Journal of Chromatography, 133 (1977) 390–395

CHROM. 9771

Note

Separation of methylated basic amino acids

R. HELM
Physiological Institute Czechoslovak Academy of Sciences, Prague (Czechoslovakia)
O. VANČÍKOVÁ
Research Institute for Food Industry, Prague (Czechoslovakia)
K. MACEK
3rd Medical Department, Medical Faculty, Charles University, Prague (Czechoslovakia)
and
Z. DEYL
Physiological Institute Czechoslovak Academy of Sciences, Prague (Czechoslovakia)
(Received September 23rd, 1976)

In spite of the vast amount of information on the ion-exchange chromatography of amino acids (for reviews, see refs. 1–4), there is still a lack of knowledge regarding the separation of methylated basic amino acids. However, there is an urgent need for the separation of these amino acids because of their involvement in both the structure and function of some proteins: methylated lysines were found in myosin[5–7], flagellin[8] and histones[9]; methylated arginines, in addition to muscle proteins, were reported[10] in basic encephalitogenic protein in myelin[11] and in histones[12]; and methylated histidines occur in myosin[13–15], actin[16], histones from various types of cells[17–19] and actomyosin-like protein of the adrenal medulla[20]. The determination of 3-methylhistidine in urine, where it indicates the rate of breakdown of muscle proteins[21], is a routine analysis, but the biomedical implications of the other methylated amino acids have not yet been sufficiently elucidated. This situation, however, is expected to change in the near future, as some of these amino acids have been shown to serve as markers for some categories of proteins and to exhibit quantitative changes with pathological and physiological conditions, as can be demonstrated by the alterations of methylated amino acids in muscle with age[10,22].

These facts prompted our efforts to obtain a more detailed knowledge of the separation of these amino acids by automated ion-exchange chromatography. Another reason was that irregularities in their chromatographic behaviour have been observed both in our preliminary tests and as recorded in the literature[23,24].

EXPERIMENTAL

Aminex A-5, Beckman UR and Ostion KS LG 0802 ion-exchange resins were selected on the basis of their suitability for the separation of N^{ε}-monomethyllysine (MML), dimethyllysine (DML), trimethyllysine (TML), 1-methylhistidine (1-Me-

His), 3-methylhistidine (3-Me-His), asymmetrical dimethylarginine (asym.-DMA) and symmetrical dimethylarginine (sym.-DMA), with regard to the pH of the buffer used and temperature at which the column was operated.

1- and 3-Me-His, MML and DML were purchased from Calbiochem (Los Angeles, Calif., U.S.A.) and Cyclo Chem (Los Angeles, Calif., U.S.A.). TML was synthesized by alkylation of the copper complex of lysine. Methylated arginines (sym.-DMA and asym.-DMA) were prepared by the method of Kakimoto and Akazawa[25] as modified by Reporter and Corbin[10], (urea was replaced with thiourea).

The separations were carried out with a home-assembled amino acid analyzer, most parts of which were Technicon components. A 30 × 0.9 cm jacketted column was packed with resin and run at a constant temperature in the range 37–55°. Citrate buffers (0.35 *N*) of the appropriate pH (4.26, 5.16, 5.28, 5.38 and 5.79) served as mobile phases. No gradient elution was used. Separations were performed at a flow-rate of 60 ml/h in the sample line and the ninhydrin flow-rate was set at 30 ml/h.

RESULTS AND DISCUSSION

As indicated in Table I, complete separations of all methylated basic amino acids can be achieved with any of the ion exchangers under investigation provided that the pH and temperature are properly chosen. All amino acids other than those under investigation are eluted as a single peak at the front of the chromatogram and do not interfere with the separations described. The differences in the retention volumes

