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Metabolic Difference between 3,4-Dihydroxyphenylpyruvic Acid (DHPP) and 3-Methoxy-4-hydroxyphenylpyruvic Acid (MHPP)

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3,4-Dihydroxyphenylpyruvic acid (DHPP) was mainly metabolized to L-3,4-dihydroxyphenylalanine (L-DOPA) through transamination, while 3-methoxy-4-hydroxy-phenylpyruvic acid (MHPP) was mainly metabolized to 3-methoxy-4-hydroxyphenyllactic acid (MHPL) through reduction. In order to explain the metabolic difference between these two aromatic α-keto acids related with DOPA, the metabolism of ^{14}C-labeled DHPP and MHPP was compared *in vitro*. There was no significant difference between the two α-keto acids in the initial rate of transamination to the corresponding amino acids using rat liver mitochondrial fraction. The K_m values to mitochondrial transaminase were calculated to be 8 mM and 6 mM for DHPP and MHPP, respectively. NADH-dependent activity for reducing DHPP and MHPP to the corresponding aromatic α-hydroxy acids was the highest in the heart followed by the kidney, muscle and liver and was not inhibited with oxamate (5 mM), the inhibitor of lactate dehydrogenase, indicating that aromatic α-keto acid reductase (AKAR) predominantly participates in this reaction. The initial rate of DHPP reduction with purified rat liver AKAR preparation was, however, only about 1/10 of that of MHPP reduction. The K_m value of DHPP (3.6 mM) was about two-fold larger than that of MHPP (1.5 mM), demonstrating a lower affinity of DHPP to AKAR than that of MHPP.

Keyword——3,4-dihydroxyphenylpyruvic acid; 3-methoxy-4-hydroxyphenylpyruvic acid; 3,4-dihydroxyphenylalanine; aromatic α-keto acid reductase; lactate dehydrogenase; transaminase; metabolism

During the course of our studies on the metabolism of D and L isomers of 3,4-dihydroxyphenylalanine (DOPA), we found two aromatic α-keto acids related with DOPA as metabolic intermediates, *i.e.*, 3,4-dihydroxyphenylpyruvic acid (DHPP)[2] and 3-methoxy-4-hydroxyphenylpyruvic acid (MHPP).[3] The former is formed from D-DOPA in rat kidney through oxidative deamination with D-amino acid oxidase and the latter is formed from L-3-methoxy-4-hydroxyphenylalanine (3-O-methylDOPA) in rat liver through transamination with tyrosine aminotransferase. The metabolism of these two α-keto acids differs significantly each other when administered to rats. Various metabolites of L-DOPA; dopamine, its conjugate, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were detected mainly in the urine after administration of DHPP-2-^{14}C.[4] On the other hand, HVA and 3-methoxy-4-hydroxyphenyllactic acid (MHPL) were detected mainly after administration of MHPP-2-^{14}C.[5] These results show that DHPP is first metabolized to L-DOPA by transamination *in vivo*, but MHPP is directly metabolized to HVA and MHPL without transamination to 3-O-methylDOPA. We have also found[5] that NADH-dependent reduction of MHPP to MHPL is catalyzed by lactate dehydrogenase (LDH) or aromatic α-keto acid reductase (AKAR) or

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2) H. Shindo and T. Maeda, *Chem. Pharm. Bull.*, (Tokyo), **22**, 1721 (1974).
3) H. Shindo, K. Nambu, T. Maeda, K. Hattori, K. Tanaka, M. Hioki, N. Miyakoshi and M. Tanaka, Seventh Symposium on Drug Metabolism and Action, Sapporo, Oct. (1975).
4) T. Maeda and H. Shindo, *Chem. Pharm. Bull.* (Tokyo), **25**, 1992 (1977); T. Maeda, N. Miyakoshi and H. Shindo, *Chem. Pharm. Bull.* (Tokyo), **25**, 2001 (1977).
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both and that AKAR is predominantly responsible for this change. In this paper, some additional experiments were performed in an attempt to give an explanation to the metabolic difference between these two α-keto acids.

Experimental

Determination of Transamination Rate of DHPP and MHPP to L-DOPA and 3-O-Methyl DOPA with Rat Liver Mitochondoria——The mitochondria mitochondrial fraction was obtained according to Johnson, *et al.*[6] Four male rats of Wistar-Imamichi strain weighing 160 g were bled to death and the liver was excised and homogenized in 9-fold volume of 0.25 M sucrose in a teflon homogenizer. The liver homogenate (25 ml) was laminated on the 20 ml of 0.34 M sucrose and centrifuged at 700 *g* for 10 min to remove cell debris and blood cells. The upper phase (0.25 M sucrose layer) was collected and centrifuged at 9000 *g* for 10 min. The pellet was resuspended and centrifuged again at 9000 *g* for 10 min. The washed pellet was suspended to the original volume and dialyzed against 0.25 M sucrose overnight.

