Tyrosine Formation from Phenylalanine by Ultraviolet Irradiation

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When phenylalanine was irradiated at ultraviolet (UV) light, *p*-tyrosine, *m*-tyrosine and *o*-tyrosine were identified as hydroxylated products. From *p*-tyrosine and *m*-tyrosine, the formation of L-3,4-dihydroxyphenylalanine (DOPA) was observed. The hydroxylation of phenylalanine was prevented by radical scavengers, *e.g.*, catalase, superoxide dismutase, sodium thiocyanate, mannitol, potassium iodide and thiourea. Replacement of air with nitrogen gas prevented the hydroxylation, but did not depress it completely. The addition of H_2O_2 increased significantly the hydroxylation of phenylalanine. These results suggest that the hydroxylation of phenylalanine by UV irradiation may be caused by ·OH formed during the decomposition of H_2O.

Keywords UV irradiation; phenylalanine; *p*-tyrosine; *m*-tyrosine; *o*-tyrosine; hydroxyl radical

It is well known that aromatic amino acids such as phenylalanine, 4-hydroxyphenylalanine (*p*-tyrosine) and tryptophan are photooxidized by near-ultraviolet (UV) light to colored products that are bound very tightly to protein amino groups.[1] In 1971, Pirie reported that sunlight in the presence of air caused oxidative cleavage of the indole rings of tryptophan to give *N'*-formylkynurenine.[2] It has also been observed that UV irradiation of phenylalanine and *p*-tyrosine result in formation of the hydroxylated products, *p*-tyrosine and L-3,4-dihydroxyphenylalanine (DOPA).[3] However, although these hydroxylated products have been analyzed by paper partition chromatography and a colorimetric method, detailed analysis of the hydroxylation reaction has not yet been carried out.

It has been reported that active oxygen species such as ·OH, O_2^- and H_2O_2 are produced from photolysis of H_2O under an aerobic condition.[4,5] Attack of ·OH radicals upon aromatic compounds under physiological conditions results largely in the formation of hydroxylated products.[6] We have also demonstrated with chemical systems that ·OH can mediate the formation of *p*-tyrosine, 3-hydroxyphenylalanine (*m*-tyrosine) and 2-hydroxyphenylalanine (*o*-tyrosine) from phenylalanine, but that H_2O_2, O_2^-, and $^1O_2$ can not.[7]

In this paper, we examined in detail the hydroxylation of phenylalanine by UV irradiation.

Experimental

Materials The following chemicals were purchased from Sigma Chemical Company: L-phenylalanine, L-*p*-tyrosine, DL-*m*-tyrosine, DL-*o*-tyrosine, DOPA, catalase from bovine liver and superoxide dismutase from bovine blood. All other chemicals used were of the highest purity commercially available.

UV Light Irradiation An aqueous solution containing phenylalanine (50 μmol), *p*-tyrosine (5 μmol), or *m*-tyrosine (5 μmol) in 5 ml of 0.1 M phosphate buffer was irradiated. After irradiation, 0.5 ml of hydrochloric acid (0.5 M) was added, and 100 μl of the mixture was directly injected into high-performance liquid chromatography (HPLC). A 15 W UV lamp, UVGD-15 (UVP, Inc. San Gabriel, CA, U.S.A.), that has maximal energy output at 254 nm at which the intensity is 440 μW/cm² at a distance of 15 cm, was used as the UV source. This lamp emits UV light with a wavelength between 220 and 280 nm. Phenylalanine, *p*-tyrosine and *m*-tyrosine solutions were placed in glass tubes (6 × 4.2 cm i.d.), and irradiated at 37 °C from a distance of 14 cm with a UV lamp. Nitrogen (N_2) gas was used after passing through a column of 2% pyrogaroll in 1 M sodium hydroxide to remove any trace of O_2.