TABLE I

RETENTION TIMES OF METHYLATED AMINO ACIDS ON DIFFERENT ION EXCHANGERS AT VARIOUS pH VALUES

Amino acid	*pH*	*Time of elution of peak maximum (min)*		
		Beckman UR (50°)	*Aminex A-5 (37°)*	*Ostion KS LG 0802 (37°)*
Lysine	5.79	240	—	61
	5.38	263	—	74
	5.32	291	—	76
	5.28	309	103	90
	5.16	335	113	92
	4.26	—	120	129
Monomethyllysine	5.79	265	—	73
	5.38	285	—	80
	5.32	300	—	89
	5.28	317	112	102
	5.16	341	122	106
	4.26	—	127	135–140
Dimethyllysine	5.79	282	—	77
	5.38	298	—	84
	5.32	307	—	81
	5.28	325	118	95
	5.16	345	126	98
	4.26	—	130	135–140

TABLE I *(continued)*

Amino acid	*pH*	*Time of elution of peak maximum (min)*		
		Beckman UR (50°)	*Aminex A-5 (37°)*	*Ostion KS LG 0802 (37°)*
Trimethyllysine	5.79	300	—	80
	5.38	310	—	87
	5.32	314	—	85
	5.28	333	—	95
	5.16	345	—	98
	4.26	—	100	135–140
	5.79	300	—	80
	5.38	310	—	87
	5.32	314	—	85
	5.28	333	—	95
	5.16	345	—	98
	4.26	—	100	135–140
Histidine	5.79	323	—	86
	5.38	328	—	95
	5.32	328	—	109
	5.28	343	—	124
	5.16	352	—	125
	4.26	—	145	135–140
1-Methylhistidine	5.79	340	—	91
	5.38	342	—	104
	5.32	345	—	118
	5.28	349	125	131
	5.16	355	135	133
	4.26	—	136	135–140
3-Methylhistidine	5.79	360	—	96
	5.38	355	—	108
	5.32	355	—	125
	5.28	356	138	139
	5.16	360	152	141
	4.26	—	160	135–140
Arginine	5.79	—	—	120
	5.38	—	—	131
	5.32	—	—	145
	5.28	—	214	147
	5.16	—	255	152
	4.26	—	265	155–160
Asym.-dimethylarginine	5.79	382	—	125
	5.38	385	—	136
	5.32	—	—	149
	5.28	—	165	152
	5.16	—	202	154
	4.26	—	205	155–160
Sym.-dimethylarginine	5.79	388	—	130
	5.38	400	—	144
	5.32	—	—	156
	5.28	—	182	155
	5.16	—	214	154
	4.26	—	216	155–160

vary, however. The best resolution was obtained with the Beckman UR resin at a high pH (5.79) at 50°. This separation is insensitive towards temperature variations. Methylated lysines are eluted in order to increasing number of methyl groups: 1-Me-His precedes 3-Me-His and asym.-DMA precedes sym.-DMA. At pH 5.38 the separation is still complete, but the differences in retention volumes are smaller. At pH 5.32 methylated lysines often give a complex combined peak.

With Aminex A-5, the separation is complete when operating the column at pH 4.26, but the elution of individual amino acids is different to that on Beckman UR resin. Here, TML is fastest, followed by MML, DML, 1-Me-His, 3-Me-His and methylated arginines. Adequate separations were obtained at a lower temperature (37°).

The optimum pH for the separation of the methylated amino acids on the Ostion KS LG 0802 resin appears to be 5.32. Although a complete separation can be obtained when the analyzer is carefully operated, the differences between the retention volumes are much smaller than with the other types of resins. The advantage of this resin is, however, the time of analysis that is more than one third shorter than with Aminex A-5 and Beckman UR ion exchangers. The order of elution of individual amino acids is strongly dependent on the pH of the buffer used and the identities of the individual peaks have to be checked carefully against appropriate standards. Thus, for example, at pH 5.79 methylated lysines are eluted in the order MML, DML and TML while at pH 5.38 the order is DML, TML and MML.

