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ION-EXCHANGE THIN-LAYER CHROMATOGRAPHIC SEPARATION OF AMINO ACIDS ON ALGINIC ACID

D. COZZI, P. G. DESIDERI, L. LEPRI AND V. COAS
Institute of Analytical Chemistry, University of Florence (Italy)
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SUMMARY

Alginic acid was tested as an adsorbent in the ion-exchange chromatography of 31 amino acids on thin layers, the eluents being hydrochloric acid, acetic acid, water, potassium nitrate solutions and aqueous organic solvents. The behaviour of the amino acids on alginic acid was compared with their behaviour on carboxymethylcellulose. An ion-exchange mechanism operates in the case of basic amino acids. As regards the neutral amino acids, their affinity for alginic acid seems to be influenced by steric hindrance.

INTRODUCTION

Ion-exchange chromatography is simpler to perform and gives more reproducible results than partition chromatography, and it is particularly suitable for the analytical separation of organic ions with similar acidic or basic properties. Those ion-exchangers which have a high selectivity and therefore respond to small differences in these properties are of particular interest. Alginic acid is known[1–3] to show such a selectivity in the case of numerous inorganic ions, and has now been tested in the field of organic ions. The present work was limited to amino acids, which had been studied before on ion-exchange papers[4], and are suitable for testing the selectivity of ion-exchangers.

EXPERIMENTAL

Unlike pure alginic acid, plates coated with a 4:1 (w/w) mixture of alginic acid and cellulose retain their ion-exchange capacity practically unchanged; they also ensure a better reproducibility than pure alginic acid plates. In order to coat four 20 × 20 cm plates, 6 g of alginic acid and 1.5 g of cellulose were suspended in 40 ml of water, and the suspension was applied (thickness 300 μ) with the aid of an automatic Chemetron applicator. Other plates were prepared with a suspension of 5 g of carboxymethylcellulose (Na^+ form) in 50 ml of water, and with a suspension of 4.5 g carboxymethylcellulose (H^+ form) in 40 ml of water. The plates were dried overnight at 18–22° prior to use.

Each amino acid was dissolved separately to form a 1% solution in 10% iso-

propanol. Some amino acids, such as tyrosine and cystine, required 0.1 *N* HCl as solvent. The amount of each sample (*cf.* Table I) was decided on the basis of the size of its spot and sensitivity of the visualizing agent, found in preliminary experiments. The samples were applied to the plates at the start line, 1.5 cm from the lower edge. The plates were then developed to a height of 11 cm, in about 30–35 min, by the ascending technique with hydrochloric acid, acetic acid, water and potassium nitrate solutions. With aqueous isopropanol the developing time was about 50–60 min. The visualizing reagent described by MOFFAT AND LYTLE[5] proved particularly suitable.

TABLE I

R_F VALUES OF AMINO ACIDS ON ALGINIC ACID THIN LAYERS

Amino acids	*HCl*		CH_3COOH	*Amount*
	0.01 mole/l	*0.05 mole/l*	*(1 mole/l)*	*(μg)*
Arg	0.02	0.10	0.00	2.0
Lys	0.02	0.14	0.02	2.0
Orn	0.03	0.14	0.02	2.0
His	0.03	0.12	0.02	2.7
$(Cys)_2$	0.06	0.16	0.07	1.7
Gly (NH_3)	0.12	0.39	0.09	1.5
Try	0.14	0.28	0.13	2.5
γ-AnB	0.14	0.50	0.10	3.0
β-AiB	0.16	0.54	0.11	3.5
β-Ala	0.16	0.50	0.10	1.5
Gly (OCH_2CH_3)	0.18	0.50	0.13	2.0
Cit	0.22	0.43	0.19	2.0
Dopa	0.22	0.46	0.22	1.3
Tyr	0.23	0.54	0.23	0.8
Gly	0.23	0.49	0.19	1.5
Glu	0.28	0.58	0.27	0.4
Glu (NH_2)	0.28	0.54	0.27	0.5
Phe	0.30	0.58	0.27	1.3
Ser	0.30	0.59	0.27	1.2
Met	0.31	0.58	0.28	1.2
Ala	0.33	0.61	0.26	1.3
α-AnB	0.34	0.65	0.29	1.0
Thr	0.35	0.66	0.30	1.7
Sar	0.35	0.62	0.32	3.0
Asp	0.35	0.60	0.33	2.0
Val	0.36	0.68	0.29	0.9
α-AiB	0.36	0.72	0.32	3.5
Ile	0.36	0.69	0.35	1.2
Pro	0.40	0.66	0.40	3.0
β-Cl-Ala	0.47	0.64	0.44	2.3
Tau	0.96	0.97	0.86	5.0

