# Photoionization of aromatic amino acid at different pH values*

SONG Qinhua (宋钦华), LI Quanxin (李全新), SUN Haihong (孙海虹),

XU Yeping (徐业平), CHEN Congxiang (陈从香), YU Shuqin (俞书勤),

MA Xingxiao (马兴孝),

(Open Laboratory of Band-selective Chemistry, Department of Chemical Physics, University of Science and

Technology of China, Hefei 230026, China)

YAO Side (姚思德) and LIN Nianyun (林念芸)

(Shanghai institute of Nuclear Research, Chinese Academy of Sciences, Shanghai 201800, China)

**Abstract**    The photoioization of aromatic amino acid in solutions of different pH values were investigated by 248 nm laser flash photolysis. The results showed that the photoionizations of tyrosine and tryptophan were affected by pH value of solution, but phenylalanine was not. The ionization was in favor of monophotonic process at high pH value and of biphotonic process at low pH value. The mechanisms of pH effect on photoionization of tyrosine and tryptophan were the deprotonation of phenolic hydroxyl group and the deprotonation of > NH on the indole ring respectively. Our results explain the discrepancy of values for the yield of hydrated electron obtained by other investigators.

**Keywords:   laser flash photolysis, aromatic amino acid, photoionization, pH effect.**

Three aromatic amino acids, tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe), exhibit significant absorption in the UVB (280—320 nm) region, their chromophores are substituting aromatic groups. The UV photochemistry of these three amino acids, especially Trp, has been investigated intensively during last three decades[1—5]. Various photoionization measurements have been carried out employing either low intensity[6—9] or high intensity[10—19] laser UV excitation. But great variation in the values of quantum yield $\Phi_{e-}$ of photoionization exists, even in those values obtained by one group[13,15,17]. The mechanism of photoionization was reported to be monophotonic in some studies[12,13,15,19] but biphotonic (two-step) in others[14,16]. In this work, the pH effect on the photoionization of aromatic amino acid was investigated by laser (KrF 248 nm) flash photolysis. The variation in the values for quantum yield of photoionization is explained.

## 1   Experimental

DL-tyrosine(Tyr), D-tryptophan (Trp) and L-phenylaanine (Phe) obtained from Sigma Co. were used as received. The pKa values of Tyr, Trp and Phe are listed in table 1. Aromatic amino acids (symbolized by HA) in aqueous solutions exist as $H_2A^+$ at pH 1.4, HA at pH 6.4 and $A^-$ (Trp、Phe) or $A[-H^+]^{2-}$ (Tyr) at pH 11.4 respectively. All samples were prepared in triple distilled water and deaerated by high purity nitrogen for 20 min. Laser photolysis was performed using

an apparatus described in reference [20].

Table 1    Laser power density index of the absorption intensities of photoionization of
aromatic amino acid by 248 nm laser light in solutions

| Aromatic amino acid | pKa[10—12] | Laser power density index[a] | | |
|---|---|---|---|---|
| | —COOH, —NH$_3^+$, —OH | 1.2[b] | 6.5[b] | 11.4[b] |
| Tyr | 2.2, 9.1, 10.1 | 1.7[c] | 1.5 | 1.0 |
| Trp | 2.4, 9.4 | 1.6[d] | 1.4 | 1.2 |
| Phe | 1.8, 9.1 | 1.9[e] | 1.9 | 1.8 |

a) Estimated to be accurate to ±0.1; b) pH value; c,d,e) determined at 410 nm, 330 nm and 320 nm respectively.

## 2   Results and discussion

On 248 nm laser photolysis of $N_2$-saturated neutral and alkaline aqueous solutions of aromatic amino acids relatively strong absorption changes were observed. The absorption spectra recorded immediately after laser pulse were characterized by a strong and broad band at 400—600 nm and a bleaching band at 300—400 nm (figs. 1 and 2). The absorption at 600 nm decayed in deaerated solution within 2 $\mu$s following first-order kinetic law (see insets of figs. 1, 2 and 3). The strong and broad absorptions were not observed from photolysis of $N_2O$-saturated aqueous solutions of aromatic amino acids. Hence, the strong absorption band at 400—600 nm resulted from hydrated electron $(e_{aq}^-)$ $(\lambda_{max} = 720$ nm). The appearance of $e_{aq}^-$ is the direct evidence for photoionization of aromatic amino acid. The reaction can be presented by

