Solvent Effects on Action Spectra for The Photodecomposition of N-Acetylphenylalanyl -L-Tryptophan and 3-Methyl Indole

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The UV action spectra and quantum yields for photodestruction of tryptophan (Trp) in peptide such as N-acetylphenylalanyl-L-tryptophan (NAPT) and 3-methyl indole (scatole) were determined in aerated aqueous and organic solvents. The photodestruction of aqueous NAPT was shown to be initiated by photoionization without requirement of threshold energy, as demonstrated by the similarity of fluence effect curves obtained for the action at various wavelengths and the wavelength dependence of quantum yield comparable to that reported for the photoionization of L-Trp. N-formylkynurenine (NFK)-type photoproduct, which is a photodynamic sensitizer, was not found to be involved in the photodestruction of Trp in NAPT in aqueous solution. In contrast, the action spectra of NAPT and scatole in organic solvents have revealed evidences for the significant role of internal photosensitization by NFK-type photoproduct in photolysis of Trp in peptide.

Introduction

Since the report[1] of involvement of tryptophan (Trp) photolysis in UV-induced deactivation of protein, the photochemical role of Trp in photobiological process has been well recognized. A number of investigators, using continuous irradiation, have extensively studied the photochemistry and photophysics of Trp in aqueous solution.[2-9] As a result, N-formylkynurenine (NFK) has been identified as the major photoproduct of Trp in neutral aqueous solution, which is usually known to be a photodynamic sensitizer. These studies have proposed that the primary step of NFK formation is a photoionization, which has been also supported by laser pulse excitation experiments.[10-12]

There have been considerable disagreements about threshold requirement for the photoionization. Amouyal *et al.*[9], using N_2O to scavenge hyrated electron (e_{aq}^-) from the photolyzed tryptophan, found that photoionization quantum yield is dependent on irradiation wavelength with no observable quantum yield wavelength longer than 275 nm. However, Pigault *et al.*[13] have shown ,using immidazole as a scavenger of e_{aq}^-, that the photoionization quantum yield is nearly constant (0.015) over the first absorption band ($S_0 \rightarrow S_1$, 265-300 nm), proposing that no threshold energy is necessary for the photoionization. These results are comparable to Steen's work[14] reported in earlier paper and more recent results of Bazin *et al.*[15] which have been carried out by using protons as e_{aq}^- scavenger.

UV-induced photohemolysis of red blood cell membrane is known to occur through protein crosslinking or lipid oxidation, which is partially attributed to the initial formation of NFK from photoionization of tryptophan.[16-20] Walrant *et al.*[19] have proposed the possibility that Trp in bovine carbonic anhydrase might be photolyzed for crosslinking of the protein via internal photosensitization by NFK as well as via direct photoionization of Trp. However, this proposal is based on the studies carrried with free L-Trp, and Borkman[2] have not found any evidence for NFK-sensitization in the action spectrum for the photolysis of free L-Trp in aqueous solution. These conflicting reports indicate that NFK-sensitization or photodestruction of Trp might be affected by its microenvironments which are determined by the type of sequence of neighboring amino acids or the location of Trp-peptides in the membrane. But these microenvironmental effects on the photodestruction of Trp have not been fully understood.

Thus, in the present study, being interested in the effect of location of Trp-peptide on the photochemistry of Trp in the cell membrane, we aimed at elucidating the mechanism of photolysis of Trp-in-peptide in nonaqueous solution as well as in aqueous solution. For this purpose, N-acetylphenylalanyl-L-tryptophan (NAPT) was used as a protein-like peptide. Because of insolubility of NAPT in nonpolar solvent, 3-methyl indole (scatole) was also used as an analogy to tryptophan.[21]

Experimental Section

Materials. N-acetylphenylalanyl-L-tryptophan (NAPT) and 3-methyl indole (scatole) were used as supplied from Research Plus Lab and Sigma Chemical Co., respectively. The 0.10 m*M* solutions were made with aqueous buffer (0.1*M* HCl + 0.1*M* Tris-hydroxymethylamine methane, PH 8.00), 99.5% ethanol (Merck) or cyclohexane (Merck). In order to minimize experimental error, the solutions were used immediately after preparation.