RESULTS

Acidic eluents

The data in Table I, obtained with HCl as eluent, show that cystine and the basic amino acids were well separated from the others, but even the latter began to be separated as the concentration of HCl was increased. In particular, arginine behaved as could be expected from the most basic amino acid. The behaviour of the cystine is

due to the partial bivalent character of this amino acid. The separation of β-alanine from α-alanine and of some isomeric aminobutyric acids demonstrates the resolving power of alginic acid, as does the separation of glutamic from aspartic acid (all the more useful as other weak ion exchangers are incapable of it[4]). As expected from its marked acidity, taurine (Tau) migrated with the solvent front, unlike the other amino acids.

The replacement of HCl by HAc lowered the R_F values (which was expected) and changed the elution pattern, particularly in the case of some neutral amino acids (see Table I).

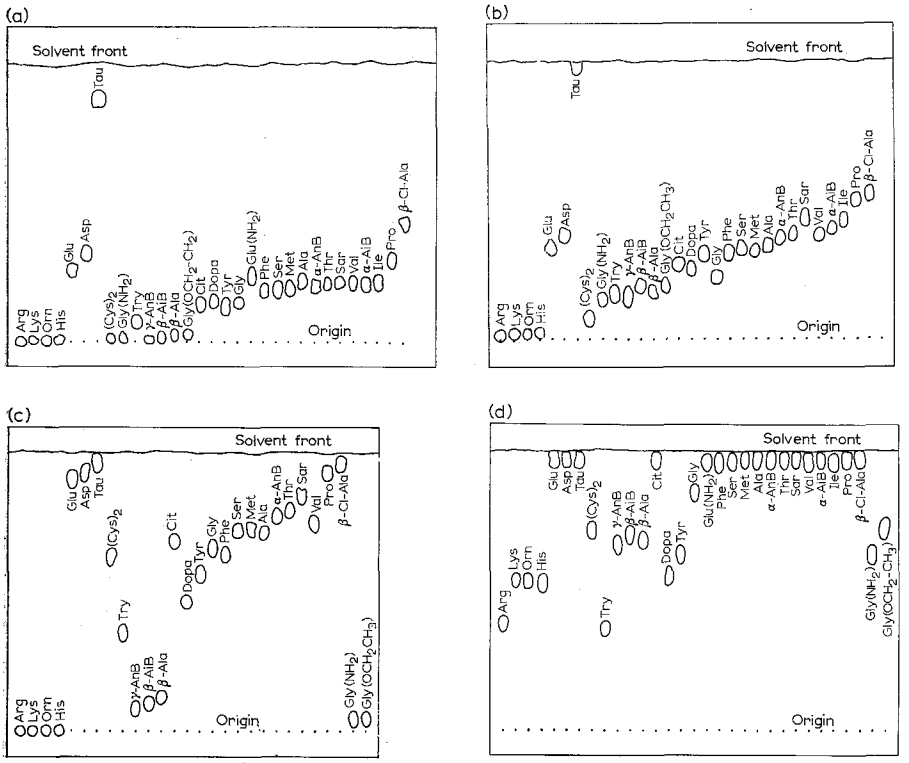


Fig. 1. Chromatograms of amino acids on alginic acid (a,b) and carboxymethylcellulose (c,d). Eluent: (a) and (c) H_2O; (b) and (d) 0.01 M KNO_3 solution.

Neutral eluents

Neutral salt solutions were tested as eluents to ascertain whether they were as efficient here as in the separation of some inorganic ions[6]. The use of water as eluent for the sake of comparison gave the chromatogram in Fig. 1a (alginic acid plate). This shows that basic amino acids, 4-aminobutyric acid (AnB), 3-aminoisobutyric acid (AiB), β-alanine and glycinamide did not migrate, an observation of analytical interest.