Analysis of Reaction Products HPLC: A Hitachi 635 high-performance liquid chromatograph was used with a Hitachi F-1000 fluorescence spectromonitor. The separations were achieved on a C_{18} reversed-phase column (Cosmosil ODS, particle size 5 μm, 250 × 4.6 mm i.d.; Nakaraitesque, Ltd., Kyoto). The eluent was 0.17 M acetic acid containing 0.17 M sodium chloride at a flow rate of 0.8 ml/min. The fluorescence was monitored with excitation at 275 nm and emission at 305 nm for *p*-tyrosine, *m*-tyrosine and *o*-tyrosine, and excitation at 280 nm and emission at 318 nm for DOPA. Peak areas were calculated by a Hitachi D-2000 chromato-integrator.

Amino Acid Analyzer: A Hitachi 835 amino acid analyzer was used. The eluent used was MCI-buffer (Mitsubishi Chemical Industries Ltd., Tokyo) for usual analysis.

Results and Discussion

Hydroxylation of Phenylalanine by UV Irradiation A solution of phenylalanine in phosphate buffer (pH 6.5) was irradiated with UV light, and three isomers (*p*-tyrosine, *m*-tyrosine and *o*-tyrosine) were found to have been formed. A typical chromatographic pattern of the solution is shown in Fig. 1. No significant hydroxylation occurred without irradiation. The peaks of *p*-, *m*- and *o*-tyrosines were identified by two methods of chromatography. First, they were identified on the basis of HPLC retention behavior and co-injection with the reference compounds. Then, the effluent corresponding to each peak was collected and subjected to ion-exchange chromatography using an amino acid analyzer. *m*-Tyrosin, *p*-tyrosine and *o*-tyrosine emerged from this system at 35, 36 and 37 min, respectively.

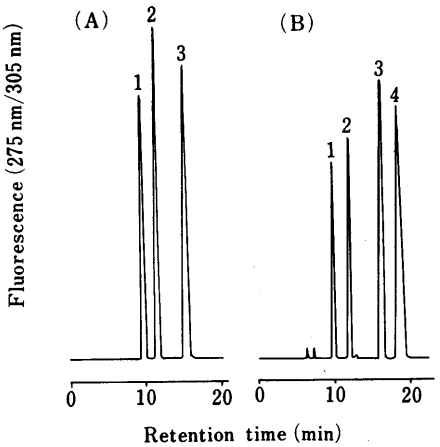

Fig. 1. High-Performance Liquid Chromatogram of the Irradiation Mixture

(A) 100 μl of solution containing approximately 5.5 nmol each of standard compounds was used for HPLC. (B) The solution, containing phenylalanine (50 μmol) in 5 ml of 0.1 M phosphate buffer (pH 6.5), was irradiated at 37 °C. After 3 h of irradiation, 0.5 ml of hydrochloric acid (0.5 M) was added. A 100 μl aliquot was directly injected into HPLC. Peaks: 1 = *p*-tyrosine; 2 = *m*-tyrosine; 3 = *o*-tyrosine; 4 = phenylalanine.

Table I. Time Course of the Hydroxylation of Phenylalanine by UV Irradiation

Irradiation time (h)	Tyrosines formed (nmol/5 ml)		
	p-	*m*-	*o*-
1	6	5	7
2	13	15	20
3	19	18	23
4	27	25	34

The solution, containing phenylalanine (50 μmol) in 5 ml of 0.1 M phosphate buffer (pH 6.5), was irradiated at 37 °C. After several times of irradiation, 0.5 ml of hydrochloric acid (0.5 M) was added, and tyrosines were determined as described in Experimental.

Table II. Time Course of the Hydroxylation of *p*-Tyrosine and *m*-Tyrosine by UV Irradiation

Irradiation time (h)	Irradiation of *p*-tyrosine	Irradiation of *m*-tyrosine
	DOPA formed (nmol/5 ml)	DOPA formed (nmol/5 ml)
1	5	70
2	10	127
3	12	157

The solution, containing *p*-tyrosine (5 μmol) or *m*-tyrosine (5 μmol) in 5 ml of 0.1 M phosphate buffer (pH 6.5), was irradiated at 37 °C. After several times of irradiation, 0.5 ml of hydrochloric acid (0.5 M) was added, and DOPA was determined as described in Experimental.

