Journal of Chromatography, 108 (1975) 391–396

CHROM. 8160

Note

Ion-exchange chromatography of some thiodiamines

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(Received December 30th, 1974)

We have been interested in the enzymic breakdown of thiodiamines, that is cystamine[1–5], lanthionamine[6] and, more recently, homocystamine, homolanthionamine[7] and cystathionamine, the 2-aminoethyl-3-aminopropyl sulphide[8]. We therefore had the opportunity to test some ion-exchange separation methods for these compounds.

A detailed study on the automated separation of amines and polyamines on a sulphonated ion-exchange resin has recently been reported by Tabor and Tabor[9], who showed that potassium salts are much more effective eluting agents than sodium salts for polyamines. Taking advantage of these observations, we have obtained some improvements in the separation of the thiodiamines of interest to us.

In this paper, we report the results obtained in the chromatographic analysis of the following thiodiamines: cystamine, homocystamine, lanthionamine, homolanthionamine and cystathionamine. The sulphoxide and the sulphone of cystathionamine have also been analyzed. It has been shown that all of these compounds can be well separated from each other and from other biologically interesting amines such as putrescine, cadaverine, spermine and spermidine, by using automated column chromatography. The only exception is putrescine, which cannot be separated from cystathionamine sulphone by the proposed elution procedures.

MATERIALS AND METHODS

Cystamine dihydrochloride was obtained from Fluka (Buchs, Switzerland); putrescine and cadaverine from Sigma (St. Louis, Mo., U.S.A.); spermine and spermidine from Calbiochem (Los Angeles, Calif., U.S.A.); and 3-bromopropylamine hydrobromide from K and K Labs. (Plainview, N.Y., U.S.A.). Homocystamine dihydrochloride was prepared as reported by De Marco and Rinaldi[10] and cystathionamine as reported by Rinaldi *et al.*[8]. Lanthionamine was prepared according to Coblentz and Gabriel[11]. Homolanthionamine was prepared by reaction of 3-bromopropylamine with sodium sulphide in aqueous solution; the details of this synthesis will be reported elsewhere. Chromatographic analyses were performed on a Bio Cal 200 amino acid analyzer. A short column (0.9 × 12 cm) filled with Aminex A-5 resin (Bio-Rad Labs., Richmond, Calif., U.S.A.), particle size 13.5 ± 2 μm, was used. The column temperature was 50° and the buffer flow-rate was 80 ml/h. The ninhydrin

solution was the standard solution in 1 *M* acetate buffer, pH 5.5, at a flow-rate of 40 ml/h. The macro-cells of the instrument, light path 3 mm, were used.

RESULTS AND DISCUSSION

The thiodiamines under consideration are not eluted from the sulphonated resin by the standard 0.35 *M* sodium citrate buffer, pH 5.28, that is commonly used for the separation of the basic amino acids in the two-column method, or with the 1.2 *M* sodium citrate buffer, pH 6.5, used in the single-column method. They can be separated, however, by washing the column with 0.2 *N* sodium hydroxide solution, and are eluted in the following order: cystathionamine sulphoxide and sulphone, lanthionamine, cystathionamine, homolanthionamine, cystamine, homocystamine. The elution profile is shown in Fig. 1. The elution is complete in 70 min, but there is overlapping between the sulphoxide and the sulphone of cystathionamine. Moreover, overlapping may also occur between lanthionamine, cystathionamine and homolanthionamine if one of them is present in excess over the others. Therefore, the elution with sodium hydroxide solution may be of little use in an initial screening for the presence of the above thiodiamines; however, it may be particularly useful for the separation of cystamine and homolanthionamine, which are eluted together in the other elution procedures proposed (see below). The elution with sodium hydroxide solution may also be advantageous, owing to the short elution time, in some special cases, for example routine quantitative analyses of samples that contain only one or other of the diamines.

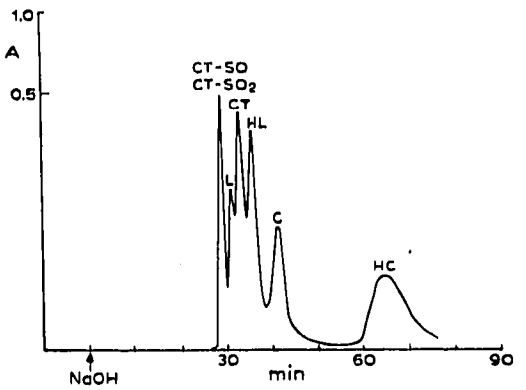

Fig. 1. Elution profile of thiodiamines obtained with 0.2 *N* NaOH as eluting agent. Amounts of 0.5 μmole of each compound were loaded on the short column of the amino acid analyzer equilibrated with 0.35 *M* sodium citrate buffer, pH 5.28, and were then eluted with 0.2 *N* NaOH. Column size: 0.9 × 12 cm. Resin: Aminex A-5. Temperature: 50°. Buffer flow-rate: 80 ml/h. Ninhydrin flow-rate: 40 ml/h. CT, CTSO, $CTSO_2$ = cystathionamine, its sulphoxide and sulphone, respectively; L = lanthionamine; HL = homolanthionamine; C = cystamine; HC = homocystamine.