Pyridoxal-5′-phosphate (0.1 mM, 0.1 ml), 0.5 ml of DHPP-2-^{14}C or MHPP-2-^{14}C (0.2, 0.5, 0.8, 1, 2, 3, 5, 10 and 15 mM) and 0.5 ml of sodium glutamate solution as an amino group donor (5 mM) were preincubated for 5 min at 37°. The substrates labeled at the carbonyl carbon were prepared from glycine-2-^{14}C as described previously.[4,5] The transamination reaction was started by the addition of 0.5 ml of mitochondrial fraction from rat liver. The reaction was terminated 15 min later by the addition of 1 N HCl followed by heat treatment (1 min at 100°). The mixture was centrifuged at 3000 rpm for 10 min and all the supernatant fluid was passed through the Dowex-1 anion exchange column (0.7×1 cm, chloride form). L-DOPA and 3-O-methyl DOPA were eluted completely by 2 ml of KCl (1 M). DHPP-2-^{14}C and MHPP-2-^{14}C were not eluted at all with this concentration of KCl. When the volume of eluate reached 10 ml, 1 ml aliquot was pipetted into the counting vial, added with 20 ml of toluene-ethanol liquid scintillator (500 ml toluene, 500 ml ethanol, 8 g PPO and 200 mg dimethylPOPOP) and measured the radioactivity in a Packard Liquid Scintillation Spectrometer Model 3380.

Determination of Reduction Rate of Pyruvate, DHPP and MHPP with Rat Tissues——Two male rats of Wistar-Imamichi strain weighing 180 g were bled to death and the heart, kidney, liver and femoral muscle were excised and homogenized in 9-fold volume of potassium phosphate buffer (pH 7.0, 50 mM) by Polytron (Kinematica Co. Ltd., Switzerland). The tissue homogenates were centrifuged at 9000 *g* for 20 min. NADH-dependent activity for reducing carbonyl groups of these α-keto acids was detected only in the 9000 *g* supernatant fraction in each tissue. To prevent transamination, endogenous amino group donors to α-keto acids were removed from the supernatant fraction by dialysis against the potassiun phosphate buffer overnight. Protein concentration of the dialysate was determined by Lowry's method.[7] The initial rate of disappearance of NADH which is the essential cofactor for the reduction of pyruvate, DHPP and MHPP to lactic acid and the corresponding aromatic α-hydroxy acids, respectively, was measured by tracing the decrease of optical density at 340 mμ in a Shimazu Multipurpose Spectrophotometer.[8] The potassium phosphate buffer (0.5 ml), 2.5 ml of the substrate solution (pyruvate: 1 mM, DHPP and MHPP: 2 mM) and 0.1 ml of NADH solution (0.1 mM) were added to the reference cell (total 3.1 ml). To the sample cell, the potassium phosphate buffer (0.4 ml), 2.5 ml of the substrate solution and 0.1 ml of NADH solution were added and then the reaction was started by addition of 0.1 ml of the dialyzed 9000 *g* supernatant fraction from each tissue homogenate (total 3.1 ml). In the experiment to examine the inhibitory effect of oxamate which is the specific inhibitor of lactate dehydrogenase (LDH),[5] 0.4 ml of oxamate (5 mM) was added to the sample cell instead of the potassium phosphate buffer.

Results and Discussion

The rate of transamination was compared between DHPP and MHPP using rat liver mitochondrial fraction. The mitochondrial fraction contains a high transaminase activity as well as the supernatant fraction, while it is completely devoid of the enzyme activity reducing DHPP and MHPP, *i.e.*, lactate dehydrogenase (LDH) and aromatic α-keto acid reductase (AKAR). Aromatic amino acid decarboxylase activity is also deficient in the mitochondrial fraction. Therefore, transamination of MHPP and DHPP was compared without affecting the equilibrium conditions of transamination system. As shown in Fig. 1, there was no significant difference in the initial rate of transamination between MHPP and DHPP. The K_m

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values were calculated to be 6 mM and 8 mM for MHPP and DHPP, respectively, demonstrating that two α-keto acids have a similar affinity to the transaminase.