Methods. Continuous monochromatic light irradiations were performed on 3 ml of sample solutions in 1 cm quartz cell at room temperature. The light source was air-cooled mercury lamp (Bausch & Lomb model SP 200, 200W) fitted to a Bausch & Lomb UV monochromator. The monochromatic light was focused on the quartz cell located 3cm apart from the exit slit (6 mm width) so that the irradiation area was 0.8 cm^2. The solution was stirred magnetically during irradiation, which was also performed in black box designed to avoid stray light.

The solutions were irradiated at 10 nm interval from

240 to 310 nm for periods up to 180 minutes. The photodecomposition of Trp in NAPT or scatole at each period was monitored by analysis of absorption spectra which were measured on Beckman UV-5260 spectrophotometer. Fluence effect curves were obtained for each irradiation wavelength by plotting absorbance changes at 280, 320 or 250 nm *vs.* irradiation time. An half time to saturation of changes in absorption was determined at each wavelength in order to make quantum correction for the action, *i.e.* photodecomposition. Plotting the reciprocal of photon fluxes for an half time (action/photon) *vs.* irradiation wavelength resulted in action spectra for the photodecompostiion. The photon fluxes absorbed by the solution were measured by the chemical actinometry using ferrioxalate solution as developed by Hatchard and Parker.[22]

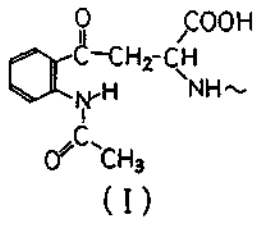
The quantum yields (ϕ_p) for photochemical destruction of NAPT or scatole were calculated from the following equation (1).

$$\phi_p = \frac{\Delta A_{280} \times V}{e_{280} \times d \times 0.96 \times I_\lambda^0 \times \bar{A}_\lambda \times \Delta t} \quad (1)$$

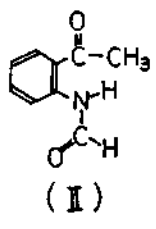
In this equation, ΔA_{280} represents the change in absorption at 280 nm resulted from irradiation for Δt minutes. V is the volume of irradiated sample solution with light pathlength, d and e_{280} is molar extinction coefficient of the sample at 280 nm. Finally, I_λ^0 is fluence rate (quantum flux) of monochromatic light (mole/min), which is subsequently corrected by the average absorption at irradition wavelength, $\bar{A}_\lambda$ during irradiation for Δt min and transmission efficiency of the sample cell, 0.96.

Results and Discussion

In order to monitor the monochromatic light-induced decomposition of NAPT and scatole in various solvents, absorption spectra were determined at each period of irradiation. Figures 1 and 2 show the typical absorption spectra of 0.10 m*M* aqueous NAPT and 0.10 m*M* scatole in ethanol solution, respectively after 0 to 60 min and 0 to 180 min of irradiation at 280 nm. Similar results were obtained for other solvent systems as well as for other irradiation wavelengths (Data not shown). The common aspect of these spectral changes is that the decreased absorption at 280 nm upon irradiation is followed by the increase of absorption at 295–350 nm or at 240–265 nm. These changes are consistent with those reported for the photodestruction of L-Trp[5], other Trp-peptides[4,7,20] and scatole[23,24], suggesting the production of new chromophore such as N-formylkynurenine (NFK)-type photoproducts (I) and (II) from NAPT and scatole, respectively, as shown below.



