Journal of Chromatography, 151 (1978) 424–427

CHROM. 10,677

Note

Quantitative isolation of N^{τ}-methylhistidine by ion-exchange paper and column chromatography

N. NISHIZAWA, T. NOGUCHI and S. HAREYAMA
Department of Agricultural Chemistry, Iwate University, Morioka, Iwate (Japan)
and
R. FUNABIKI
Department of Agricultural Chemistry, Tokyo Noko University, Fuchu-shi, Tokyo (Japan)
(Received September 26th. 1977)

Since the potential usefulness of urinary N^{τ}-methylhistidine (3-methylhistidine, 3-MeHis) for measuring the catabolic rate of skeletal muscle protein was suggested[1], confirmatory evidence has been reported by several workers[2–6]. Catabolic rates of muscle proteins under various nutritional conditions have been estimated by this method[6–10]. For further studies on the catabolic rate of muscle proteins or the metabolism of 3-MeHis, it is necessary to isolate pure 3-MeHis quickly and quantitatively from animal tissues and natural products. Although many workers have reported the separation of 3-MeHis by thin-layer chromatography, paper chromatography and paper electrophoresis[11–16], these methods give a poor resolution of 3-MeHis from N^{π}-methylhistidine (1-methylhistidine, 1-MeHis). On the other hand, ion-exchange chromatography has sometimes proved successful[3,17,18]. In an extension of these studies, we report here convenient methods for the quantitative isolation of 3-MeHis from animal tissues and other natural products by ion-exchange paper and column chromatography.

EXPERIMENTAL

Separation of 3-MeHis by ion-exchange paper chromatography (method A)

Ion-exchange chromatography was carried out with 20 × 20 cm carboxymethylcellulose paper (Toyo Scientific Instrument Co., Tokyo, Japan). Two microlitres of mixtures containing approximately 5 μg each of 3-MeHis, histidine (His) and 1-MeHis were placed on the paper at a distance of 1.5 cm between each. These amino acids were developed by the ascending technique with 0.1 *M* α-picoline in a glass chamber. Development to a distance of 11 cm at room temperature required 70 min. The paper was then dried to remove the solvent, sprayed with a mixture of 50 ml of 0.1% ninhydrin in ethanol, 2 ml of collidine and 15 ml of glacial acetic acid[19] and finally heated for 2 min at 100°.

Isolation of 3-MeHis by ion-exchange column chromatography (method B)

Ion-exchange chromatography was performed using 200–400-mesh Dowex

50-X8 (pyridine form). The resin was packed by gravity into a column (19 × 1.2 cm I.D.) to give a bed height of 12.2 cm and a column volume of 15 ml. The column had previously been equilibrated with 0.2 *M* pyridine. The hydrolyzates, corresponding to 1–2 g of fresh tissues, 1 g of de-fatted skin of rats[20] or 0.5–1 g of feedstuffs[21], were evaporated to dryness and the residues were dissolved in 5 ml of 0.2 *M* pyridine and applied to the column. The acidic and neutral amino acids were eluted with 150 ml of 0.2 *M* pyridine. 3-MeHis was then eluted with 55 ml of 1 *M* pyridine. The flow-rate of the eluent was *ca.* 0.75 ml/min. The 3-MeHis fraction thus obtained was evaporated to dryness. When the radioactivity in the 3-MeHis fraction was measured, the fraction was re-dissolved in 5 ml of 0.2 *M* pyridine and the above procedure for isolating 3-MeHis was repeated in order to reduce quenching in a liquid scintillation spectrometer. The 3-MeHis thus obtained was finally dissolved in 2 ml of 0.01 *M* hydrochloric acid and an aliquot of 1.5 ml was used for measurement of the radioactivity. The remainder of the sample was used for the determination of 3-MeHis. The 3-MeHis fraction was found by amino acid analysis to contain only 3-MeHis.

RESULTS AND DISCUSSION

A typical chromatogram obtained with method A is shown in Fig. 1. 3-MeHis was separated completely from His and 1-MeHis. The R_F values of 3-MeHis, His and 1-MeHis were 0.82, 0.71 and 0.65, respectively.

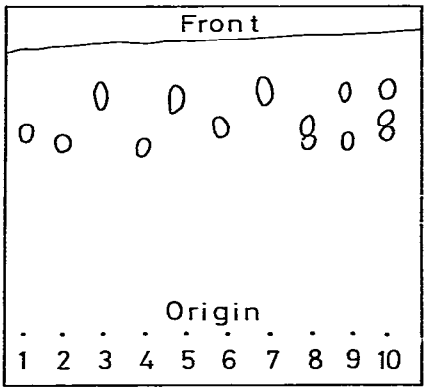

Fig. 1. Separation of 3-MeHis from His and 1-MeHis using carboxymethylcellulose paper developed with 0.1 *M* α-picoline. 1 = His; 2 = 1-MeHis; 3 = 3-MeHis; 4 = 1-MeHis; 5 = 3-MeHis; 6 = His; 7 = 3-MeHis; 8 = mixture of His and 1-MeHis; 9 = mixture of 3-MeHis and 1-MeHis; 10 = mixture of 3-MeHis, His and 1-MeHis.

