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Note

The advantages of carboxymethylcellulose for the separation of amines from amino acids

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The successful separation of amines from biological material by paper chromatography, paper electrophoresis, etc., requires that the sample be relatively free of amino acids, salts, and other water soluble compounds. Awapara *et al.*[1] obtained such samples by adsorbing basic nitrogenous compounds onto a weak cation-exchange resin (Amberlite CG50) in the H^+-form, washing it free of neutral and acidic amino acids with water, and subsequently eluting the amines and basic amino acids with acetic acid. This method was used by Perry *et al.*[2] for the recovery of amines from urine.

However, recent work in this laboratory has shown that a carboxymethylcellulose ion exchanger, CM52 (H^+) has certain advantages over conventional resins.

EXPERIMENTAL

The ion exchangers studied were Zeokarb 226 [4.5% divinylbenzene (DVB), 200 mesh], Amberlite CG50 (type 2, 200 mesh), and Whatman CM52 (microgranular carboxymethylcellulose). Before activation, the Zeokarb 226 and Amberlite CG50 were purified by cycling between 5 *N* NaOH and 5 *N* HCl; CM-cellulose breaks down under such treatment, and was therefore purified according to the manufacturers' recommendations. The exchangers were converted to the H^+-form with 2 *N* HCl (0.5 *N* HCl for CM52) and packed into columns 10 × 1 cm I.D.

To investigate the ability of the H^+-forms of the various exchangers to discriminate between amines and amino acids, individual compounds (1 mg) in 1 ml of water were applied to columns and eluted with water at a flow-rate of 1 $ml \cdot cm^{-2} \cdot min^{-1}$ until 50 ml had been collected. The bound basic compounds were then eluted with 4 × 25 ml of 2 *N* HCl (again 0.5 *N* HCl for CM52), the acid eluates evaporated to dryness *in vacuo* at 50°, and the residues redissolved in 50 ml of water. Aliquots (2 ml) of both the washings and the acid eluates were analysed with Folin's reagent (Table I) and the colour intensity compared with that produced by the original sample at equivalent dilution.

RESULTS

Table I shows that although most neutral and acidic amino acids were readily

TABLE I

THE BEHAVIOUR OF AMINES AND AMINO ACIDS ON WEAK CATION EXCHANGERS

Results are the average of three separate experiments for each compound. Recoveries were quantitative within experimental error (100 ± 0.5%). — = not detected. % (Water) = Percentage of compound washed from column with 50 ml of water; % (HCl) = percentage of compound eluted from column with 4 × 25 ml of HCl. Estimation was by heating 2-ml aliquots of each compound (see text) with 2 ml of a 1:1 (v/v) mixture of 0.0625% aqueous Folin's reagent (1,2-naphthaquinone-4-sulphonic acid) and 0.05 *M* borax buffer (pH 11.0) for 10 min at 50°, and measuring the resulting colour intensity at 500 nm.

Compound (1 mg)	*Ion exchanger*					
	Zeokarb 226		*Amberlite CG50*		*Whatman CM52*	
	% (Water)	*% (2 N HCl)*	*% (Water)*	*% (2 N HCl)*	*% (Water)*	*% (0.5 N HCl)*
Glycine	100.5	—	99.6	—	99.9	—
Alanine	100.1	—	100.5	—	99.8	—
Threonine	99.7	—	100.1	—	100.1	—
Serine	100.4	—	99.5	—	100.4	—
Leucine	100.0	—	99.6	—	99.5	—
Phenylalanine	99.7	—	99.9	—	100.2	—
Glutamine	99.8	—	100.0	—	100.1	—
Asparagine	99.6	—	99.9	—	100.5	—
Proline	100.2	—	99.7	—	100.5	—
Valine	100.2	—	100.1	—	99.6	—
Cysteine	99.5	—	99.8	—	100.3	—
Taurine	100.0	—	99.5	—	100.1	—
Tyrosine	—	100.3	—	99.8	99.9	—
Tryptophan	—	100.1	—	100.4	100.3	—
Arginine	—	100.0	—	100.4	—	100.2
Histidine	—	100.0	—	99.8	—	99.6
Lysine	—	100.2	—	100.1	—	99.8
Ethylamine	—	99.7	—	100.5	—	100.4
Diethylamine	—	99.6	—	99.6	—	99.8
n-Butylamine	—	100.4	—	99.9	—	100.4
Isobutylamine	—	99.6	—	100.2	—	100.3
sec.-Butylamine	—	99.7	—	99.9	—	100.0
n-Amylamine	—	99.8	—	100.0	—	100.2
Ethanolamine	—	100.3	—	100.4	—	99.5
1,2-Diaminopropane	—	100.5	—	99.8	—	100.0
1,3-Diaminopropane	—	100.0	—	99.6	—	99.5
Spermine	—	100.4	—	100.5	—	100.0
Histamine	—	99.7	—	99.9	—	100.2
Pyrollidine	—	100.2	—	99.8	—	100.4
Phenylethylamine	—	99.5	—	100.5	—	99.6
Tryptamine	—	99.6	—	100.2	—	100.4
5-Hydroxytryptamine	—	99.8	—	99.8	—	99.6
Tyramine	—	99.6	—	99.5	—	100.0

