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Photoalkylation of Aromatic Amino Acids as a Model of Photochemical Modification of Protein with Chloroacetamide

TATSUO HAMADA and OSAMU YONEMITSU

Faculty of Pharmaceutical Sciences, Hokkaido University[1]

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On irradiation of tryptophan and tyrosine in aqueous solutions with the 2537 Å light in the presence of chloroacetamide, photoalkylations of both amino acids proceeded with the same quantum yield of 0.10. Quenching rate constants (k_q) of the fluorescences of tryptophan and tyrosine with chloroacetamide were determined to be 4.0—4.5×10^9 and $4.0 \times 10^9\ \text{M}^{-1}\text{sec}^{-1}$, respectively. Almost no difference between both k_q can explain the same reactivity of tryptophan and tyrosine. In an equimolar mixture, the reactivity of tryptophan was about 7 times that of tyrosine, and this reflects the ratio of extinction coefficients of both amino acids at 2537 Å. In a mixture of several amino acids, if the photolysis was stopped when tryptophan disappeared less than 20%, all amino acids except tryptophan were recovered almost quantitatively. Wavelength dependence of this photolysis was finally examined. On irradiation of the 303 nm light, quantum yield for disappearance of tryptophan was also 0.10, whereas that of tyrosine was almost nil.

Keywords——amino acid; photoalkylation; quantum yield; quenching of fluorescence; wavelength dependence

There are not so many applications of organic photochemistry to chemical modification of protein. Most of the research is concerned with dye-sensitized singlet oxygen oxidation of histidine, methionine, tryptophan and cystein, and only a few examples gave selective oxidation.[2] A unique photochemical modification of protein, which consisted in photolysis of diazoacetylated proteins, was published by Westheimer, *et al.*[3] However, a complex mixture generated by many reactions such as Wolf rearrangement, insertion into C–H bonds, cycloaddition to C–C double bonds and electrophilic substitution to oxygen of OH groups was obtained as was to be predicted from the reactivity of intermediate carbenes.

Since the photocyclization of N-chloroacetyltryptophan in aqueous solution was found,[4] this photoreaction has been extensively studied from viewpoints of its mechanism[5] and applications to organic synthesis[6] including intermolecular photoreactions between chloroacetamide and electron-rich aromatics, indole, phenol, anisole, *etc.*[7] Aromatics without electron donating groups were recovered unchanged under these photochemical conditions. A common mechanism involving an electron transfer from excited electron-rich aromatic rings to electron-deficient chloroacetamide has been proposed.[5]

When protein is irradiated in the presence of chloroacetamide, it is expected that only tryptophan and tyrosine residues having electron-rich aromatic rings are alkylated. In the

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[1] Location: *Kita-12, Nishi-6, Kita-ku, Sapporo, 060, Japan.*

[2] G. Jori, G. Galiazzo, and E. Scoffone, *Biochemistry*, **8**, 2868 (1969), and references cited therein.

[3] J. Shafer, P. Baronowsky, R. Laursen, F. Finn, and F.H. Westheimer, *J. Biol. Chem.*, **241**, 421 (1966); C.S. Hexter and F.H. Westheimer, *ibid.*, **246**, 3928 (1971).

[4] O. Yonemitsu, P. Cerrutti, and B. Witkop, *J. Am. Chem. Soc.*, **88**, 3941 (1966).

[5] *a*) T. McCall, G.S. Hammond, O. Yomenitsu, and B. Witkop, *J. Am. Chem. Soc.*, **92**, 6991 (1970); *b*) O. Yonemitsu, H. Nakai, Y. Okuno, S. Naruto, K. Hemmi, and B. Witkop, *Photochem. Photobiol.*, **15**, 509 (1972); *c*) S. Naruto, O. Yonemitsu, N. Kanamaru, and K. Kimura, *J. Am. Chem. Soc.*, **93**, 4053 (1971); *d*) S. Naruto and O. Yonemitsu, *Tetrahedron Letters*, **1975**, 3399.

