Inhibition of Melanoma Tyrosinase by Fusaric Acid

Fusaric acid (5-butylpicolinic acid) was isolated during the screening of dopamine β-hydroxylase inhibitors from microbial origin. This compound was found to be a potent inhibitor of dopamine β-hydroxylase in vitro and in vivo, and a potent hypotensive agent to various mammals[1,2]. Fusaric acid had been reported to inhibit polyphenol oxidase from tomato plant tissues by BOSSI[3]. Since these enzymes contain copper, fusaric acid appears to be an inhibitor of copper enzymes. The inhibitory effect of fusaric acid on mammalian tyrosinase, which is also a copper protein, is described in this communication.

Harding-Passey mouse melanoma tissue was homogenized in 3 volumes of water, and the homogenate was centrifuged at $1{,}000 \times g$ for 10 min. The supernatant was used as enzyme. Three different methods were used to measure the inhibitory effect of fusaric acid on melanoma tyrosinase.

In the assay 1, the activity was measured by the oxidation of DOPA to dopachrome[4]. Incubation mixture contained: 800 µmoles of phosphate buffer, pH 6.9; 0.15 ml of the enzyme solution; 4 µmoles of DOPA; and water to 3.5 ml. The reaction was started by adding DOPA. Incubation was continued at 26 °C in a cuvette. DOPA was omitted for the sample in the reference cuvette. Increase in the absorbance at 480 nm was recorded in a Hitachi EPS-3T self-recording spectrophotometer with an attachment of an integrating sphere.

In the assay 2, the tyrosine hydroxylase activity of tyrosinase[5] was assayed by tritium release from L-tyrosine-3, 5-^{3}H. Incubation mixture contained: L-tyrosine-3, 5-^{3}H (4×10^4 cpm), 0.2 µmole; L-DOPA, 0.1 µmole; potassium phosphate buffer, pH 6.9, 200 µmoles; 0.1 ml of the 10-fold diluted supernatant as enzyme, and water to 1.0 ml. Incubation was carried out for 4 h at 30 °C. The tritiated water was collected by Dowex 50-H^+ column and counted in a liquid scintillation spectrometer.

In the assay 3, melanin formed from L-tyrosine-U-^{14}C was assayed[6]. Incubation mixture contained: L-tyrosine-U-^{14}C (3×10^4 cpm), 0.2 µmole; L-DOPA, 0.01 µmole; 0.1 ml of the 10-fold diluted supernatant as enzyme; penicillin G, 140 µg; potassium phosphate buffer, pH 6.9, 100 µmoles; and water to 1.0 ml. Incubation was carried out at 30 °C for 16 h. Melanin was isolated by filtrating through a membran filter and counted in a liquid scintillation counter.

As shown in the Table, fusaric acid inhibited melanoma tyrosinase at concentrations between 0.1 mM and 10 mM when the activity was measured by 3 different methods. When the activity was measured by the DOPA oxidation (assay 1), fusaric acid produced 100% inhibition at 10 mM, and 50% inhibition at 0.3 mM. A Lineweaver-Burk plots against the concentration of DOPA in the absence and presence of fusaric acid, 0.25 mM, showed that fusaric acid inhibits melanoma tyrosinase in competition to DOPA. Ki value of fusaric acid obtained was $2 \times 10^{-4} M$. The inhibiton of melanoma tyrosinase was pH-dependent. Inhibition by fusaric acid was more pronounced at pH 6.0 than at pH 6.9. These results are similar to the report by BOSSI[3] on the inhibition of polyphenol oxidase from tomato plant tissues by fusaric acid.

These results indicated that fusaric acid inhibits mammalian tyrosinase. However, the inhibitory effect of fusaric acid toward tyrosinase is weak when compared to its inhibition toward dopamine β-hydroxylase. The latter enzyme was inhibited by 50% at a concentration of $3 \times 10^{-8} M$ of fusaric acid[2]. Fusaric acid inhibits dopamine β-hydroxylase in competition with a cofactor, ascorbic acid, whereas it inhibits tyrosinase in competition with a substrate, DOPA. It should be noted, however, that DOPA was identified as cofactor for the tyrosine hydroxylase activity of melanoma tyrosinase[7]. The inhibitory mechanism of fusaric acid on dopamine β-hydroxylase and tyrosinase may be due to the chelating action on the copper contained in the enzymes. However, the present results suggest that fusaric acid is a specific inhibitor of dopamine β-hydroxylase in vivo, since it inhibits the enzyme at very low concentrations[8].

Zusammenfassung. Nachweis, dass Fusarinsäure die DOPA-Oxydation durch Melanom-Tyrosinase kompetitiv mit DOPA hemmt.

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Fusaric acid (M)	Tyrosinase activity (% of control)		
	DOPA oxidation	^{3}H release from L-tyrosine-3, 5-^{3}H	Melanin formation from L-tyrosine-U-^{14}C
—(control)	100	100	100
1×10^{-5}	99	100	87
1×10^{-4}	78	100	89
1×10^{-3}	27	60	41
1×10^{-2}	0	15	2

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