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Short Communication

Role of metal ions in the ligand-exchange separation of amino acids

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ABSTRACT

Amberlite IR-120 resin was impregnated with transition metal ions such as Mn(II), Fe(II), Fe(III), Cu(II), Zn(II) and Mo(VI) and used for the ligand-exchange separation of amino acids available in the hydrolysates of toria and mustard oilcakes. Among the various metal ions studied, Mn(II) and Fe(II) cannot be employed for separation. The elution volume and selectivity of amino acids with the metal forms of the resin decrease in the order Cu(II) $\approx$ Fe(III) $>$ Mo(VI) $>$ Zn(II).

INTRODUCTION

Ligand-exchange chromatography has been employed for the separation and purification of amino acids [1,2]. The establishment of ligand-exchange equilibrium between the resin and the external solution depends on numerous factors, *e.g.*, the nature of the support, the composition of the eluent, the complex-forming ability of amino acids towards the cation present and the structure of the molecule, *i.e.*, the nature, number, position and properties of the functional groups they contain.

The influence of the eluent composition and the nature of the ion exchanger has been discussed by Doury-Berthod *et al.* [3]. In this work, the influence of the nature of the metal cation on the ligand-exchange separation of amino acids was studied. Amberlite IR-120 impregnated with transition metal ions such as Mn(II), Fe(II), Fe(III), Cu(II), Zn(II) and Mo(VI) was used for the ligand-exchange separation of amino acids in the hydrolysates of toria and mustard oilcakes.

EXPERIMENTAL

The mustard and toria oilcakes, after removal of residual oil and toxic materials, were hydrolysed with 6 *M* hydrochloric acid at 110°C to yield a mixture of amino acids and peptides. Amberlite IR-120 resin was converted into the metal form resin by stirring with 0.1 *M* solution of the chlorides or sulphates of Mn^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+} and Zn^{2+} for 24 h; with Mo^{6+}, molybdic acid solution in dilute nitric acid was used.

The operation of the column was the same as reported earlier [4]. Glass columns (90 cm $\times$ 0.9 cm I.D.) were packed with the metal forms of the resin to a depth of 60 cm. The resin was then equilibrated with 0.1 *M* ammonia solution until the effluent pH was *ca.* 10. About 8–10 h are required for equilibration of the column. The flow-rate was set to 5–6 drops per min or 5 ml in 10 min.

A known amount of the sample after adjusting its pH to 9.5–10 with concentrated ammonia solution was loaded on to the top of column. The sample was

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allowed to flow into the resin and elution was started immediately with 0.1 *M* ammonia solution, followed by elution with 1, 2 and 3 *M* ammonia solution. Generally, 25 ml of each of the first three solutions were used but the elution was continued with 3 *M* ammonia solution up to complete elution of the amino acids. However, when using 2 *M* ammonia solution with the copper column for the hydrolysate of toria oilcake and 1 *M* ammonia solution with the zinc column for the hydrolysate of mustard oilcake, the total volume of eluent applied had to be increased to 26 ml as the elution of the respective amino acids was still continuing. The eluates were collected in the order of five fractions each of 2 ml, ten fractions each of 1 ml and ten fractions each of 0.5 ml.

Thin-layer chromatography (TLC) of all of the eluate fractions was carried out on silica gel G-coated plates by using modified ninhydrin reagent [5] for colour development and the total volumes required for the respective amino acids were determined. The elutions were repeated with the required volumes for the respective amino acids to arrive at the final results given in Tables I and II.

RESULTS AND DISCUSSION

Amberlite IR-120 resin, containing sulphonic acid groups as its active sites, binds transition metal ions by the formation of ionic bonds. Ligand-exchange equilibrium for amino acid separations on a ligand-exchange column containing a metal form of the resin has been reported earlier [4]. The crude proteinaceous matter obtained from toria and mustard oilcakes on acid hydrolysis with 6 *M* hydrochloric acid yielded a mixture of amino acids and peptides. The amino acids present in the hydrolysates were detected by TLC. The hydrolysed samples were applied to columns containing Mn(II), Fe(II), Fe(III), Cu(II), Zn(II) and Mo(VI) forms of the resin. Generally the peptides were eluted as metal chelates in the first 20 ml. The amino acids were subsequently eluted with 0.1, 1, 2 and 3 *M* ammonia solutions.

When Mn(II) and Fe(II) forms of the resin were used in the column after application of an amino acid mixture, the resin turned black on addition of ammonia solution. The eluted solution gave a precipitate of metal hydroxide, indicating degrada-

TABLE I

LIGAND-EXCHANGE CHROMATOGRAPHY OF HYDROLYSATE OF TORIA OILCAKE

Free amino acids from ligand-exchange columns containing resin in the Cu(II), Zn(II), Fe(III) and Mo(VI) forms.