The separation of methylated histidines can be improved by using an elevated temperature (Fig. 1), and the same holds to a lesser extent for DML + TML and

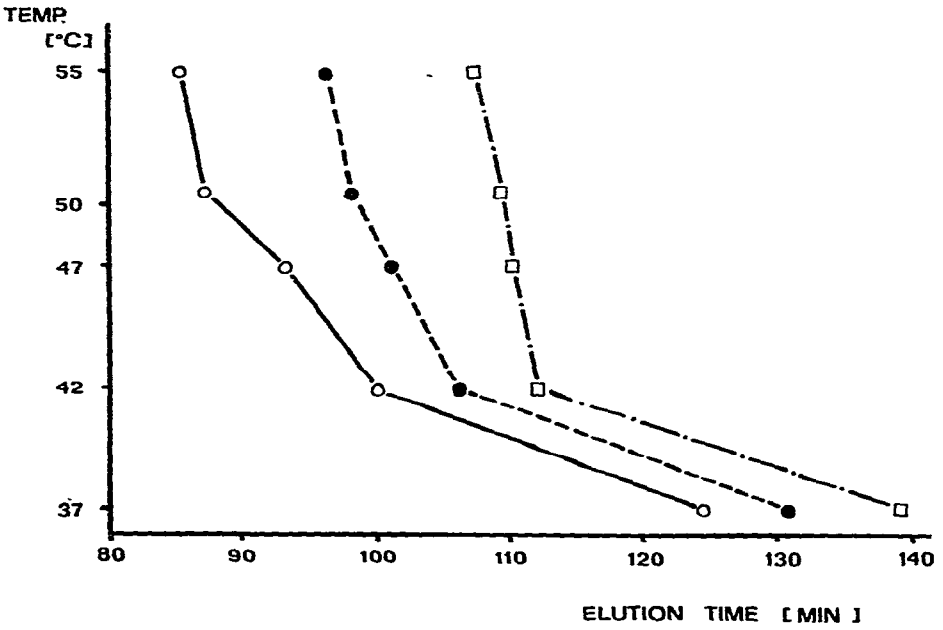

Fig. 1. Dependence of elution of histidine and its methylated derivatives on temperature. Ion exchanger, Ostion KS LG 0802; pH, 5.28. ○, His; ●, 1-Me-His; □, 3-Me-His.

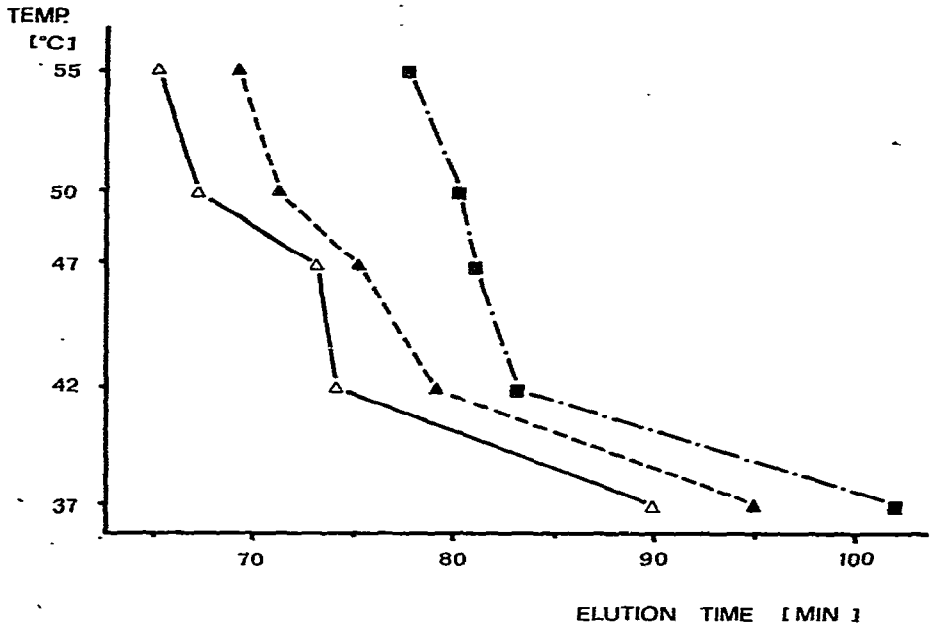

Fig. 2. Dependence of elution of lysine and its methylated derivatives on temperature. Ion exchanger, Ostion KS LG 0802; pH, 5.28. △, Lys; ▲, DML + TML; ■, MML.

TABLE II

OPTIMAL CONDITIONS FOR THE SEPARATION OF METHYLATED BASIC AMINO ACIDS ON COLUMNS OF ION EXCHANGERS

MML = monomethyllysine; DML = dimethyllysine; TML = trimethyllysine; 1-Me-His = 1-methylhistidine; 3-Me-His = 3-methylhistidine.

