Journal of Chromatography, 566 (1991) 223–227
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5798

Short Communication

Simple and sensitive determination of plasma N$^{\tau}$-methylhistidine by high-performance liquid chromatography using pre-column derivative formation with *o*-phthalaldehyde–2-mercaptoethanol

TAKASHI NAGASAWA*, TADASHI SAKAI and RYOJI ONODERA

Faculty of Agriculture, Miyazaki University, Miyazaki-shi, Miyazaki 889-21 (Japan)

(First received November 15th, 1990; revised manuscript received December 21st, 1990)

ABSTRACT

A simple, rapid and sensitive assay method for plasma N$^{\tau}$-methylhistidine by isocratic high-performance liquid chromatography has been developed. The deproteinized plasma was treated with *o*-phthalaldehyde–2-mercaptoethanol. The derivatives were separated on a LiChrospher 100 RP-18 column within 10 min. The detection limit for N$^{\tau}$-methylhistidine was 0.5 pmol. The plasma N$^{\tau}$-methylhistidine content of beef cattle and dairy cows was 0.038 ± 0.004 and 0.017 ± 0.002 nmol/ml/kg, respectively.

INTRODUCTION

The amino acid, N$^{\tau}$-methylhistidine (3-methylhistidine; τMHis), is present in actin and myosin, which are major proteins in skeletal muscle [1]. Urinary τMHis is known to be a good index of skeletal msucle protein degradation, since it is not reutilized for protein synthesis [2,3] and is quantitatively excreted in rats [3], cattle [4] and humans [5]. Numerous studies have been performed to measure the rate of muscle protein degradation from urinary τMHis. Recently attempts have been made to evaluate muscle protein degradation by measuring blood plasma τMHis [6,7]. However, it seems to be necessary to develop specific analytical methods for plasma τMHis [8–12], because the concentration of plasma τMHis is much lower than that of other amino acids.

This paper describes a simple, rapid and sensitive method for the determination of τMHis in cattle plasma by high-performance liquid chromatography (HPLC), using *o*-phthalaldehyde–2-mercaptoethanol (OPA–2-ME) pre-column derivatization.

0378-4347/91/$03.50 © 1991 Elsevier Science Publishers B.V.

EXPERIMENTAL

Equipment

A Model 880PU pump unit (Japan Spectroscopic, Tokyo, Japan) with a SSC-E1E 005 injector (Senshu Science, Tokyo, Japan) was used. Chromatographic separations were performed using a LiChrospher 100 RP-18 column (250 mm × 4 mm I.D., Kanto Chemical, Tokyo, Japan). The column eluent was monitored using 340 nm excitation and 455 nm emission wavelengths by a Model 721FP fluorescence detector (Japan Spectroscopic).

Materials

All reagents were of the highest purity available. Histidine, OPA and amino acid standard solution (Type H; mixture of seventeen amino acids and ammonium chloride) were purchased from Wako (Osaka, Japan). N^{π}-Methylhistidine (πMHis) and τMHis were obtained from Behring Diagnostics (La Jolla, CA, U.S.A.).

Samples

Plasma (heparinized) were collected from five Japanese Black beef cattle weighing *ca.* 200 kg and six dairy cows (Holstein) weighing *ca.* 300 kg. All samples collected were kept at −80°C until analysis.

Methods

A 1-ml volume of heparizined plasma was deproteinized with an equal volume of 20% (w/v) trichloroacetic acid (TCA). TCA was extracted with 2 ml of diethyl ether from 1 ml of the supernatant, and the sample was then made up to 5 ml with water for reaction with OPA.

The OPA reagent was prepared by dissolving 25 mg of OPA in 0.5 ml of methanol and adding 1.5 ml of 0.4 *M* borate buffer (pH 12.0). To this mixture, 25 μl of 2-ME were added.

The samples for HPLC were prepared by adding 10 μl of the OPA reagent to 0.2 ml of filtered sample. The solution was mixed thoroughly for 10 s, and 5 μl were injected into the HPLC column 1 min after mixing. The mobile phase consisted of 13% (v/v) acetonitrile in 50 m*M* sodium acetate buffer (pH 5.0). The flow-rate was 1.5 ml/min.