(I)



(II)

The formation of NFK-type photoproducts is indeed done by a photooxidation, as evidenced by the observation that the rate of photodecomposition diminished significantly

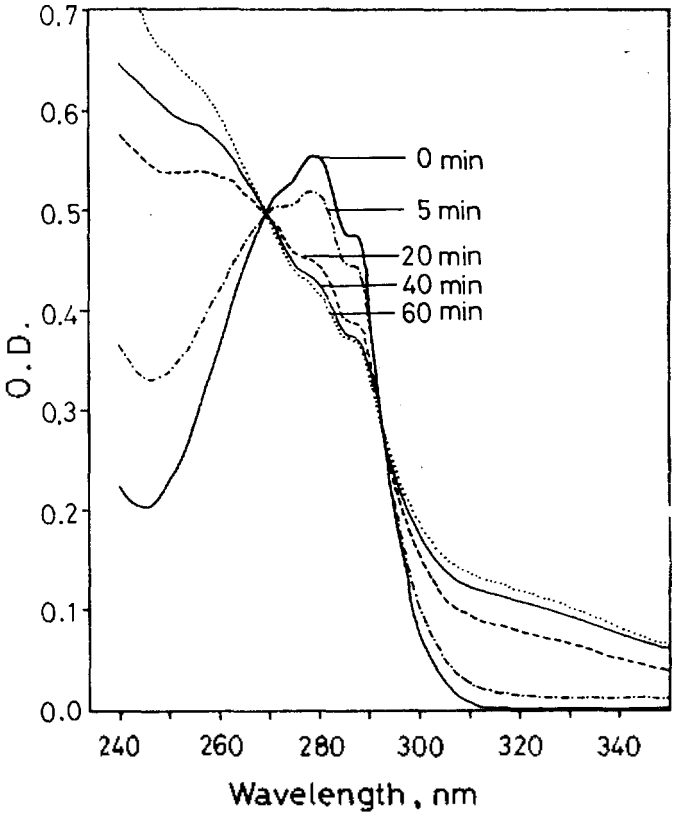


Figure 1. UV absorption spectra of 0.10mM NAPT in aerated aqueous solution at 25°C for various periods of exposure to 280nm irradiation.

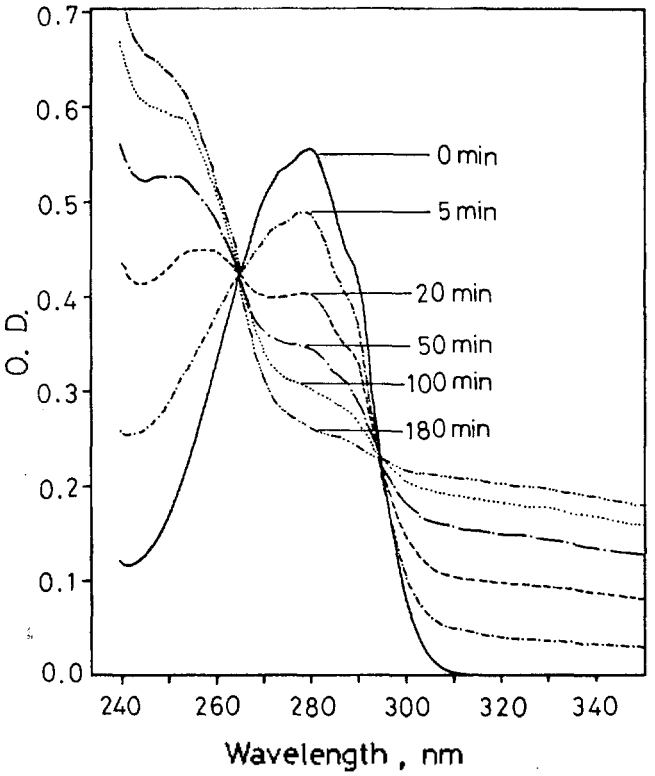


Figure 2. UV absorption spectra of 0.10m*M* scatole in aerated ethanol solution at 25°C for various periods of exposure to 280nm irradiation.

when the solution was N_2-saturated. The photooxidation of aqueous NAPT appears to be achieved by the same mechanism at all excitaiton wavelengths, since the fluence effect curves for photodestruction of Trp look similar at all wavelengths, as shown in Figure 3; they are almost superimposible if the fluence is multiplied by a certain factor. The same trends were observed for the fluence effect curves obtained by monitoring the formation of new photoproduct.