NADH-dependent reduction of two α-keto acids and pyruvate was then compared. As shown in Fig. 2, the rate of reduction of pyruvate, *i.e.*, lactate dehydrogenase activity (LDH), was high in four tissues in the following order: muscle>heart>liver>kidney (open bar). The LDH activity was inhibited by oxamate (5 mM) over 97% (shaded bar). On the other hand, as shown in Fig. 2, the reducing activity for MHPP was 1/20—1/30 of that for pyruvate and was not inhibited by oxamate (—20%) in the every tissue. Furthermore, the distribution of

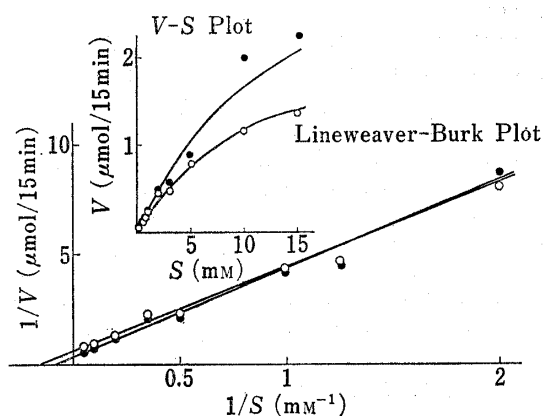

Fig. 1. Comparison of Transamination Rate of MHPP and DHPP with Rat Liver Mitochondria

K_m: DHPP (—○—): 8 mM, MHPP (—●—): 6 mM, Amino group donor: Sodium glutamate (5 mM).

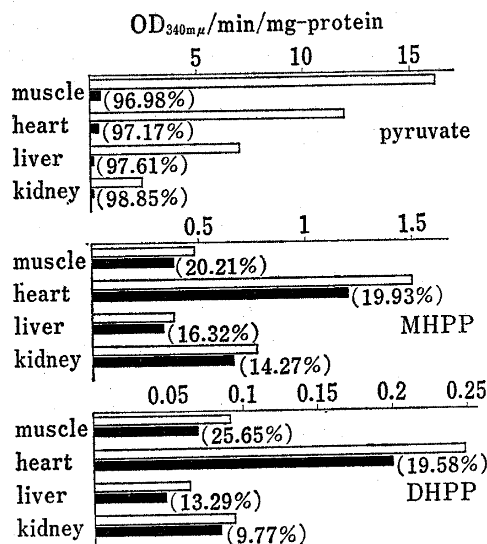

Fig. 2. Comparison of Reduction Rate of Pyruvate, MHPP and DHPP with Rat Tissues

Figures bracketed: % inhibition with oxamate (5 mM).
□: control, ■: with 5 mM oxamate.

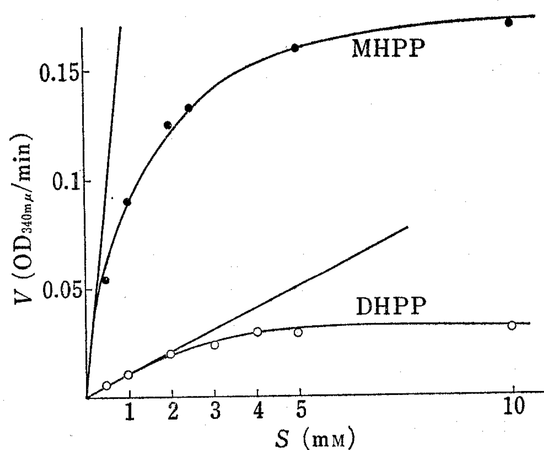

Fig. 3. Comparison of Reduction Rate of MHPP and DHPP with Purified Rat Liver AKAR (*V*–*S* Plot)

—●—: MHPP, —○—: DHPP, MHPP/DHPP=10.63, Protein: 136 μg.

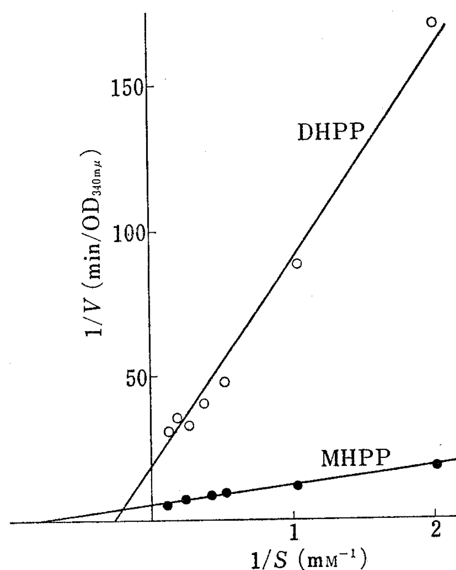

Fig. 4. Comparison of K_m Values of MHPP to Purified Rat Liver AKAR (Lineweaver-Burk Plot)

K_m: DHPP: 3.6 mM, MHPP: 1.5 mM, Protein: 136 μg.