[6] Y. Okuno and O. Yonemitsu, *Chem. Pharm. Bull.* (Tokyo), **23**, 1039 (1975), and references cited therein.

[7] *a*) S. Naruto and O. Yonemitsu, *Chem. Pharm. Bull.* (Tokyo), **20**, 2163 (1972); *b*) O. Yonemitsu and S. Naruto, *ibid.*, **19**, 1158 (1971).

present paper, the photochemical reactivity of each amino acid has been examined as a model experiment for the chemical modification of protein with chloroacetyl derivatives.

Experimental

Materials——Commercially available amino acids were used without further purification. Chloroacetamide was purified by twice recrystallizations from ethanol.

Samples for Irradiation——Three mM solutions of each amino acid and of mixture of amino acids containing chloroacetamide (30 mM) in 1/15 M phosphate buffer (pH 5.8) were prepared in volumetric flasks. Then 5.0 ml portions of these solutions were transferred to 15 mm$\phi \times$10 cm quartz tubes. The samples were degassed by three freeze-pump-thaw cycles and sealed at 10^{-5} Torr when necessary.

Irradiation of Samples and Quantum Yields——Irradiations were performed with the 2537 Å light from a 60 W low pressure mercury lamp (PIL-60, Eikosha, Osaka) on a merry-go-round apparatus (Rayonet MGR-500). All quantitative analyses for disappearance of amino acids were performed with an automatic amino acid analyzer (JEOL JL C-5 AH). Quantum yields for the disappearance of amino acids were determined relative to the formation of ferrous ion from a 12 mM potassium ferrioxalate solution as an actinometer[8] photolyzed simultaneously. The light intensities absorbed by the sample solutions were determined as shown in Table I and II.

Irradiation with a monochromatic light at various wavelengths was carried out with a spectro-irradiator (JASCO-FA). The light intensities were shown in Table III.

Absorption and Emission Spectra——Absorption spectra were obtained on 323 Hitachi Recording Spectrophotometer. Fluorescence spectra were measured by using a Hitachi Fluorescence Spectrophotometer MPF-2A.

Results and Discussion

A 3 mM solution of tryptophan in 1/15 M phosphate buffer (pH 5.8) was degassed and irradiated in the presence of ten-fold excess of chloroacetamide with a 60 W low pressure mercury lamp on a merry-go-round apparatus. After 5, 10, and 20 min irradiations, the recovered tryptophan was analyzed with an automatic amino acid analyzer. A quantum yield for the disappearance of tryptophan was measured relative to a 12 mM ferrioxalate actinometer irradiated simultaneously and calculated to be 0.10. As we reported previously, introduction of an acetamide group must have occurred at all positions in the indole ring.[7a] This is expected to become an advantage in cases of chemical modification of protein.

Similarly, tyrosine was irradiated and its quantum yield of disappearance was also 0.10. The photoreaction must have occurred at *ortho* positions with reference to the OH group in tyrosine.[7b]

No difference in reactivity (quantum yield) between tryptophan and tyrosine probably reflects similar electron-donating abilities and lifetimes of their singlet excited states. In fact,

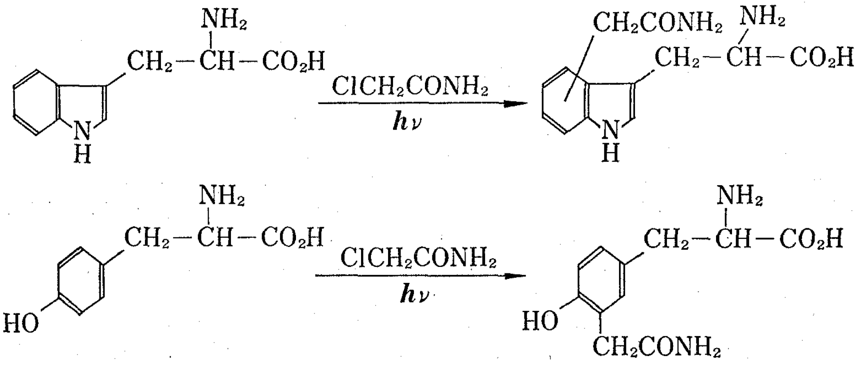

Chart 1

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8) C.G. Hatchard and C.A. Parker, *Proc. Roy. Soc.* (London) **A**, **235**, 518 (1956).