Figure 4 shows action spectrum for the photodecomposition of aqueous NAPT, which was obtained by plotting average action/photon vs. wavelenth determined from the fluence-effect curves of Trp destruction (circle symbols) and formation of photoproduct (triangle and square symbols). This

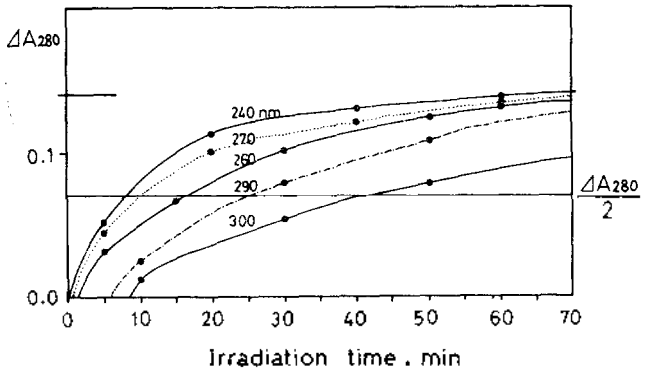


Figure 3. Fluence effect curves of 0.10m*M* NAPT in aerated aqueous sulution irradiated at monochromatic wavelengths at 25°C, which were monitored by observing absorbance changes at 280nm (ΔA_{280}) *vs.* irradiation time.

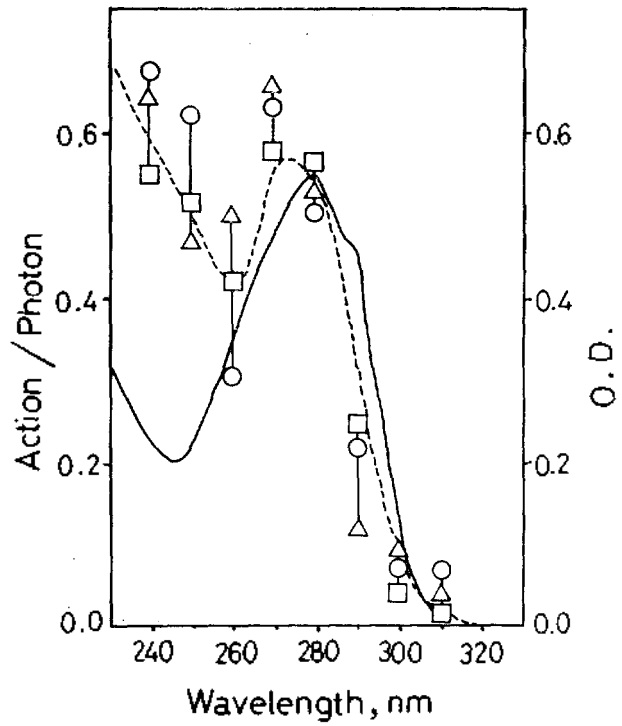


Figure 4. Action (dotted line) and absorption spectra (solid line) of 0.10m*M* NAPT in aerated aqueous solution. Action spectra were monitored by observing decrease in absorption at 280nm (circle), increase in absorption at 320nm (triangle) and increase in absorption at 250nm (square).

action spectrum follows the absoroptin spectrum (solid line) in the range of 270–310 nm within experimental error. However, it starts to deviate substantially from the absorption over the wavelength shorter than 260 nm. This is consistent with the fact that the quantum yields are the same (0.045) in the wavelength range, 270–310 nm and they inreeasc sharply from 260 nm as shown in Table 1. The wavelength dependence of quantum yields for the photodecomposition of NAPT is well matched with for the photoionization of L-Trp obseı ved by Pigault *et al.*[13] and Steen[14] (See Table 1). This comparison, with similarity in the shapes of fluence effect curves (Figure 3). indicates that the primary process of photooxidation of NAPT is a photoionzation without threshold energy requirement. This is in agreement with the results for L-Trp of Bazin *et al.*[15], but not with those of Amouyal *et al.*[9]. The sharp increase of quantum yield for