RESULTS AND DISCUSSION

Fig. 1A shows a typical chromatogram of 5 pmol each of τMHis, πMHis and His. The separation was complete when 13% acetonitrile was present in the mobile phase. When the acetonitrile concentration was increased above 13%, τMHis was eluted more rapidly. However, the resolution from τMHis was incomplete. As shown in Fig. 1B, τMHis was separated from the standard amino acids.

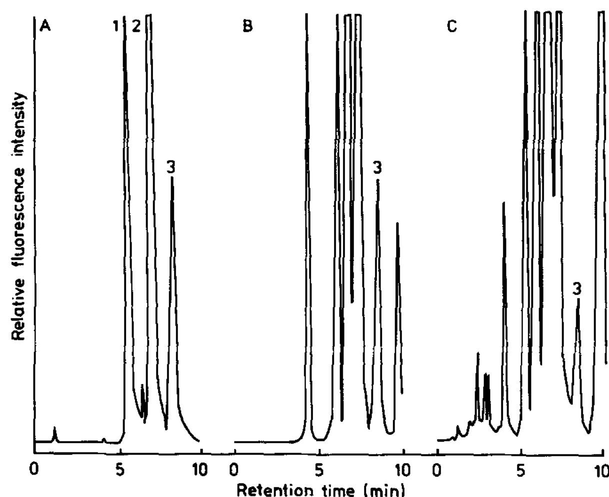

Fig. 1. Chromatograms of (A) a standard solution (1 nmol/ml each) of His (1), πMHis (2) and τMHis (3), (B) a standard solution of an amino acid mixture and τMHis and (C) a plasma sample from dairy cow (sample corresponding to 0.5 μl of plasma).

The relation between the fluorescence intensity and the concentration was linear from 0.5 to 500 pmol of τMHis. Generally, the OPA reagent is mixed with an equal volume of the sample solution [13]. In the present method, the volume of the OPA reagent was only 5% of that of the sample solution. Because of the instability of amino acid-OPA derivatives [9,14], the area of τMHis peak decreased to 90% of the initial area 10 min after the reaction was started. The reproducibility of the peak area of τMHis was 1–3% (triple determinations).

Fig. 1C shows the chromatogram of the TCA-soluble fraction of diary cow plasma. τMHis was clearly separated within 10 min from other peaks, under isocratic conditions used. The recovery of τMHis added to plasma of beef cattle was 94.5 ± 1.9% (n = 5).

Since little reabsorption and efficient clearance of τMHis by the kidney [15] increases urinary τMHis relative to other amino acids, measurement of urinary τMHis is easy and has been well described [16–20]. In contrast, plasma τMHis is very low, less than 20 nmol/ml of plasma [6–8], and it is difficult to separate τMHis from other amino acids. There are several HPLC methods available for the determination of plasma τMHis [7,10–12]. These methods required special equipment, such as a gradient maker or sample processors. In the present method only simple equipment and a single solvent was used.

Table I shows plasma τMHis concentrations in beef cattle and dairy cows. These values are slightly lower than those reported by Blum *et al.* [6] 10–20 nmol/ml. The τMHis concentrations were different between individual animals, but they were close when expressed on the basis of body weight (cattle, 0.038 ± 0.004 nmol/ml/kg; dairy cows, 0.017 ± 0.002 nmol/kg/kg). Recently, plasma τMHis values have been used as an index of muscle protein degradation instead

TABLE I

τMHis CONTENT IN PLASMA OF BEEF CATTLE AND DAIRY COWS

	Body weight (kg)	τMHis content	
		nmol/ml of plasma	nmol/ml/kg
Beef cattle			
No. 909	181	6.14	0.0339
No. 910	249	9.19	0.0369
No. 911	222	7.69	0.0346
No. 912	212	8.25	0.0390
No. 915	238	10.58	0.0446
Mean ± S.D.	220 ± 26	8.4 ± 1.7	0.038 ± 0.004
Dairy cows			
No. 1	313	4.90	0.0157
No. 2	359	5.33	0.0148
No. 3	284	4.87	0.0171
No. 4	318	5.25	0.0165
No. 5	286	4.31	0.0151
No. 6	303	6.05	0.0200
Mean ± S.D.	311 ± 27	5.1 ± 0.6	0.017 ± 0.002

of urinary τMHis [6,7]. When muscle protein degradation is evaluated from urinary τMHis, 24-h urine is required. Collection of 24-h urine is difficult in large animals, but collection of plasma is much easier. Furthermore, plasma τMHis can be indicative of acute changes of muscle protein degradation [12]. Plasma τMHis values measured by this method are accurate. Also the proposed method will enable the measurement of lower concentrations of τMHis from perfused hindquarters [21–24] or incubated muscle [25,26].