$$HA \xrightarrow{nh\nu} A^{\cdot} + e_{aq}^- + H^+ \quad (n = 1 \text{ or } 2).\qquad(1)$$
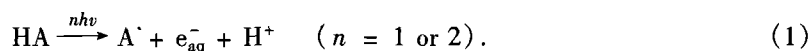
In addition, photodissociation of aromatic amino acids can also occur, and produce the radical $A^{\cdot}$,
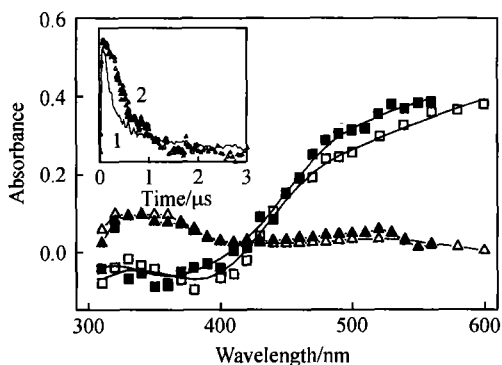
$$HA \xrightarrow{h\nu} A^{\cdot} + H^{\cdot}.\qquad(2)$$



Fig. 1.   Transient absorption spectra from photolysis of $10^{-3}$ mol·dm$^{-3}$ Trp aqueous solution. pH 6.5: □, 80 ns (saturated with $N_2$), △, 0.1 $\mu$s (saturated with $N_2O$); pH 11.4: ■, 50 ns (saturated with $N_2$), ▲, 0.1 $\mu$s (saturated with $N_2O$). Inset: dependence on time of the absorbance at 560 nm, pH 6.5 (1), pH 11.4 (2).
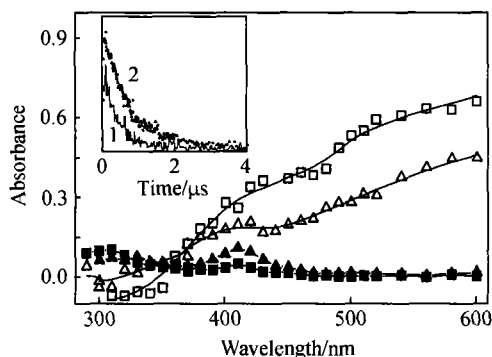


Fig. 2.   Transient absorption spectra from photolysis of $10^{-3}$ mol·dm$^{-3}$ Tyr aqueous solution. pH 6.5: △, 80 ns (saturated with $N_2$), ▲, 0.1 $\mu$s (saturated with $N_2O$), □, 50 ns (saturated with $N_2$); pH 11.4: ■, 0.1 $\mu$s (saturated with $N_2O$). Inset: dependence on time of the absorbance at 600 nm, pH 6.5 (1), pH11.4(2).
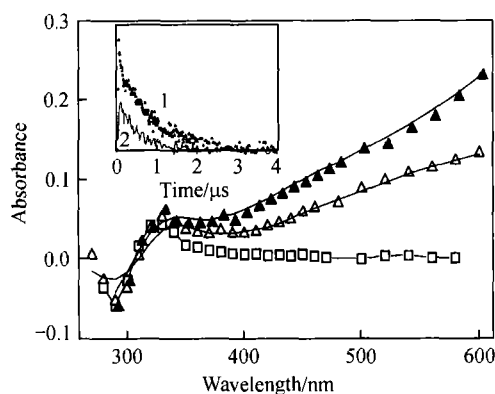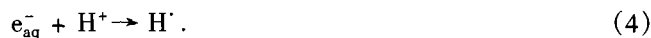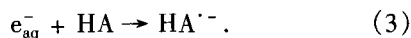
Fig. 3.    Transient absorption spectra from photolysis of $10^{-3}$ mol·dm$^{-3}$ Phe aqueous solution saturated with N$_2$ at pH 1.2 (□ 0.1 $\mu$s), pH 6.5 (△ 0.1 $\mu$s) and pH 11.4 (▲ 0.1 $\mu$s). Inset: dependence on time of the absorbance at 600 nm, pH 11.4 (1), pH 6.5 (2).

The decay of hydrated electron is due to its reaction with substrate (untransformed aromatic amino acid),

$$e_{aq}^- + HA \rightarrow HA^{\cdot-}. \tag{3}$$

If photoionization is a monophotonic process, the amount of hydrated electrons increases linearly with incident laser intensity ($I_L$), namely the quantum yield $\Phi_{e^-}$ is constant. The amount of $e_{aq}^-$ is in proportion to $I_L$ squared, and $\Phi_{e^-}$ varies with laser intensity, if photoionization is a biphotonic process.

