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Formation of 3-Hydroxy-4-methoxyphenylalanine from 3,4-Dihydroxyphenylalanine by Rat Liver Homogenate

The O-methylation of 3,4-dihydroxyphenylalanine with rat liver homogenate was investigated in the presence of S-adenosylmethionine and $MgCl_2$ *in vitro*. By catechol-O-methyltransferase, it was found that 3,4-dihydroxyphenylalanine was methylated to 3-hydroxy-4-methoxyphenylalanine.

Keywords——enzymatic methylation of 3,4-dihydroxyphenylalanine; 3-methoxy-4-hydroxyphenylalanine; 3-hydroxy-4-methoxyphenylalanine; rat liver; high-performance liquid chromatography

The methylation of 3,4-dihydroxyphenylalanine (DOPA) by catechol-O-methyltransferase (COMT) to 3-methoxy-4-hydroxyphenylalanine (3-O-methylDOPA) is known as the main metabolite.[1,2] However, the methylation to 3-hydroxy-4-methoxyphenylalanine (4-O-methylDOPA) in mammalian tissues has not been found. In this communication, it would

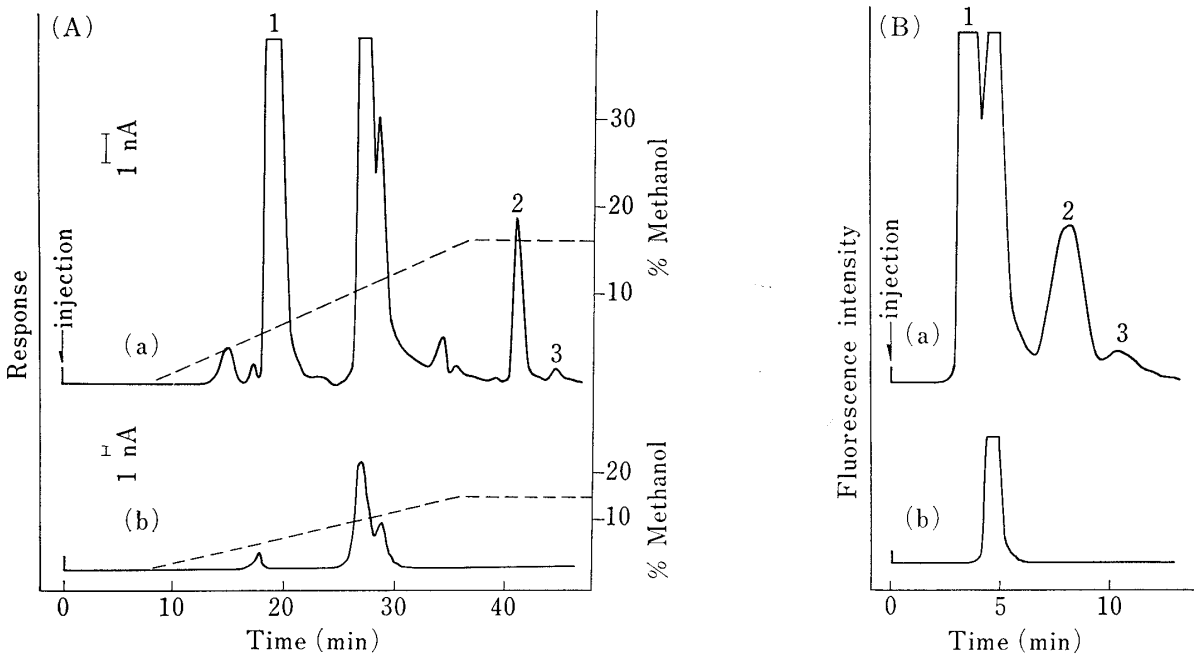

Fig. 1. High Performance Liquid Chromatograms of the Reaction Mixture equipped with a Voltammetry Detector (A) and a Fluorimetric Detector (B)

Injection sample: (a) After reaction of 3,4-dihydroxyphenylalanine with rat liver homogenate, S-adenosylmethionine and $MgCl_2$ for 30 min under the conditions described in the text. Injection sample: (b) Same as (a), except of 3,4-dihydroxyphenylalanine.