Table I shows the complete separation of 3-MeHis from His and 1-MeHis by method B. 3-MeHis was eluted with 1–4 column volumes of 1 *M* pyridine, which was contrary to the findings of Haverberg *et al.*[3], who reported that 3-MeHis appeared after applying 7–8 column volumes of 1 *M* pyridine. The mean recovery of 3-MeHis was 97 ± 0.7% (standard error; $n = 8$) within the range 0.166–6.92 μmole. Lysine, methyllysines, arginine and methylarginines were not eluted from the column, even after applying 300 ml of 1 *M* pyridine and an additional 600 ml of 2 *M* pyridine. Creatinine was eluted together with acidic and neutral amino acids.

TABLE I

RETENTION VOLUMES OF 3-MeHIS, HIS AND 1-MeHIS WITH A COLUMN OF DOWEX 50-X8 (PYRIDINE FORM)

After elution of acidic and neutral amino acids with 0.2 *M* pyridine, 3-MeHis, His and 1-MeHis were eluted with 1 *M* pyridine.

Amino acid	*Retention volume (ml)*
3-MeHis	15– 55
His	110–170
1-MeHis	180–240

Method A for the separation of 3-MeHis is rapid and efficient, especially when many samples have to be analysed at the same time, *e.g.*, eluates from a column. If a fluorogenic reagent [16,22–28]was used for the detection of the amino acids, method A could be more useful because of its simplicity and high sensitivity.

Method B is convenient and efficient for the quantitative isolation of 3-MeHis. By employing method B, we found the contribution rate of skin and gastrointestinal tract to urinary excretion of 3-MeHis in the rat[20]. Further, analyses with this method showed that feedstuffs, *e.g.*, maize, soybean meal, oat, wheat and rice brans and lucerne meal, contain significant amounts of 3-MeHis. This indicates that dietary 3-MeHis should be determined before calculating catabolic rates of skeletal-muscle proteins of domestic animals from urinary excretion of 3-MeHis [21].

The methods reported here for the rapid and/or quantitative isolation of 3-MeHis could be useful for studies of the rates of catabolism of muscle proteins.

REFERENCES

1 A. M. Asatoor and M. D. Armstrong, *Biochem. Biophys. Res. Commun.*, 26 (1967) 168.
2 V. R. Young, S. D. Alexis, B. S. Baliga, H. N. Munro and W. Muecke, *J. Biol. Chem.*, 247 (1972) 3592.
3 L. N. Haverberg, H. N. Munro and V. R. Young, *Biochim. Biophys. Acta*, 371 (1974) 226.
4 L. N. Haverberg, P. T. Omstedt, H. N. Munro and V. R. Young, *Biochim. Biophys. Acta*, 405 (1975) 67.
5 C. L. Long, L. N. Haverberg, V. R. Young, J. M. Kinney, H. N. Munro and J. W. Geiger, *Metabolism*, 24 (1975) 929.
6 N. Nishizawa, M. Shimbo, S. Hareyama and R. Funabiki, *Brit. J. Nutr.*, 37 (1977) 345.
7 N. Nishizawa, R. Funabiki and S. Hareyama, *J. Nutr. Sci. Vitaminol.*, 21 (1975) 383.
8 V. R. Young, L. N. Haverberg, C. Bilmazes and H. N. Munro, *Metabolism*, 22 (1973) 1429.
9 L. N. Haverberg, L. Deckelbaum, C. Bilmazes, H. N. Munro and V. R. Young, *Biochem. J.*, 152 (1975) 503.
10 R. Funabiki, Y. Watanabe, N. Nishizawa and S. Hareyama, *Biochim. Biophys. Acta*, 451 (1976) 143.
11 I. Smith, L. J. Rider and R. P. Lerner, *J. Chromatogr.*, 26 (1967) 449.
12 R. S. Ratney, M. F. Godshalk, J. Joice and K. W. James, *Anal. Biochem.*, 19 (1967) 357.
13 M. Reporter and J. L. Corbin, *Biochem. Biophys. Res. Commun.*, 43 (1971) 644.
14 H. Hassall, *Methods Enzymol.*, 17B (1971) 99.
15 J. N. A. Van Balgooy and E. Roberts, *Biochem. Pharmacol.*, 22 (1973) 1405.
16 S.-C. Lee and S.-J. Yin, *J. Chromatogr.*, 129 (1976) 482.
17 I. R. McManus, *J. Biol. Chem.*, 235 (1960) 1398.
18 R. T. Markiw, *Biochem. Med.*, 13 (1975) 23.
19 A. L. Levy and D. Chung, *Anal. Chem.*, 25 (1953) 396.

20 N. Nishizawa, T. Noguchi, S. Hareyama and R. Funabiki, *Brit. J. Nutr.*, 38 (1977) 149.
21 N. Nishizawa, Y. Toyoda, T. Noguchi, S. Hareyama and R. Funabiki, in preparation.
22 W. B. Shelley and L. Juhlin, *J. Chromatogr.*, 22 (1966) 130.
23 T. D. Turner and S. L. Wightman, *J. Chromatogr.*, 32 (1968) 315.
24 D. Aures, R. Fleming and R. Håkanson, *J. Chromatogr.*, 33 (1968) 480.
25 L. Edvinsson, R. Håkanson, A. L. Rönnberg and F. Sundler, *J. Chromatogr.*, 67 (1972) 81.
26 A. M. Felix and M. H. Jimenez, *J. Chromatogr.*, 89 (1974) 361.
27 E. G. G. Lindeberg, *J. Chromatogr.*, 117 (1976) 439.
28 H. Nakamura and J. J. Pisano, *J. Chromatogr.*, 121 (1976) 79.