Neutral eluents were also useful in the comparison of alginic acid with carboxymethylcellulose, an ion exchanger of wide chromatographic use. This comparison helped to explain the behaviour of amino acids on alginic acids plates. The chromato-

grams in Fig. 1a–1d were obtained on alginic acid and on carboxymethylcellulose, with water and 0.01 *M* KNO_3 solution as eluents. The two adsorbents are seen to differ considerably in their ability to resolve the amino acids examined.

Mixed eluents

Aqueous organic solvents, recently used to elute organic[7] and inorganic[8,9] substances on ion exchangers, were found useful. This is particularly the case with aqueous isopropanol, which surpassed the reference eluent in resolving power on alginic acid thin layers. (*cf* R_F values in Table II and the chromatogram in Fig. 2). The selectivity increased and the R_F values decreased (except for some amino acids) when isopropanol was included in the eluent. The elution of amino acids with aqueous organic solvents is affected not only by ion exchange, but also by other factors such as liquid–liquid partition phenomena and a reduction in the polarity of the eluent in the exchanger[10].

TABLE II

R_F VALUES OF AMINO ACIDS ON ALGINIC ACID THIN LAYERS

Eluents: (1) 0.01 *M* HCl in 10% isopropanol; (2) 0.01 *M* HCl in 50% isopropanol; (3) 0.05 *M* HCl in 10% isopropanol; (4) 0.05 *M* HCl in 50% isopropanol.

Amino acids	*Eluent*			
	1	*2*	*3*	*4*
Arg	0.01	0.00	0.09	0.07
Lys	0.02	0.00	0.12	0.07
Orn	0.02	0.00	0.12	0.04
His	0.02	0.00	0.11	0.04
$(Cys)_2$	0.04	0.00	0.12	0.02
$Gly(NH_2)$	0.12	0.09	0.37	0.23
Try	0.14	0.33	0.32	e.s.[a]
γ-AnB	0.14	0.13	0.51	0.49
β-AiB	0.17	0.16	0.58	0.57
β-Ala	0.17	0.14	0.51	0.37
$Gly(OCH_2CH_3)$	0.19	0.18	0.56	0.48
Cit	0.20	0.16	0.43	0.39
Dopa	0.22	0.23	0.46	0.62
Tyr	0.23	0.29	0.54	0.74
Gly	0.19	0.09	0.49	0.37
Glu	0.28	0.20	0.58	0.58
$Glu(NH_2)$	0.28	0.16	0.58	0.57
Phe	0.30	0.36	0.58	0.74
Ser	0.30	0.15	0.59	0.50
Met	0.30	0.30	0.59	0.68
Ala	0.33	0.24	0.60	0.53
α-AnB	0.34	0.26	0.64	0.64
Thr	0.34	0.21	0.64	0.60
Sar	0.32	0.22	0.62	0.57
Asp	0.33	0.22	0.58	0.50
Val	0.36	0.35	0.68	e.s.
α-AiB	0.36	0.29	0.74	0.77
Ile	0.35	0.42	0.69	e.s.
Pro	0.37	0.31	0.62	0.56
β-Cl-Ala	0.46	0.22	0.63	0.47
Tau	0.85	0.54	0.90	0.56

[a] e.s. = Elongated spot.

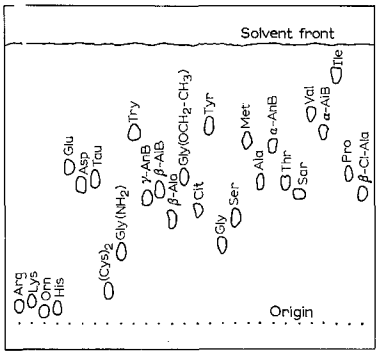


Fig. 2. Chromatogram of amino acids on alginic acid. Eluent: 0.05 *M* KNO_3 in 50 % isopropanol.