TABLE 1: Quantum Yields for Photochemical Destruction of 0.1 m*M* NAPT and Scatole in aerated Aqueous and Organic Solution at 25°C

Wavelenth, nm	NAPT		Scatole	
	H_2O	C_2H_5OH	C_2H_5OH	C_6H_{12}
240	0.090 (0.008)	0.064	0.013	0.058
250	0.090 (0.005)	0.069	0.017	0.046
260	0.071 (0.004)	0.068	0.013	0.022
270	0.055 (0.003)	0.064	0.010	0.037
280	0.045 (0.003)	0.054	0.010	0.027
290	0.045 (0.003)	0.064	0.010	0.045
300	0.045 (0.003)	0.058	0.032	0.252
310	0.045 (—)	0.110	0.114	0.593

( ): Data for the photoionizatiom quantum yields of L-tryprophan taken from Pigault *et al.*[13]

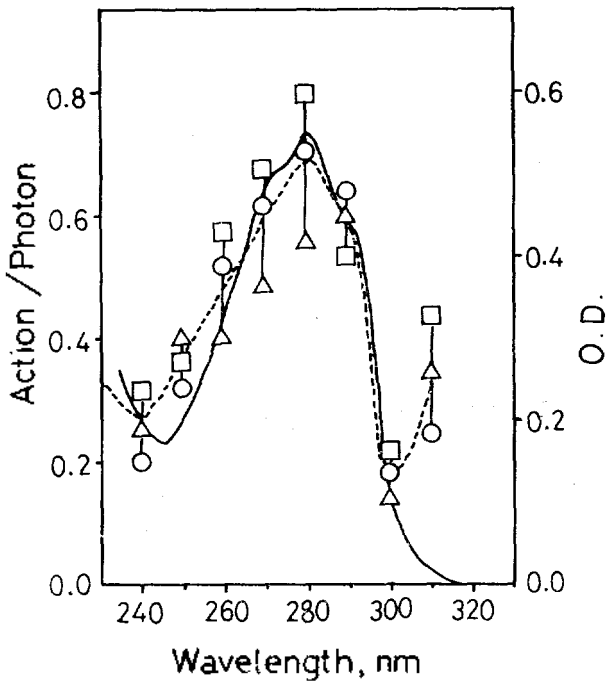


Figure 5. Action and absorption spectra of 0.10m*M* NAPT in aerated ethanol solution. The detailes for monitoring methods are referred to Figure 4.

photodecomposition within the second absorption band (S_2 state) is attributable to the faster ionization reaction in competition with internal conversion from S_2 to S_0 state, as has been suggested by Steen.[14]

The action for the photodecomposition of aqueous NAPT was not observed in the wavelength range, 310–350 nm where NFK-type photoproduct absorbs light more strongly than Trp. The action spectral data sets are also identical within experimental error, both for photodestruction of Trp and formation of photoproduct (Figure 4). These results suggest that secondary photoreaction such as an internal photosensitization by NFK-type photoproduct is not significant in the photodecomposition of NAPT in aqueous solution. Also, the rate of photodecomposition was observed to be unaffected upon addition of 10 m*M* sodium azide, a well known singlet oxygen ($^1O_2$) quencher. If NFK-type photoproduct is involved efficiently in the internal photosensitization of Trp-oxidation, one would not observe this fact, since NFK derivatives are known to generate $^1O_2$.[18] All of these results show similarity to those from Borkman's action spectral data[2] for free L-Trp in aqueous solution, implying that the photochemical action might not be affected by peptide bonding.