Ammonia solution eluent concentration (*M*)	Cu(II) column		Zn(II) column		Fe(III) column		Mo(VI) column	
	Elution volume (ml)	Amino acid eluted	Elution volume (ml)	Amino acid eluted	Elution volume (ml)	Amino acid eluted	Elution volume (ml)	Amino acid eluted
0.1	25	Glutamic acid + aspartic acid	14	Glutamic acid + asparic acid	18	Glutamic acid + aspartic acid	17	Glutamic acid + aspartic acid
1	20	Methionine	10	Methionine	15	Methionine	14	Methionine
	5	Cystine	8	Phenylalanine	10	Phenylalanine	10	Phenylalanine
			7	Histidine				
2	15	Cystine	10	Cystine	10	Histidine	10	Lysine
	11	Phenylalanine	15	Lysine	15	Cystine	10	Cystine
							5	Histidine
3	15	Lysine	20	Arginine	18	Lysine	5	Histidine
	15	Histidine			20	Arginine	18	Arginine
	16	Arginine						

tion of the metal form of the resin. The elution volume and the corresponding amino acids eluted with different ligand-exchange columns containing Fe(III), Cu(II), Zn(II) and Mo(VI) are reported in Tables I and II.

The elution volumes are different for the same amino acids for the hydrolysates of toria and mustard oilcakes. The metal ion loading of the resin was the same in both instances. This difference in the elution volumes is due to the different amounts of amino acids present in two types of oilcake.

The order of elution of amino acids for both toria and mustard oilcakes for the Cu(II) form of the resin is glutamic acid ≈ aspartic acid > methionine > cystine > phynylalanine > lysine > histidine > arginine. For the Mo(VI) form of the resin, the sequence remains the same except that cystine is eluted after lysine. The Zn(II) and Fe(III) forms of the resin show the trend glutamic acid ≈ aspartic acid > methionine > phenylalanine > histidine > lysine > arginine. The general trend of the elution sequence is that a group of acidic amino acids (glutamic acid and aspartic acid) are eluted first, followed by a group of neutral amino acids (methionine, phenylalanine) and a group of basic amino acids (lysine, histidine and arginine). In all instances except with the Cu(II) column cystine is eluted in the group of basic amino acids. This order is in agreement with that reported previously [3,6].

The results showed that the general elution sequence of amino acids does not change appreciably. However, the basic amino acids show more differentitated behaviour. The elution volumes and thereby the selectivity of ligand-exchange chromatography are greatly affected. The elution volumes are notably smaller with the Zn(II) form of the resin. The decreases in the retention volume and in the selectivity that are observed on passing from the Cu(II) to the Zn(II) form of the resin have been reported [7,8] for the separation of aliphatic amines. The chromatographic behaviour for the separation of three amino acids, glycine, alanine and leucine, has been discussed [3]. The elution volume when using the Mo(VI) form of the resin was lower than those with the Cu(II) and Fe(III) forms, but greater than that with the Zn(II) form. With the Fe(III) form of the resin, the elution volume for some of the amino acids is greater and for others it is less than that with the Cu(II) form of the resin. Generally, the acidic amino acids are eluted in a smaller volume

TABLE II

LIGAND-EXCHANGE CHROMATOGRAPHY OF HYDROLYSATE OF MUSTARD OILCAKE

Free amino acids from ligand-exchange columns containing resin in the Cu(II), Zn(II), Fe(III) and Mo(VI) forms.

Ammonia solution eluent concentration (*M*)	Cu(II) column		Zn(II) column		Fe(III) column		Mo(VI) column	
	Elution volume (ml)	Amino acid eluted	Elution volume (ml)	Amino acid eluted	Elution volume (ml)	Amino acid eluted	Elution volume (ml)	Amino acid eluted
0.1	25	Glutamic acid + aspartic acid	13	Glutamic acid + asparic acid	22	Glutamic acid + aspartic acid	18	Glutamic acid + aspartic acid
1	15	Methionine	11	Methionine	13	Methionine	12	Methionine
	10	Cystine	7	Phenylalanine	12	Phenylalanine	10	Phenylalanine
			8	Histidine				
2	10	Cystine	10	Lysine	12	Histidine	10	Lysine
	10	Phenylalanine	10	Cystine	13	Lysine	10	Histidine
	5	Lysine	5	Arginine				
3	10	Lysine	15	Arginine	20	Cystine	12	Cystine
	10	Histidine			24	Arginine	15	Arginine
	20	Arginine						

with the Fe(III) form of the resin. The elution volumes and the selectivity of amino acids with the metal forms of the resin decrease in the order Cu(II) $\approx$ Fe(III) $>$ Mo(VI) $>$ Zn(II).

The differences in the chromatographic behaviour of the different metallic forms of the resin are probably due to the differences in the stabilities of the mixed complexes formed by amino acids with the metal cations. The order is in accordance with the values of the stability constants of the metal complexes reported for various amino acids.

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