the fluorescences of tryptophan and tyrosine in water were quenched quite efficiently by chloroacetamide and the spectral distribution of the unquenched fluorescences remained unchanged. Linear Stern-Volmer plots were obtained as shown in Fig. 1, and $k_q\ \tau$ of tryptophan and tyrosine derived from these plots were 12.1 and 14.5 M^{-1}, respectively. Lifetimes of the fluorescences were already reported as 2.7—3.0 nsec for tryptophan[9] and 3.6 nsec for tyrosine.[10] Therefore, quenching rate constants (k_q) for tryptophan and tyrosine were calculated as 4.0—4.5 $\times 10^9$ and 4.0 $\times 10^9\ \text{M}^{-1}\ \text{sec}^{-1}$, respectively.[5] Almost no difference between both quenching rate constants can explain the same quantum yields for disappearance of both amino acids in the presence of chloroacetamide.

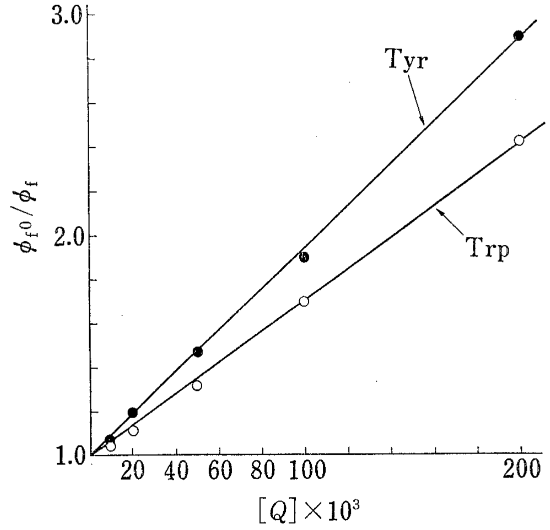

Fig. 1. Stern-Volmer Plots for the Quenching of Fluorescence of Tryptophan and Tyrosine by Chloroacetamide in Water

emission max. of Trp: 350 nm (excitation at 288 nm
emission max. of Tyr: 310 nm (excitation at 280 nm)

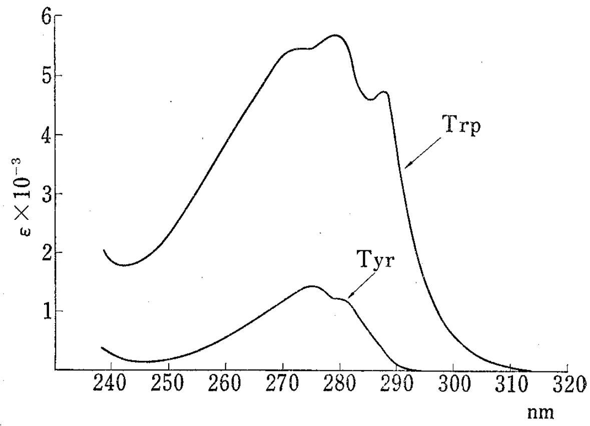

Fig. 2. Absorption Spectra of Tryptophan and Tyrosine in Water

Table I. Recovery (%) and Quantum Yield for Disappearance of Tryptophan and Tyrosine on Irradiation of their Equimolar Mixture in the Presence of Chloroacetamide[a]

	Recovery, % (quantum yield)	
	Control[b]	Presence of chloroacetamide[c]
Trp	100	65(0.10)
Tyr	101	95(0.014)

a) A 3 mM solution of Trp and Tyr was irradiated with a 60 W low pressure lamp for 20 min. The light intensity absorbed by the sample solution was determined to be 2.8×10^{-6} einstein/min.
b) absence of chloroacetamide
c) Concentration of chloroacetamide was 30 mM.