In contrast to the aqueous solution, the action for the photodecomposition of NAPT in ethanol solution was observed to be deviated from the absorption at wavelength longer than 300 nm, as shown in Figure 5. Indeed, the quantum yield for the photodecomposition at 310 nm was found to be 2–3 times higher than at shorter wavelength (See Table 1). These results imply that the mechanism of photodecomposition of NAPT in ethanol solution might be different from that in aqueous solution. Actually, it has been reported[9,21] that the quantum yield for photoionization of Trp is zero or tremendously lower in ethanol solution than in aqueous solution. Therefore, if the photodecomposition is initiated only by photoionization, the quantum yield for photodecomposition of NAPT would be also lower in ethanol solution than in aqueous solution. Howveer, the quantum yields in ethanol solution are more or less the same as in aqueous solution, as shown in Table 1, and the overall action spectrum looks similar to the absorption spectrum of NFK[18] rather than that of Trp (See Figure. 5). Similar actions were observed for the photodecomposition of scatole, a Trp-analogy, in ethanol and cyclohexane solutions as shown in Figures 6 and 7, respectively. These results indicate that internal photosensitization by NFK-type photoproducts (I) and (II) is significantly involved in the photodecomposition of Trp and scatole in organic solvents. It is noteworthy that the deviation of the action spectral data for scatole destruction (circles) from the corresponding data for chromophore production (triangles and squares) is more substantial especially in the long wavelength region, compared to that of the action spectral data of NAPT. This might suggest that the secondary photochemical reaction like the internal photosensitization is more significantly involved in the generation of photoproducts of scatole than in the case of NAPT. The photodecomposition rate was found to be diminished greatly upon addition of 10 m*M* sodium azide, indicating that the photodecomposition is partially done through generation of $^1O_2$ from the excited NFK-type photoproducts.

The quantum yield for the photodestruction of scatole at 310 nm was found to be increased as the polarity of sol-

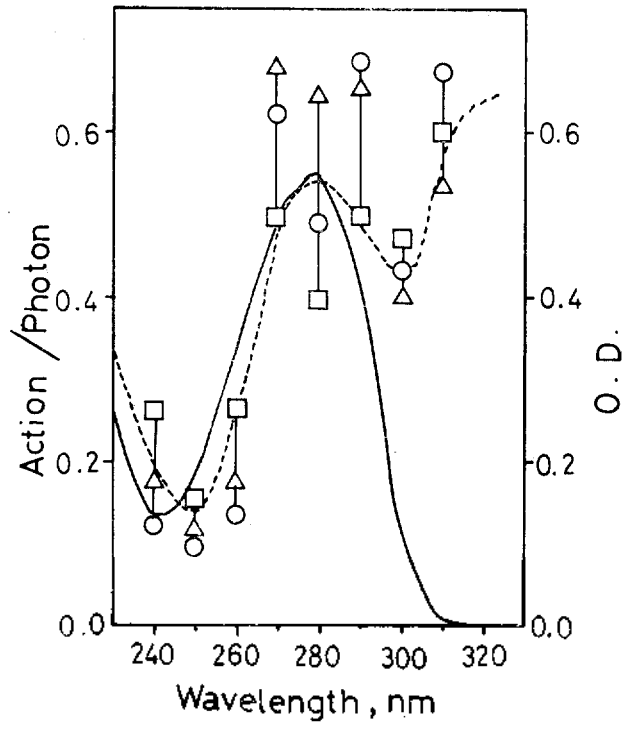


Figure 6. Action (dotted line) and absorption spectra (solid line) of 0.1 m*M* scatole in aerated ethanol solution. The monitoring methods are the same as in Figure 4.