Under various laser intensities, the absorbances at 650 nm ($A_{650}$) for photolysis of Tyr solution were determined at 0.1 $\mu$s after the pulse. In alkaline solution, the plot of $A_{650}$ against the laser intensity is a straight line as shown in fig. 4. According to equation $\lg A_{650} = n \lg I_L$, the calculated laser power density index ($n$) was 1.5 for neutral solution and 1 for alkaline solution respectively. It is obvious that both the mono- and biphotonic pathways exist in the process of photoionization in neutral solution of Tyr, but only monophotonic pathway exists in alkaline solution of Tyr. So, the pathway of photoionization is changed by pH value of solution. In addition, it has been found that the photoionization of Tyr in neutral solution is a monophotonic pathway under low intensity laser, a biphotonic pathway under high intensity laser (see fig. 4). The same results were obtained from Trp and Phe, suggesting laser intensity can affect the process of photoionization of aromatic amino acid.

The strong absorptions in the range 400—600 nm were not observed from photolysis of N$_2$-saturated acid aqueous solutions of aromatic amino acids. This is due to the fast reaction of $e_{aq}^-$ with proton (H$^+$) forming hydrogen radical (H$^\cdot$),



Fig. 4.    Dependence on laser intensity of the absorbance of Tyr at 650 nm 0.1 $\mu$s after laser pulse. pH 6.5 (curve 1), pH 11.4 (curve 2).

$$e_{aq}^- + H^+ \rightarrow H^\cdot. \tag{4}$$

The absorbances at $\lambda_{max}$ of the radicals of aromatic amino acid were determined by changing laser intensity. Laser power density indexes obtained are shown in table 1. These indicate that photoionization pathways of aromatic amino acid are mainly biphotonic. By determining the absorbance at $\lambda_{max}$ of A$^\cdot$, the obtained values of laser power density index of photoionization should be those of photolysis (including photoionization and photodissociation, eq. (2)), thus the experimental values may be a

little smaller than actual ones.

Using the same method, laser power density indexes of Trp and Phe photoionization were obtained in neutral and alkaline solutions respectively, and are listed in table 1. Although pKa values of $NH_3^+$ for three aromatic amino acids are in the range of pH 6.5—11.4, only changes of laser power density index of Tyr are marked with the increased pH value. This may be due to the pKa (10.1) of substituting aromatic group of Tyr is in the range of pH 6.5—11.4.

Cowgill studied pH effects on fluorescence quantum yield ($\Phi_F$) of aromatic amino acid and their derivatives. The results showed that, with increasing pH, $\Phi_F$ of Trp and Phe increased acutely at pKa of —$NH_3^+$ and —COOH, and that of Tyr increased at pKa of —COOH, but decreased at pKa of —$NH_3^+$ and —OH (in the range of pH 9—10)[21]. We suggested that —OH (deprotonation) induced decrease conceals —$NH_3^+$ induced increase of Tyr's $\Phi_F$. Trp's $\Phi_F$ starts to decrease at pH 11.0 resulting from excited > NH of indole ring deprotonated[21]. The density of electron cloud of the excited molecule is lower than that of the ground, thus acidity enhances and pKa decreases. Therefore, when the substituting aromatic groups deprotonate, the fluorescence quantum yields of aromatic amino acid decrease. We consider that an additional deactivation pathway might exist for the excited aromatic amino acid, namely, photoionization via a monophotonic process.

It has been demonstrated that when guanine deprotonates from N1 (chromophore) the ionization potential decreases, thus the transformation of photoionization process from bi- to monophoton under 248 nm laser pulses will occur[22,23]. DeFelippis et al.[24] determined the oxidation potentials of Tyr and Trp in pH 7 and 11 solutions, and the values in pH 11 solution are much smaller than that of pH 7 solution. This is consistent with our results of photoionization of Trp and Tyr. The pH doesn't change the process of photoionization of Phe because there is no active hydrogen on its substituting aromatic group.

In conclusion, besides the wavelength of excited light, pH value of solution and laser intensity will affect the processes of photoionization of aromatic amino acid and cause variation in the measurement of quantum yield of photoionization. Therefore, only under the same condition, the same quantum yields of photoionization of aromatic amino acid can be obtained.

## References

1  Creed, D., The photophysics and photochemistry of the near-UV absorbing amino acids I. Tryptophan and its simple derivatives, Photochem. Photobiol., 1984, 39: 537.

2  Creed, D., The photophysics and photochemistry of the near-UV absorbing amino acids II. Tyrosine and its simple derivatives, Photochem. Photobiol., 1984, 39: 563.

