was achieved with 6 *M* ammonium hydroxide as eluent.

Chromatography on cobalt– and zinc–Chelex 100

Ligand-exchange chromatography on these complexes at pH 8.3–9.5 showed that cobalt–Chelex 100 and zinc–Chelex 100 have properties similar to those of nickel–Chelex at low-alkaline pH (ref. 10). In both cases, only the basic amino acids

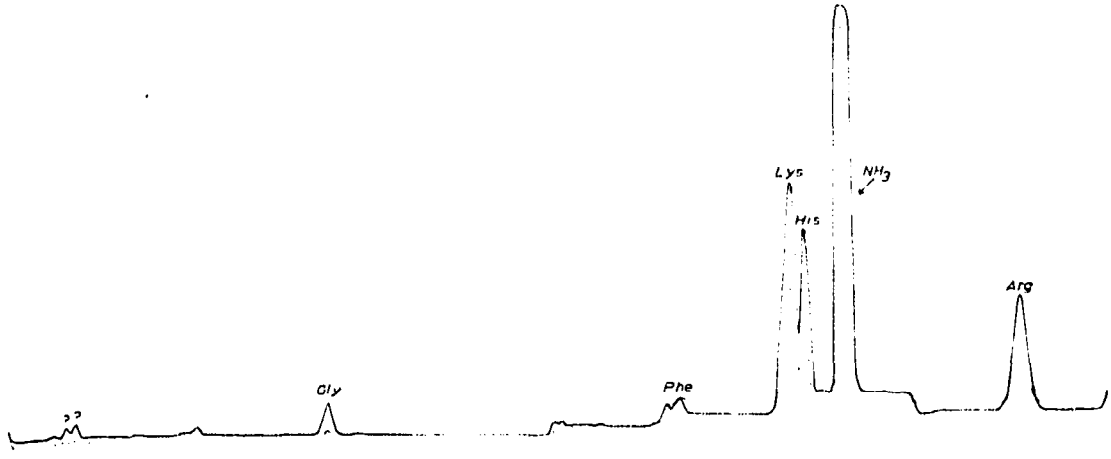

Fig. 2. Ion-exchange chromatogram of amino acids removed from a mixture of the common amino acids by binding to cobalt–Chelex 100 at pH 8.3. ———, Absorbance at 570 nm; · · · · · ·, absorbance at 440 nm.

lysine, histidine and arginine were quantitatively attached to the metal resins. The amino acid chromatogram of a test performed on cobalt–Chelex 100 at pH 8.3 is shown in Fig. 2. Although 3 *M* ammonium hydroxide (50 ml) was sufficient for the elution of these amino acids from the zinc–Chelex, the elution of histidine and arginine from cobalt–Chelex 100 was not quantitative. Complete displacement of the amino acids from the cobalt–Chelex was accomplished with 6 *M* ammonium hydroxide, which also caused slight leakage of the metal; no such leakage was observed during elution with 3 *M* ammonium hydroxide. This may be taken as an indication that there is greater affinity between cobalt and the basic amino acids than between nickel and these amino acids.

In conclusion, it can be stated that, although ligand-exchange on copper–Chelex 100 serves as an excellent chromatographic method for the removal of most of the common amino acids from dilute solutions, its short-comings are due to its very low affinity for aspartic and glutamic acids. The situation could to a certain extent be improved by the use of buffers at higher pH (see Table II), but sorption of these amino acids was not quantitative. Chromatography on cobalt– or zinc–Chelex 100 at low-alkaline pH is a suitable selective method for the concentration and removal of the basic amino acids in the presence of the other amino acids.

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