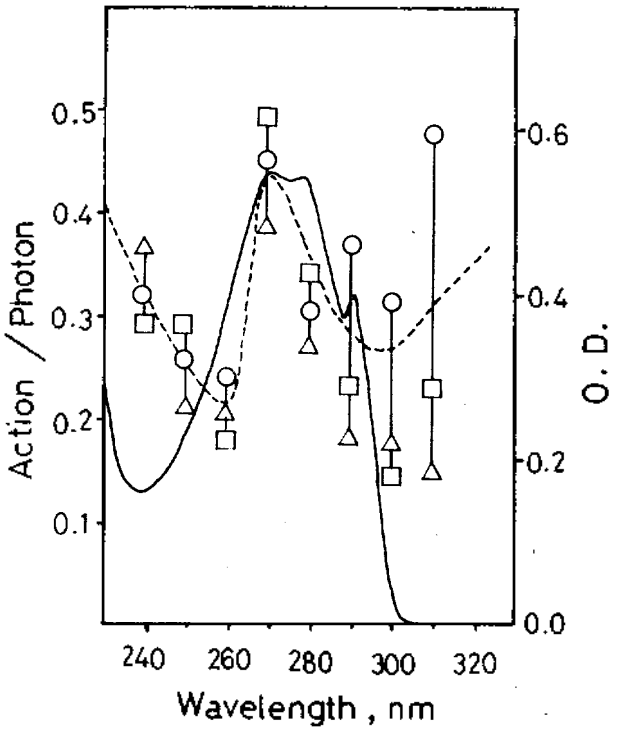


Figure 7. Action and absorption spectra of 0.10m*M* scatole in cyclohexane solution. The monitoring methods are referred to Figure 4.

vent decreases (from ethanol to cyclohexane), as shown in Table 1. This may be interpreted to be simply due to the longer lifetime of $^1O_2$ generated from excited state of NFK derivative in cyclohexane, because the lifetime of $^1O_2$ is known to depend on the polarity of solvent.[23] However, actual lifetime of $^1O_2$ in cyclohexane is only 1.6 times longer than in ethanol solvent (1.0×10^{-6} sec), which is not comparable to 6 times higher quantum yield for the photodestruction of scatole in cyclohexane than in ethanol. Thus, this interpretation could not be accepted without reservation. Alternatively, NFK derivatives in organic solvent are known to form intramolecular hydrogen bond between the N-H proton of formamide and the ortho carbonyl on side chain.[24,25] The intramolecular hydrogen bonding becomes more feasible as the polarity decreases. This fact also matches well with the highly increased quantum yield for the photodestruction at 310 nm in nonpolar solvent. Thus, the photosensitizing ability of NFK derivatives may be related to the tendency towards intramolecular hydrogen bonding. Pileni *et al.*[24] has found that the intramolecular hydrogen bonding causes to change the photophysical processes and enhance the formation of excited triplet state. Therefore, one would expect that energy transfer from the excited triplet state of NFK derivative to molecular oxygen could be enhanced, and the efficiency of $^1O_2$ production is increased to cause the enhanced efficiency of photooxidation of scatole or Trp. However, our data are not sufficient to support this idea, and further investigations would be required.

In conclusion, this study has shown that the mechanism of photooxidized destruction of Trp depends on the polarity of solvent, but not on the peptide bonding. In this experimental condition, Trp-in-pepitde in aqueous solution is photooxidized by reacting possibly with O_2^- which is generated as a result of photoionization of Trp, as shown below.

$$\mathrm{Trp} \xrightarrow{h\nu} \mathrm{Trp}^+ + e_{aq}^-$$
$$e_{aq}^- + \mathrm{O_2} \longrightarrow \mathrm{O_2^-}$$
$$\mathrm{Trp} + \mathrm{O_2^-} \longrightarrow \mathrm{NFK}$$

The secondary photochemical reaction of NFK derivative is not involved in the photodestruction of Trp in peptide in aqueous solution. On the other hand, in organic solvent, photodynamic action of NFK-type photoproduct was found to be involved in the efficient photodecomposition of Trp-in-peptide, as proposed by Walrant *et al.*[16]

$$\mathrm{Trp} \xrightarrow{h\nu} \mathrm{Trp}\cdot + \mathrm{H}\cdot$$
$$\mathrm{H}\cdot + \mathrm{O_2} \longrightarrow \mathrm{HO_2}\cdot$$
$$\mathrm{Trp} + \mathrm{HO_2}\cdot \longrightarrow \mathrm{NFK} \xrightarrow{h\nu} \mathrm{NFK^1} \longrightarrow \mathrm{NFK^3}$$
$$\mathrm{NFK} \leftarrow \mathrm{Trp} + {}^1\mathrm{O_2} \leftarrow \mathrm{O_2}$$