A better separation is obtained when the elution is carried out with 2.35 *M* potassium citrate buffer, pH 5.6, as shown in Fig. 2. In this case there is a good separation of the sulphoxide from the sulphone of cystathionamine, but there is overlapping of cystamine with homolanthionamine.

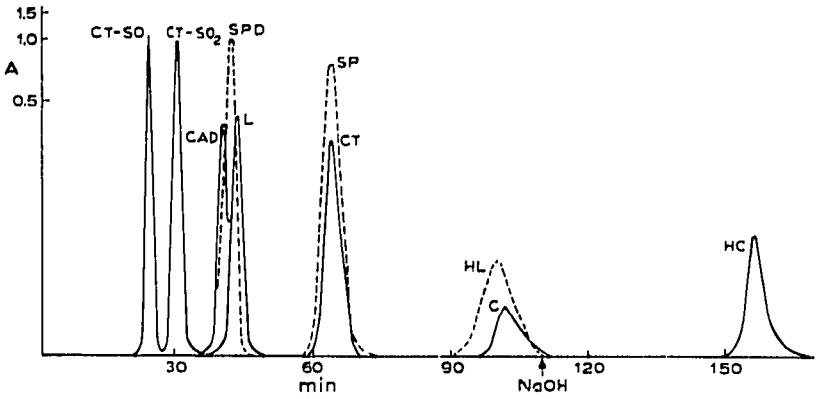

Fig. 2. Elution profile obtained using 2.35 *M* potassium citrate buffer, pH 5.6. Amounts of 0.5 μmole of each compound were loaded on the column, equilibrated and then eluted with the above buffer. At the arrow, the buffer was replaced with 0.2 *N* NaOH. All other conditions as in Fig. 1. CAD = cadaverine; SPD = spermidine; SP = spermine; other abbreviations as in Fig. 1. Putrescine is eluted with $CTSO_2$.

Homocystamine is not eluted by this buffer; it emerges when the column is washed with 0.2 *N* sodium hydroxide solution. Nevertheless, if chromatography is carried out in the presence of other non-sulphur-containing diamines, there is overlapping of cystathionamine sulphone with putrescine, of spermidine with lanthionamine and cadaverine, and of spermine with cystathionamine (Fig. 2). The elution time is almost doubled compared with the elution with sodium hydroxide solution.

A good separation between spermine and cystathionamine and between cadaverine and spermidine is obtained by using a buffer made by mixing equal volumes of 2.35 *M* potassium citrate, pH 5.6, and 2.35 *M* sodium citrate, pH 5.6. The elution profile obtained in this case is shown in Fig. 3. There is still overlapping of cystathionamine sulphone with putrescine, of spermidine with lanthionamine and of cystamine with homolanthionamine. In order to elute homocystamine, it is always

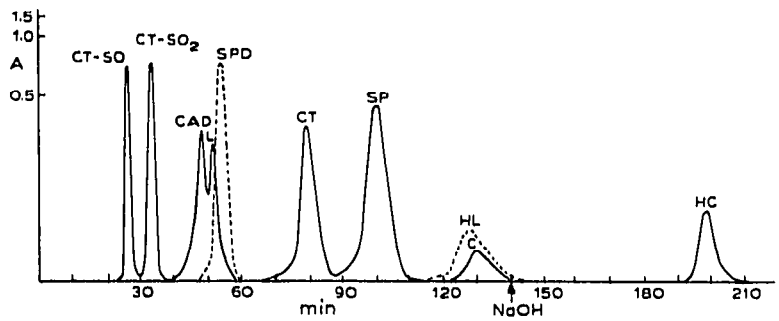

Fig. 3. Elution profile obtained using a buffer made by mixing equal volumes of 2.35 *M* sodium citrate, pH 5.6, and 2.35 *M* potassium citrate, pH 5.6. Amounts of 0.5 μmole of each compound were loaded on the column, equilibrated and then eluted with the above buffer. At the arrow, the buffer was replaced with 0.2 *N* NaOH. All other conditions as in Fig. 1. Abbreviations as in Figs. 1 and 2. Putrescine is eluted with $CTSO_2$.