The retention time of each peak was identical with that of a corresponding authentic sample.

The hydroxylation was dependent on pH. The optimal hydroxylating reaction was observed at pH 6.5 in the pH range of 6 to 8; at 6, 7, 7.5 and 8, the rates were approximately 59, 71, 57 and 54%, respectively, of that at pH 6.5. Table I shows the time courses of the formation of *p*-, *m*- and *o*-tyrosines by UV irradiation; the formation increased with irradiation time. When *p*-tyrosine and *m*-tyrosine were used as a substrate, the formation of DOPA was detected (Table II). DOPA formation from *m*-tyrosine was much faster than that from *p*-tyrosine.

Effects of Various Substances on the Hydroxylation of Phenylalanine by UV Irradition The mechanism of ·OH formation by UV irradiation has been presented as follows[5,8]:

$$H_2O \xrightarrow{h\nu} \cdot OH + \cdot H \quad (1)$$

in the presence of oxygen,

$$\cdot H + O_2 \longrightarrow HO_2^- \longrightarrow O_2^- + H^+ \quad (2)$$

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2 \quad (3)$$

$$H_2O_2 + O_2^- \longrightarrow \cdot OH + OH^- + {}^1O_2 \quad (4)$$

$$H_2O_2 \xrightarrow{h\nu} 2 \cdot OH \quad (5)$$

We examined the effect of oxygen and radical scavengers on hydroxylation of phenylalanine to obtain information about the reactive species responsible for hydroxylation (Table III). ·OH scavengers such as sodium thiocyanate, mannitol, potassium iodide and thiourea effectively prevented tyrosine formation. The addition of superoxide dismutase (SOD) and catalase also reduced the rate of tyrosine formation. Denatured SOD or catalase, which had been inactivated by boiling, had no effect on the hydroxylation of phenylalanine. When N_2 gas was bubbled through the irradiation solution, the hydroxylation of phenylalanine was reduced significantly, but not depressed to less than 33% of that under aerobic conditions. Furthermore, when N_2 gas was bubbled through the reaction, no inhibitory effect of SOD or catalase was observed. The present results may also support that ·OH formed (Eq. 1) by the decomposition of H_2O by UV irradiation is responsible for the hydroxylation of phenylalanine. On the contrary, addition of H_2O_2 was found to accelerate the hydroxylation of phenylalanine, and the amount of tyrosine formation in the presence of H_2O_2 was approximately 95 times greater than that in the absence of H_2O_2. These results suggest that the hydroxylation of phenylalanine by UV irradiation in aqueous solution may be caused partly by ·OH formed secondarily from the decomposition of H_2O_2 generated in the irradiated solution.

Table III. Effects of Various Substances on Tyrosine Formation from Phenylalanine by UV Irradiation

Substance added	Concentration (mM)	Tyrosines (*o*-, *m*- and *p*-) formed (nmol/5 ml)	
		In air	In N_2
None		48	16
+Hydrogen peroxide	200	4537	3488
+Sodium thiocyanate	200	3	
+Mannitol	200	35	
+Potassium iodide	200	6	
+Thiourea	200	9	
+Catalase	20 μg/ml	23	15
+Superoxide dismutase	20 μg/ml	28	16
+Boiled catalase[a]	20 μg/ml	46	
+Boiled superoxide dismutase[a]	20 μg/ml	47	

The mixtures contained phenylalanine (50 μmol) and the specified amount of various substances in 5 ml of 0.1 M phosphate buffer (pH 6.5). After 2 h of irradiation under aerobic or anaerobic conditions, 0.5 ml of hydrochloric acid (0.5 M) was added, and tyrosines were then determined as described in Experimental. *a*) Heated for 10 min at 100 °C.

On the basis of the results obtained in the present experiments, we concluded that the hydroxylation of phenylalanine in aqueous solution was caused by ·OH radicals produced during the UV irradiation.

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