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The Determination of *m*-Tyrosine in Human Plasma by High Performance Liquid Chromatography

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A fluorometric detector and a voltammetric detector combined with a high performance liquid chromatograph were applied to the analysis of *m*-tyrosine, *p*-tyrosine and phenylalanine in normal human plasma.

The mean value of plasma *m*-tyrosine, which has not previously been detected in mammals, was about 0.28 ± 0.05 μg/ml. The mean plasma concentrations of *p*-tyrosine (9.82 ± 1.11 μg/ml) and phenylalanine (7.34 ± 1.47 μg/ml) were in good accord with values reported by other authors.

Keywords——human plasma; *m*-tyrosine; *p*-tyrosine; phenylalanine; high performance liquid chromatography; fluorimetry; voltammetry

The hydroxylation of phenylalanine to tyrosine (*p*-tyrosine) by phenylalanine hydroxylase is well known as the main metabolic pathway.[1,2] In addition, Tong *et al.*[3] and Ishimitsu *et al.*[4] recently reported the formation of *m*-hydroxyphenylalanine (*m*-tyrosine) from phenylalanine *in vitro* by beef adrenal and rat brain preparations, respectively.

On the other hand, many authors have reported the presence of *m*-tyramine[5] and *m*-hydroxyphenylacetic acid[6] in human urine, and it has recently been demonstrated that all of these compounds can be derived from *m*-tyrosine in the human body.[7,8]

The above findings suggest that *m*-tyrosine may be formed in mammals. However, the existence of *m*-tyrosine in mammals has not yet been reported.

This paper describes the determination of *m*-tyrosine in human plasma by high performance liquid chromatography (HPLC).

Experimental

Apparatus——(A) A Hitachi 635 high speed liquid chromatograph and a Hitachi 650-10S fluorimetric detector were used.

(B) A Yanagimoto L-2000 high speed liquid chromatograph and a Yanagimoto VMD 101 voltammetric detector were used.

Chromatographic Conditions——(A) Fluorescence: Flow rate, 0.6 ml/min; fluorimeter sensitivity, 1.0; recorder range, 0.2 V. The other chromatographic conditions were as described in our previous paper.[9]

(B) Voltammetry (VMD): Yanapak ODS was packed in a 4.0×250 mm i.d. stainless steel column; column temperature, 25°C; mobile phase, 0.1 M phosphate buffer (pH 3.1) and 16% methanol in 0.1 M phosphate buffer (pH 3.1); flow rate, 0.56 ml/min; applied potential, 0.90 V *vs.* Ag/AgCl.

Plasma——The subjects were 10 persons (5 men, 5 women) of our college staff and students. The blood samples (venous blood) were obtained from the subjects between 10 and 11 a.m. A 12 ml sample of blood was collected into a syringe with 0.1 ml of sodium heparin (1000 U/ml) and centrifuged immediately at $1000 \times g$ for 5 min at 4°C. A 2 ml sample of plasma was deproteinized by the addition of 0.5 ml of 1.0 M trichloroacetic acid. The plasma was centrifuged at $10000 \times g$ for 10 min, and the supernatant was used.

Results and Discussion

Determination of phenylalanine and hydroxyphenylalanines of plasma samples from normal persons was carried out by systematic quantitative HPLC analysis with fluorometric and voltammetric detectors as described in "Experimental." Typical chromatograms are shown in Fig. 1. These chromatographic analyses indicated the presence of a significant

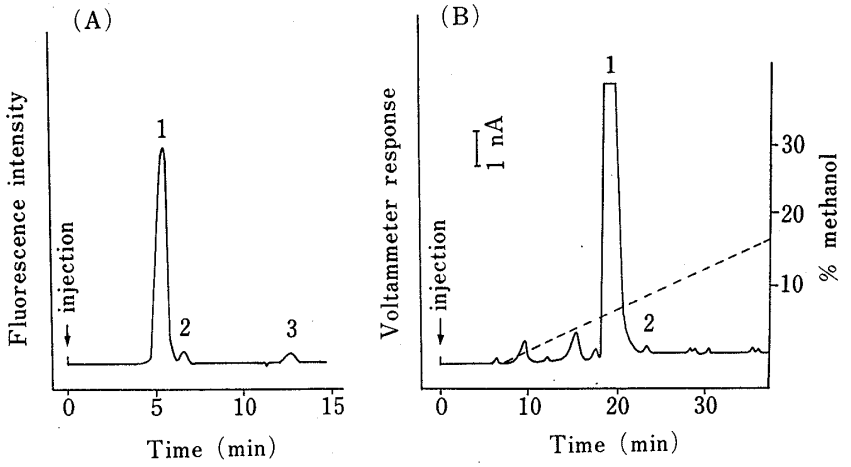

Fig. 1. High Performance Liquid Chromatograms of Human Plasma obtained with a Fluorometric Detector (A) and a Voltammetric Detector (B)

Injection: One μl (A) or 5 μl (B) of the sample as described in Methods was injected into the HPLC machine.
Elution: For method B, 0.1 M phosphate buffer (pH 3.1) was used for the first 6 min, then a linear gradient of increasing methanol concentration in the above buffer was applied over 6—36 min. The other chromatographic conditions were as described in Experimental.
Peaks: 1=*p*-tyrosine; 2=*m*-tyrosine; 3=phenylalanine.

TABLE I. Contents of *m*-Tyrosine, *p*-Tyrosine and Phenylalanine in the Plasma (Fluorometric Detector)

Age	Sex[a)]	*m*-Tyrosine (μg/ml)	*p*-Tyrosine (μg/ml)	Phenylalanine (μg/ml)
34	M	0.26	11.42	6.74
28	M	0.37	10.01	7.62
21	M	0.29	9.73	10.93
19	M	0.30	9.59	7.39
18	M	0.24	8.71	6.05
22	F	0.18	9.36	7.21
21	F	0.21	9.16	6.67
21	F	0.30	8.12	5.29
18	F	0.30	11.65	8.03
18	F	0.30	10.45	7.52
Mean ± S.D.		0.28±0.05	9.82±1.11	7.34±1.47

a) M, male; F, female.

amount of *m*-tyrosine, besides *p*-tyrosine, in the plasma sample. *o*-Hydroxyphenylalanine, however, was not detected.

The amounts of *m*-tyrosine in plasma samples from 10 normal persons were assayed by the present methods. Table I gives the results obtained by the fluorometric method. *m*-Tyrosine levels were 0.28±0.05 μg/ml (range 0.37—0.18). No significant difference in the concentration of *m*-tyrosine was observed between the sexes.

The average values of plasma *m*-tyrosine thus obtained are not very different from these obtained by the voltammetric method, in which the chromatographic conditions differed from those used in the fluorometric method. The regression equation for *m*-tyrosine was $y=0.924x+0.039$, where y is the result obtained from VMD data and x is the result from the fluorometric data; the correlation coefficient (r) was 0.978. In addition, the mean values of phenylalanine and *p*-tyrosine in these subjects were determined to be 7.34±1.47 μg/ml (range 10.93—5.29) and 9.82±1.11 μg/ml (range 11.65—8.12), respectively, by using the fluorometric

method (Table I). The values obtained in this experiment are similar to those described by many authors.[10] These results indicate that the plasma *m*-tyrosine level of 0.28±0.05 μg/ml detected in this experiment is a reliable value.

We believe that the present paper is the first report of the determination of *m*-tyrosine in human plasma.

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