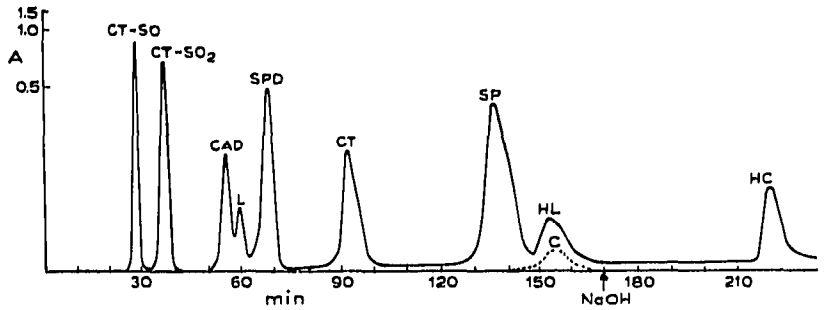

Fig. 4. Elution profile obtained using a buffer made by mixing 80 volumes of 2.35 *M* sodium citrate, pH 5.6, and 20 volumes of 2.35 *M* potassium citrate, pH 5.6. Amounts of 0.5 μmole of each compound were loaded on the column, equilibrated and then eluted with the above buffer. At the arrow, the buffer was replaced with 0.2 *N* NaOH. All other conditions as in Fig. 1. Abbreviations as in Figs. 1 and 2. Putrescine is eluted with $CTSO_2$.

necessary to wash the column with sodium hydroxide solution. The elution time is even longer than above.

A complete separation between spermidine and lanthionamine is achieved when the elution is carried out with a buffer made by mixing 80 volumes of 2.35 *M* sodium citrate, pH 5.6, and 20 volumes of 2.35 *M* potassium citrate, pH 5.6. Nevertheless, there is always overlapping of cystathionamine sulphone with putrescine and of cystamine with homolanthionamine. The elution profile is shown in Fig. 4.

No better results were obtained by further variations in the ratios between sodium and potassium citrate in the eluting buffers.

Table I gives the elution times observed with the four elution procedures reported above. In Table II are reported the colour constants (C_{IIW}) and the ratios $A_{440}:A_{560}$ for the compounds examined, calculated on chromatograms obtained with the potassium citrate buffer.

TABLE I

ELUTION TIMES, IN MINUTES, OBTAINED WITH DIFFERENT 2.35 *M* CITRATE BUFFERS AND WITH 0.2 *M* SODIUM HYDROXIDE SOLUTION

Compound	*2.35 M citrate buffers, pH 5.6*			*0.2 N NaOH*
	100% K⁺	*50% K⁺*	*20% K⁺*	
Putrescine	30	33	36	
Cadaverine	41.5	48.5	55	
Spermine	66.5	100	139	
Spermidine	42	54	68	
Cystathionamine	67	81	92	33
Homocystamine*	50	50	50	65
Lanthionamine	43	52	60	31
Homolanthionamine	100	128	155	36
Cystamine	105	131	153	41.5
Cystathionamine sulphoxide	24	26.5	28.5	29
Cystathionamine sulphone	31	33.5	36	29

* For homocystamine, the elution times are referred to the change of the buffer with NaOH.

TABLE II

COLOUR CONSTANTS AND $A_{440}:A_{560}$ RATIOS CALCULATED ON CHROMATOGRAMS OBTAINED USING AS ELUENT 2.35 *M* POTASSIUM CITRATE BUFFER, pH 5.6

Compound	*Colour constant,* C_{UW},	$A_{440}:A_{560}$
Putrescine	44.6	0.28
Cadaverine	23.6	0.30
Spermine	79.6	0.22
Spermidine	62	0.24
Cystathionamine	37	0.19
Homocystamine*	18.7	0.90
Lanthionamine	29	0.22
Homolanthionamine	22.3	0.21
Cystamine	9.7	0.33
Cystathionamine sulphoxide	31.8	0.20
Cystathionamine sulphone	39.4	0.19

* For homocystamine, the values are referred to the elution with 0.2 *N* NaOH.

In conclusion, all of the thiodiamines considered, with the exception of cystamine and homolanthionamine, can be well separated from each other in about 160 min on the short column of the amino acid analyzer, using as eluting agent 2.35 *M* potassium citrate buffer, pH 5.6, followed by 0.2 *N* sodium hydroxide solution (Fig.2). Cystamine and homolanthionamine can be separated by eluting the column directly with 0.2 *N* sodium hydroxide solution.

Incidentally, as regards cystamine and homolanthionamine, it should be emphasized that other simple analytical methods may be exploited for their differentiation: for example, the common reactions for the disulphides, which obviously will be given by cystamine but not by homolanthionamine, as the Folin–Marenzi reaction after addition of hydrogen sulphite[12], which may be also performed on paper chromatograms[13].

In the presence of other more common non-sulphur-containing diamines or polyamines, one or the other of the proposed elution procedures may be helpful, according to the particular problem under study.

ACKNOWLEDGEMENT

This work was supported in part by a grant from the Consiglio Nazionale delle Ricerche, Rome.

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