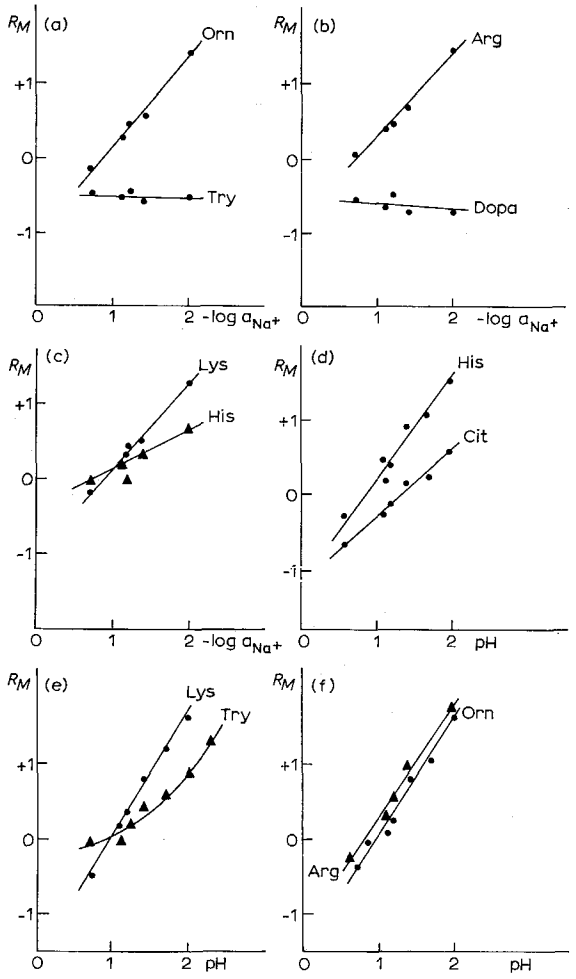


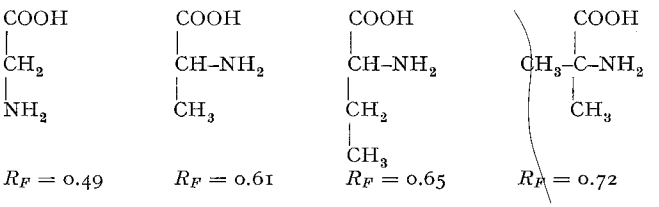
Fig. 3. R_M values *vs.* $-\log a_{Na^+}$ for some amino acids on sodium carboxymethylcellulose (a,b,c) and *vs.* pH on alginic acid thin layers (d,e,f). Eluents: NaCl and HCl solutions.

Retention mechanism

The good resolution of amino acids within a given group on alginic acid suggests that ion exchange is not the only factor affecting the retention. However, as regards the basic amino acids, non-ionic interactions found in cellulose-based ion exchangers[4] are secondary to ion exchange. The use of the expression pH = R_M + const. (ref. 11) gave the curves in Fig. 3 (a–c refer to carboxymethylcellulose and d–f to alginic acid). In the case of carboxymethylcellulose (Na^+ form), the slope of the straight lines for basic amino acids (which is equal to 1, except for histidine) indicates a purely ion-exchange mechanism. The behaviour of histidine, for which the slope is 0.53, is explained by the fact that the pH of the measurements was close to the isoelectric point of histidine, and so the R form predominated over the R^+ form. In the case of alginic acid as adsorbent, the slope of the straight lines (1.4–1.6) indicates that basic amino acids behave as divalent ions in the pH region examined. The slope of citrulline (Cit) (0.87) suggests a single charged cation. The discrepancy between the theoretical and the experimental values of the slope is probably due to a pH gradient along the plate[1].

The curves for the neutral amino acids, tryptophan and 3,4-dihydroxyphenylalanine (Dopa) in Fig. 3 indicate the complete absence of ion exchange on carboxymethylcellulose (Na^+ form). The curve for tryptophan on alginic acid shows that adsorption on the latter is not determined by ion exchange alone, another factor may be steric hindrance, due to the side chains of the amino acids and the adjacent OH groups of alginic acid, which influences the reaction between the ionic groups of the amino acid and the adsorbent.

The following values, obtained for some representative amino acids with 0.05 *N* HCl, reveal considerable differences in the R_F, according



to whether the NH_2 group is attached to a primary, a secondary, or a tertiary carbon atom. These differences are attributable to differences in the steric hindrance of the side chains. Provided that this observation is of general validity, it can be utilized to separate substances with similar acidities or basicities but different degrees of steric hindrance.

CONCLUSIONS

The present results have confirmed that alginic acid is a useful stationary phase for chromatography of organic and inorganic ions. Alginic acid exhibits a selectivity which makes it different from other weak ion exchangers and renders it suitable for analytical work. It is also better than carboxymethylcellulose in this respect.

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