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the activity among tissues agreed with that of AKAR,[9] being high in heart and kidney but low in muscle and liver. These results indicate that MHPP is reduced to MHPL mostly by aromatic α-keto acid reductase (AKAR), as discussed already in the previous paper.[5] The reducing activity for DHPP was found to be much lower than that of MHPP in the every tissue (Fig. 2). The tissue distribution of the activity, however, coincided well with that of MHPP and the activity was not inhibited by oxamate. These results indicate that AKAR participate predominantly also in the reduction of DHPP.

Subsequently, AKAR was purified about 10-fold from 105000 *g* supernatant fraction of rat liver by the procedure as reported previously.[5] The final AKAR preparation showed no LDH activity at all. Using this preparation, the initial rate of DHPP reduction was shown to be less than 1/10 of that of MHPP, as shown in Fig. 3. The K_m value of DHPP (3.6 mM) was about 2-fold larger than that of MHPP (1.5 mM), demonstrating a weaker affinity of DHPP to AKAR than MHPP (Fig. 4).

Considering from all these results in combination, the previous results[4,5] that MHPL was found in rat urine after administration of MHPP-2-^{14}C, while the corresponding metabolite of DHPP, 3,4-dihydroxyphenyllactic acid, was not found in the urine after administration of DHPP-2-^{14}C, can be explained well in the following may (Chart 1). DHPP is a poor substrate of AKAR and is mainly transaminated to L-DOPA, which is eliminated immediately from the equilibrium system by decarboxylation to dopamine. On the other hand, 3-O-methylDOPA is not decarboxylated easily,[10] and the transamination equilibrium is shifted far to MHPP side. Therefore, MHPP is mainly metabolized to MHPL since MHPP is a good substrate of AKAR. In fact, as described before,[4,5] over 80% of DHPP was immediately converted to L-DOPA and dopamine with rat liver homogenate, while only less than 30% of MHPP was converted to 3-O-methylDOPA even after a long incubation as 2 hr.

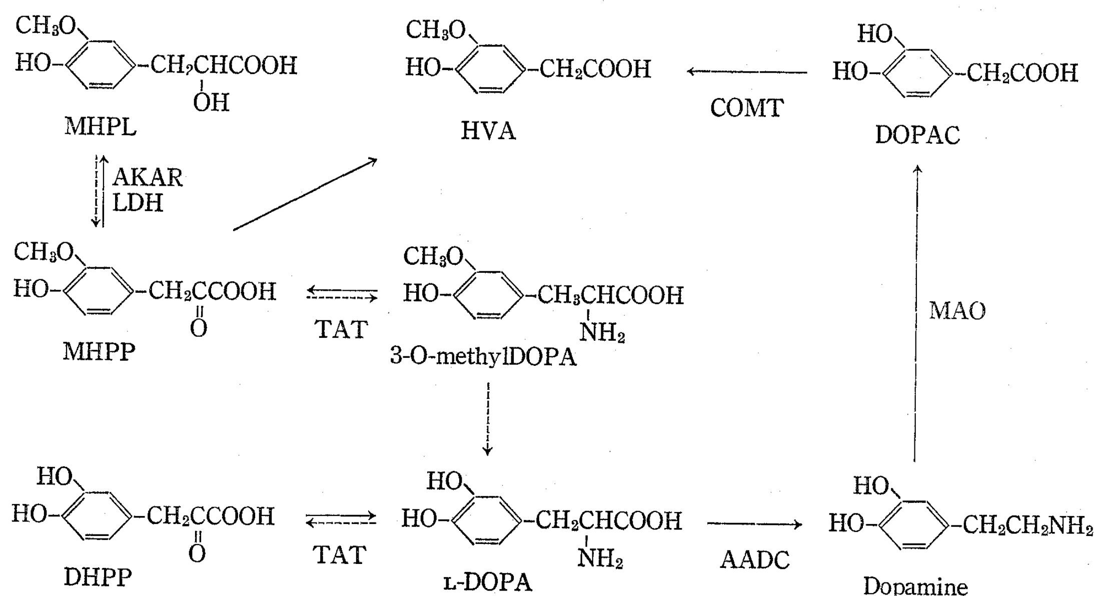

Chart 1. Main Metabolic Pathway of MHPP and DHPP in Rat

AADC: Aromatic amino acid decarboxylase, AKAR: Aromatic α-keto acid reductase, COMT: Catechol O-methyl transferase, LDH: Lactate dehydrogenase, MAO: Mono amine oxidase, TAT: Tyrosine amino transferase.
⟶: Main metabolic pathway, ⇢: Minor metabolic pathway.

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