washed from columns of Zeokarb 226 and Amberlite CG50 with 50 ml of water, the aromatic amino acids tryptophan and tyrosine were quantitatively bound along with the amines and basic amino acids. CM-cellulose, under the same conditions, did not show this undesirable behaviour.

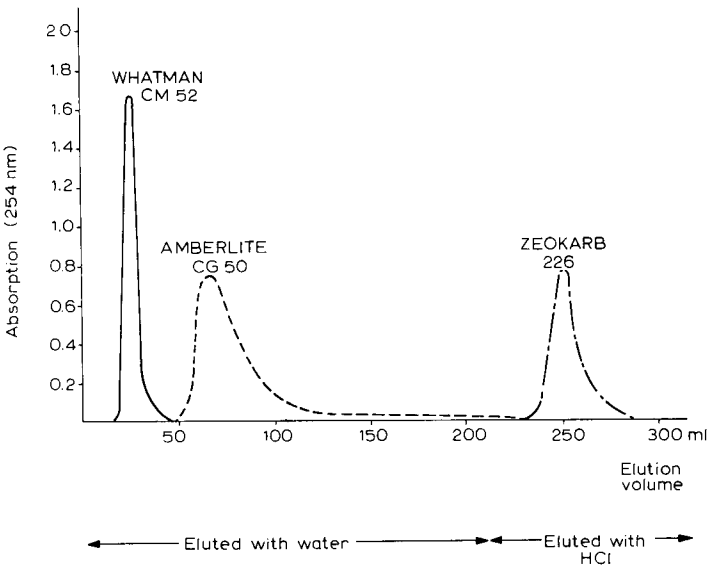

Fig. 1. The behaviour of 1 mg tryptophan on weak cation exchangers. Tryptophan in the column effluent was monitored with a Bausch and Lomb Spectronic 700 spectrophotometer at 254 nm using a flow-through cell.

However, since Awapara *et al.*[1] had reported that tryptophan and tyrosine could in fact be washed from Amberlite CG50 with water, the binding of tryptophan (1 mg) to the three types of exchanger was further investigated by monitoring the effluents at 254 nm.

The results, shown in Fig. 1, indicate that tryptophan is strongly bound to Zeokarb 226, but can be washed from Amberlite CG50 with approx. 25 bed volumes of water. However, the amino acid can be quantitatively recovered from Whatman CM52 with only 7 bed volumes of water. It seems likely that the retention of tryptophan and tyrosine by Zeokarb 226 and to a lesser extent by Amberlite CG50 is due, at least in part, to interaction between the aromatic nucleus of the amino acids and the aromatic groupings of the DVB residues used to cross-link these resins, though it is interesting that phenylalanine could be washed quite readily from both resins.

DISCUSSION

It appears that carboxymethylcellulose in the H^+-form can be used for the separation of amines from all but the basic amino acids, with less risk of contamination by the aromatic amino acids tryptophan and tyrosine. These have a tendency to be retained by Amberlite CG50, and were certainly bound by the batch of Zeokarb 226 studied.

The suitability of the method for the recovery of amines from biological material is being further investigated by a study of basic nitrogenous compounds in aquatic macrophytes, results of which will be published later.

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