If energy transfer between two amino acids can be neglected,[11] a quantum yield for each amino acid in an equimolar mixture may be proportional to its extinction coefficient at the wavelength of an arbitrary incident light. Actually, when the equimolar mixture was irradiated with the 2537 Å light, quantum yields for disappearance of tryptophan and tyrosine were

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[9] D.V. Bent and E. Hayon, *J. Am. Chem. Soc.*, 97, 2612 (1975).
[10] D.V. Bent and E. Hayon, *J. Am. Chem. Soc.*, 97, 2599 (1975).
[11] Singlet energies (Es) of indole and phenol are 99.3 and 103 kcal/mol, respectively.[12]
[12] S.L. Murov, "Handbook of Photochemistry", Marcel Dekker, New York, N.Y., 1973, p. 17.

determined to be 0.10 and 0.014, respectively. The ratio (0.10/0.014=7.1) is fairly good agreement with that of extinction coefficients (2580/320=8.0) in the ultraviolet spectra which are shown in Fig. 2.

Next, a mixture of several amino acids in the presence and absence of chloroacetamide was irradiated (Table II). As expected from the reaction mechanism, all amino acids except tryptophan and tyrosine were found to be inert to the photolysis. When the disappearance of tryptophan was less than 20%, that of tyrosine was not observed. Degassing of sample solutions is not necessarily required in cases of irradiation of amino acids.[13]

Finally, in order to learn the wavelength dependence of this photolysis, each solution of tryptophan and tyrosine was irradiated with a spectroirradiator. As can be seen from Fig. 2,

TABLE II. Recovery (%) and Quantum Yield for Disappearance of Amino Acid[a]

	Recovery % (quantum yield)			
	Control[b]		Presence of chloroacetamide[c]	
	9 min	14 min	9 min	14 min
Trp	100	101 100[d]	88 (0.09)	82 (0.11) 81 (0.11)[d]
Tyr	100	99 100[d]	101	99 100[d]
His	100	100 99[d]	100	101 101[d]
Met	101	100 100[d]	98	99 101[d]
Cys	103	100 100[d]	102	101 100[d]
Arg	101	100	98	100
Asp	100	99	101	103
Thr	101	101	102	103
Pro	103	102	99	101
Gly	102	98	100	99
Val	103	102	101	100

a) A 3 mM solution of a mixture of amino acids was irradiated with a 60 W lamp. The light intensity was 2.3×10^{-6} einstein/min.
b) absence of chloroacetamide
c) Concentration of chloroacetamide was 30 mM.
d) A degassed sample solution was irradiated.

TABLE III. Wavelength Dependence in the Photolysis of Tryptophan and Tyrosine in the Presence of Chloroacetamide[a]

	Quantum yield					
	254 nm		276 nm		303 nm	
	10 min	20 min	10 min	20 min	10 min	20 min
Trp	0.10	0.10	0.10	0.12	0.11	0.10
Tyr	0.10	0.10		0.08	<0.006	<0.007

a) A solution of each amino acid (3 mM) with chloroacetamide (30 mM) was irradiated. Light intensities at 254, 276, and 303 nm were 1.9×10^{-8}, 6.4×10^{-8} and 1.3×10^{-7} einstein/min, cm^3, respectively.

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13) When proteins are irradiated under this photochemical conditions, degassing is usually essential.[14]
14) Y. Abe and T. Hamada, unpublished results.

irradiation with a light above 295 nm was expected to be effective only to tryptophan, not to tyrosine. In fact, quantum yields for disappearance of tryptophan at 276 and 303 nm were the same as that at 254 nm, whereas the reactivity of tyrosine at 303 nm was nearly nil as shown in Table III.

An application of the photolysis presented in this paper for photochemical modification of protein and enzyme is now under investigation.