ACKNOWLEDGEMENTS

We thank Dr. Y. Yamauchi, Japan Spectroscopic Co. Ltd., for helpful discussions and Dr. H. Katayama, Associate Professor of Miyazaki University for technical advice on animal handling.

REFERENCES

1 A. M. Asatoor and M. D. Armstrong, *Biochem. Biophys. Res. Commun.*, 26 (1967) 168.
2 V. R. Young, B. S. Baliga, S. D. Alexis and H. N. Munro, *Biochim. Biophys. Acta*, 199 (1970) 297.
3 V. R. Young, S. D. Alexis, B. S. Baliga and H. N. Munro, *J. Biol. Chem.*, 247 (1972) 3592.
4 C. I. Harris and G. Milne, *Br. J. Nutr.*, 45 (1981) 411.
5 C. L. Long, L. N. Haverberg, V. R. Young, J. M. Kenney, H. N. Munro and J. W. Greiger, *Metabolism*, 24 (1975) 929.
6 J. W. Blum, T. Reding, F. Jans, M. Wanner, M. Zemp and K. Bachmann, *J. Dairy Sci.*, 68 (1984) 2580.

7 G. A. Quresh, A. Gutierrez and J. Bergström, *J. Chromatogr.*, 374 (1986) 363.
8 S. J. Wassner, J. L. Schlitzer and J. B. Li, *Anal. Biochem.*, 104 (1980) 284.
9 K. Bachmann, R. Galeazzi, A. Millet and A. G. Burger, *Metabolism*, 33 (1984) 107.
10 G. A. Quresh, S. van den Berg, A. Gutierrez and J. Bergström, *J. Chromatogr.*, 297 (1984) 83.
11 E. Anderson, E. Håkanson, J. Larsson and J. Mårtensson, *J. Chromatogr.*, 414 (1987) 174.
12 H. M. H. van Eijk, N. E. P. Deutz, A. J. M. Wagenmakers and P. B. Soeters, *Clin. Chem.*, 36 (1990) 556.
13 J. R. Benson and P. E. Hare, *Proc. Natl. Acad. Sci. U.S.A.*, 72 (1975) 619.
14 P. Kucera and H. Umagat, *J. Chromatogr.*, 255 (1983) 563.
15 V. R. Young and H. N. Munro, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 37 (1978) 2291.
16 N. Nishizawa, T. Noguchi, S. Hareyama and R. Funabiki, *J. Chromatogr.*, 151 (1978) 424.
17 L. C. Ward, M. Miller and S. Hawgood, *J. Chromatogr.*, 223 (1981) 417.
18 A. J. Murray, F. J. Ballard and F. M. Tomas, *Anal. Biochem.*, 116 (1981) 537.
19 P. E. Minkler, S. T. Ingalls, R. L. Griffin and C. L. Hoppel, *J. Chromatogr.*, 413 (1987) 33.
20 K. Hayashi, Y. Maeda, M. Toyomizu and Y. Tomita, *J. Nutr. Sci. Vitaminol.*, 33 (1987) 151.
21 T. Nagasawa, M. Kadowaki, T. Noguchi and H. Naito, *Agric. Biol. Chem.*, 46 (1982) 3023.
22 M. Kadowaki, T. Nagasawa, T. Hirata, T. Noguchi and H. Naito, *J. Nutr. Sci. Vitaminol.*, 31 (1985) 431.
23 A. G. Kayali, V. R. Young and M. N. Goodman, *Am. J. Physiol.*, 252 (1987) E621.
24 M. Kadowaki, N. Harada, S. Takahashi, T. Noguchi and H. Naito, *J. Nutr.*, 119 (1989) 471.
25 M. N. Goodman, *Biochem. J.*, 241 (1987) 121.
26 P.-O. Hasselgren, M. Hall-Angerås, U. Angerås, D. Benson, H. J. James and J. E. Fischer, *Biochem. J.*, 267 (1990) 37.