Resin	*Optimal pH*	*Optimal temperature*	*Combinations of amino acids causing problems in separation*
Aminex A-5	4.26	Operated at 37° but temperature variations do not influence the quality of separation	NH_3–DML; MML–DML
Beckman UR	5.79	50°	All components clearly resolved
Ostion KS LG 0802	5.32–5.38	37°	MML–DML–TML; 1-Me-His–3-Me-His

MML (Fig. 2). No improvement in the separation of DML and TML can be obtained in this way.

The reason for the alterations in the order of elution can be seen in non-specific sorption effects but we can give no definite explanation of this phenomenon. Optimal conditions for the separation of methylated basic amino acids are summarized in Table II.

ACKNOWLEDGEMENT

The authors thank Mr. M. Smrž for technical assistance.

REFERENCES

1 Z. Deyl, K. Macek and J. Janák (Editors), *Liquid Column Chromatography, A Survey of Techniques and Applications*, Journal of Chromatography Library, Vol. 3, Elsevier, Amsterdam, 1975.
2 J. L. Bailey, *Techniques in Protein Chemistry*, Elsevier, Amsterdam, 1967.
3 T. Dévény and J. Gergely, *Analytische Methoden zur Untersuchung von Aminosäuren, Peptiden und Proteinen*, Akadémiai Kiado, Budapest, 1968.
4 A. Niederwieser and G. Pataki, *New Techniques in Amino Acids, Peptide and Protein Analysis*, Ann Arbor Sci. Publ., Ann Arbor, Mich., 1971.
5 G. Huszar and M. Elzinga, *Nature (London)*, 223 (1969) 834.
6 M. F. Hardy, C. I. Harris, S. V. Perry and D. Stone, *Biochem. J.*, 120 (1970) 653.
7 W. M. Kuehl and R. S. Adelstein, *Biochem. Biophys. Res. Commun.*, 43 (1969) 59.
8 R. P. Ambler and M. W. Rees, *Nature (London)*, 184 (1959) 56.
9 K. Murray, *Biochemistry*, 3 (1964) 10.
10 M. Reporter and J. L. Corbin, *Biochem. Biophys. Res. Commun.*, 43 (1971) 644.
11 G. S. Baldwin and P. R. Carnegie, *Biochem. J.*, 123 (1971) 69.
12 M. Ki Paik and S. Kim, *Biochem. Biophys. Res. Commun.*, 40 (1970) 224.
13 G. Huszar and M. Elzinga, *J. Biol. Chem.*, 247 (1972) 745.
14 I. P. Trayer, C. I. Harris and S. V. Perry, *Nature (London)*, 217 (1968) 452.
15 G. Huszar and M. Elzinga, *Biochemistry*, 10 (1971) 229.
16 M. Elzinga, *Biochemistry*, 10 (1971) 224.
17 P. Byvoet, *Biochim. Biophys. Acta*, 238 (1971) 375a.
18 E. L. Gershey, G. W. Haslett, G. Vidali and V. G. Allfrey, *J. Biol. Chem.*, 244 (1969) 4871.
19 T. W. Borun, D. Pearson and W. P. Paik, *J. Biol. Chem.*, 247 (1972) 4288.
20 S. L. Twoomey and A. M. Poisner, *Brain Res.*, 46 (1972) 341.
21 L. N. Haverberg, P. T. Omstedt, H. N. Munro and V. R. Young, *Biochim. Biophys. Acta*, 405 (1975) 67.
22 R. Helm, Z. Deyl and O. Vančíková, *Exp. Geront.*, in press.
23 A. Beckerton, P. J. Buttery, F. J. Bailey and N. Bolton, *J. Chromatogr.*, 104 (1975) 170.
24 E. Tyihák, S. Ferenczi, I. Hazai, S. Zoltán and A. Patthy, *J. Chromatogr.*, 102 (1974) 257.
25 Y. Kakimoto and S. Akazawa, *J. Biol. Chem.*, 245 (1970) 5751.