3  Harmon, P. A., Teraoka, J., Asher, S. A., UV resonance raman saturation spectroscopy measures protein aromatic amino acid excited state relaxation rates, J. Am. Chem. Soc., 1990, 112: 8789.

4  Nikogosyan, D. N., Görner, H., Photolysis of aromatic amino acids in aqueous solution by nanosecond 248 nm and 193 nm laser light, J. Photochem. Photobiol. B: Biol., 1992, 13: 219.

5  Bensasson, R. V., Land, E. J., Truscott, T. G., Proteins and Components, in Excited States and Free Radicals in Biology and Medicine. Contributions from Flash Photolysis and Pulse Radiolysis, Oxford: Oxford University Press, 1993.

6  Grossweiner, L. I., Usui, Y., Flash photolysis and inactivation of aqueous lysozyme, Photochem. Photobiol., 1971, 13: 195.

7 Santus, R., Grossweiner, L. 1., Primary products in the flash photolysis of tryptophan, *Photochem. Photobiol.*, 1972, 15: 101.

8 Templer, H., Thistlethwaite, P. J., Flash photolysis of aqueous tryptophan, and tryptophyl alanine, *Photochem. Photobiol.*, 1976, 23: 79.

9 Amouyl, E., Bernas, A., Grand, D., On the photoionization energy threshold of tryptophan in aqueous solutions, *Photochem. Photobiol.*, 1979, 29: 1071.

10 Bent, D. V., Hayon, E., Excited state chemistry of aromatic amino acids and related peptides. I. Tyrosine, *J. Am. Chem. Soc.*, 1975, 97: 2599.

11 Bent, D. V., Hayon, E., Excited state chemistry of aromatic amino acids and related peptides. II. Phenylalanine, *J. Am. Chem. Soc.*, 1975, 97: 2606.

12 Bent, D. V., Hayon, E., Excited state chemistry of aromatic amino acids and related peptides. III. Tryptophan, *J. Am. Chem. Soc.*, 1975, 97: 2612.

13 Bryant, F. D., Santus, R., Grossweiner, L. I., Laser flash photolysis of aqueous tryptophan, *J. Phys. Chem.*, 1975, 79: 2711.

14 Lachish, U., Shafferman, A., Stein, G., Intensity dependence in laser flash photolysis experiments: hydrated electron formation from ferrocyanide, tyrosine, and tryptophan, *J. Chem. Phys.*, 1976, 64: 4205.

15 Baugher, J. F., Grossweiner, L. I., Photolysis mechanism of aqueous tryptophan, *J. Phys. Chem.*, 1977, 81: 1349.

16 Finnstrom, B., Tfibel, F., Lindqvist, L., One- and two-photon ionization of aqueous tryptophan by the harmonics of the Nd laser, *Chem. Phys. Lett.*, 1980, 71: 312.

17 Grossweiner, L. I., Brendzel, A. M., Blum, A., Multiple pathways of tryptophan photoionization, *Chem. Phys.*, 1981, 57: 147.

18 Mialocq, J. C., Amouyal, E., Bernas, A. et al., Picosecond laser photolysis of aqueous indole and tryptophan, *J. Phys. Chem.*, 1982, 86: 3173.

19 Bazin, M., Patterson, L. K., Santus, R., Direct observation or monophotonic photoionization of tryptophan excited by 300-nm radiation. A laser photolysis study, *J. Phys. Chem.*, 1983, 87: 189.

20 Zuo, Z. H., Yao, S. D., Luo, J. et al., Laser photolysis of cytosine, cytidine and dCMP in aqueous solution, *J. Photochem. Photobiol.*, *B: Biol.*, 1992, 15: 215.

21 Birks, J. B., (ed.) *The Excited States of Biological Molecules*. London: John Wiley & Elsevier, 1976.

22 Song, Q. H., Yao, S. D., Wang, W. F. et al., Monophotonic ionization of guanine components in alkaline aqueous solution by 248 nm laser light: identification of guanine-derived radical anions, *J. Photochem. Photobiol. A: Chem.*, 1997, 102: 197.

23 Song, Q. H., Yao, S. D., Li, N. Y., Monophotonic ionization of Poly[A,G] and DNA in alkaline aqueous solution by 248 nm laser light: formation of guanyl radical, *Radiat. Phys. Chem.*, 1997, 50: 435.

24 DeFelippis, M. R., Murthy, C. P., Broitman, F. et al., Electrochemical properties of tyrosine phenoxy and tryptophan indolyl radicals in peptides and amino acid analogues, *J. Phys. Chem.*, 1991, 95: 3416.