Although we have not identified the primary photochemical step of Trp in organic solvent, it is usually known to be dehydrogenation from the N-H of indole ring and formation of H.[26] This proton radical reacts with O_2 to form $HO_2\cdot$ which oxidize Trp for the production of NFK derivative.

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References

(1) A. D. MacLaren and D. Shugar, "Photochemistry of Proteins and Nucleic Acids", MacMillan, New York, 1964.
(2) R. F. Borkman, *Photochem. Photobiol.*, **26** 163 (1977).
(3) D. I. Roshchupkin, V. V. Talitsky and A. B. Pelenityk, *Photochem. Photobiol.*, **30**, 635 (1979).
(4) J. D. Tassin and R. F. Borkman, *Photochem. Photobiol.*, **32**, 577 (1980).
(5) A. Pirie, *Biochem. J.*, **125**, 203 (1971).
(6) R. S. Asquith and D. E. Rivett, *Biochim. Biophys. Acta*, **252**, 111 (1971).
(7) L. A. Holt, B. Milligan, D. E. Rivett and F. H. C. Stewart, *Biochim. Biophys. Acta*, **499**, 131 (1977).
(8) Y. Vladimirov, I. R. Schupkin and E. Fesenke, *Photochem. Photobiol.* **11**, 227 (1970).
(9) E. Amouyal, R. Bernas and D. Grand, *Photochem. Photobiol.* **29**, 1071 (1979).
(10) P. Walrant, R. Santus and L. I. Grossweiner, 3rd Annual Meeting of American Society for Photobiology, Lousville, Abstract p. 60.
(11) M. Pailthorpe, J. Bonjour and C. Nicholls, *Photchem. Photobiol*, **17**, 209 (1973).
(12) L. I. Grossweiber, A. M. Brendzel and A. Blum, *Chem. Phys.*, **57**, 147 (1981).
(13) C. Pigault, C. Hasselmann and G. Laustriat, *J. Phys. Chem.*, **86**, 1755 (1982).
(14) H. B. Steen, *J. Phys. Chem.*, **61**. 3997 (1974).
(15) M. Bazin, L. K. Patterson and R. Santus, *J. Phys. Chem.*, **87**, 189 (1983).
(16) S. V. Konev, I. D. Volotovski and L. M. Sheike, *Photochem. Photbiol.*, **27**, 289 (1978).
(17) I. E. Kochevar, *J. Invest, Dermatol.* **74**, 256 (1980).
(18) P. Walrant and R. Santus, *Photochem. Photobiol.*, **19**, 411 (1974).
(19) P. Walrant, R. Santus and L. I. Grossweiner, *Photochem. Photobiol.*, **22**, 63 (1975).
(20) I. E. Kochevar and M. Yoon, *Photochem. Photobiol.*, **37**,279 (1983).
(21) A. Bernas, D. Grand and E. Amouyal, *J. Phys. Chem.*, **84**, 1259 (1980).
(22) C. G. Hatchard and C. A. Parker, *Proc. R. Soc. London, Ser., A* **235**, 518 (1956).
(23) P. B. Merkel and D. B. Kearns, *J. Amer. Chem. Soc.*, **94**, 7244 (1972).
(24) M. P. Pileni, P. Walrant and R. Santus, *Chem. Phys. Lett.*, **42**, 89 (1976).
(25) M. P. Pileni, P. Walrant and R. Santus, *J. Phem Chem.*, **80**, 1804 (1976).
(26) I. Tatischeff and R. Klein, *Photochem. Photobiol.* **22**,, 221 (1975)