Injection: (A) Ten μl of the sample prepared as described in the text was analyzed by HPLC. (B) Fifty μl of the sample.

HPLC conditions: (A) Yanapak ODS was packed in 4.0×250 mm i.d. stainless steel column; column temperature, at room temperature; mobile phase, (1) 0.1 M phosphate buffer (pH 2.8) and (2) 16% methanol in 0.1 M phosphate buffer (pH 2.8); flow rate, 0.56 ml/min; applied potential, 0.90 V *vs.* Ag/AgCl. (B) Hitachi #3011-C resin was packed in 2.1×500 mm i.d. stainless steel column; column temperature, 45°; mobile phase, a mixture of equal volumes of 0.025 M sodium acetate and 0.05 M acetic acid; flow rate, 0.8 ml/min; detection, excitation at 282 nm and emission at 322 nm.

Peaks: 1=3,4-dihydroxyphenylalanine: 2=3-methoxy-4-hydroxyphenylalanine: 3=3-hydroxy-4-methoxyphenylalanine.

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be reported that DOPA is methylated to 3-O-methylDOPA and 4-O-methylDOPA with rat liver COMT.

Male Wister Imamichi rats (320—330 g) were killed by decapitation, and the liver was removed and homogenized with a waring blender in two part volume of cold isotonic KCl solution at pH 7.0. The COMT enzyme solution was concentrated by the modification of the method of Axelrod *et al.*[3] The initial reaction mixture contained 45 μmol of phosphate buffer (pH 7.7); 75 μmol of $MgCl_2$; 1.8 μmol of S-adenosylmethionine and 0.9 μmol of DOPA in a total volume of 2.95 ml. The reaction was initiated by the addition of enzyme. After incubation for 30 min at 37°, the reaction was terminated by the addition of 0.05 ml of 1.0 N HCl. After coagulation in boiling water and centrifugation down the precipitate, the supernatant of the reaction mixture was analyzed by the high-performance liquid chromatography (HPLC) equipped with a voltammetry and a fluorimetric detector. 4-O-MethylDOPA was synthesized by the method of Wilcox *et al.*[4]

The formation of 3-O-methylDOPA and 4-O-methylDOPA by the COMT enzyme solution from rat liver preparation in the presence of S-adenosylmethionine and $MgCl_2$ was studied in the case of presence of DOPA and absence, respectively. The elution profiles were illustrated in Fig. 1. The O-methylation of DOPA by the COMT enzyme solution was examined during 90 min from the initiation. From results of HPLC, the amount of 3-O-methylDOPA and 4-O-methylDOPA accumulated at 30 min followed with a steady increase over the next 60 min (not shown in Figure).

Table I. Enzymatic O-Methylation of 3,4-Dihydroxyphenylalanine by Rat Liver Homogenate

Modification to digest	3-O-MethylDOPA (μg/3.0 ml)	4-O-MethylDOPA (μg/3.0 ml)
Complete system[a]	23.82±0.21	1.77±0.06
–S-adenosylmethionine	N.D.*	N.D.*
$-MgCl_2$	1.41±0.08	0.21±0.05

a) Incubation mixture. see in the text for 30 minutes.
*: not detectable.
Values are means±S.D. of three experiments.

Table I shows the results by voltammetry detector for 30 min incubations of modyfying the standard incubation mixture used. It is seen that the O-methylation reaction of DOPA is essential for the addition of S-adenosylmethionine. The absence of $MgCl_2$ resulted in a decrement of the amount of O-methylDOPA to be found. Same values was obtained by fluorimetric detector of HPLC. From the results, a possibility was suggested that COMT in rat liver preparation is able to catalyze the methylation of DOPA to 4-O-methylDOPA *in vivo*.

A more detailed study is now under investigation in our laboratory, and will be the report of a future publication.

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