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Effect of pH and excitation light wavelength on the activation energy of thermal quenching of tryptophan fluorescence

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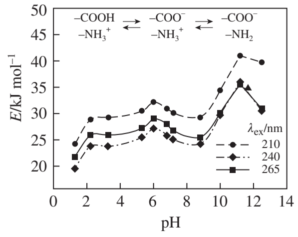
The activation energy of temperature quenching (E) of tryptophan fluorescence at pH 1–13 exhibited a maximum corresponding to the isoelectric point ($pH_i \approx 6$) of tryptophan at all exciting light wavelengths. The values of E nonmonotonically changed with the excitation wavelength. This behavior was explained by photoinduced electron transfer from the higher singlet-excited S_n ($n > 1$) states of tryptophan.



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Tryptophan (Trp) not only plays an important role in biological processes[1,2] but also exhibits fluorescence (FL) with the emission maximum at $\lambda_{FL} \approx 350$ nm and FL quantum yield (φ) of 0.13–0.14.[3,4] Tryptophan is a bipolar zwitterion at the isoelectric point ($pH_i = 5.9$),[1,5] and changes in pH significantly affect FL parameters. Changes in the states of side-chain amino or carboxyl groups affected not only the φ of FL and the maximum position of the luminescence spectrum[6] but also the FL lifetime (τ) of Trp in an electronically excited state.[7]

At pH $\leqslant$ 3, the protonization of the amino group leads to the cationic form of the amino acid $^+H_3NRCOOH$, accompanied by a decrease in φ and the lifetime τ of the excited state FL of tryptophan; at pH 3–8, the quantum yield and τ remained constant due to the dominance of the zwitterionic form $^+H_3NRCOO^-$.[6,7] The highest values of φ and τ were observed at pH > 8 in the H_2NRCOO^- state of Trp.[5,6] The temperature quenching of Trp FL was studied in neutral aqueous solutions (pH 7).[7–10] Quenching occurred according to the photoinduced electron transfer (PET) mechanism[11] with the activation energy $E = 27.5$–34 kJ mol^{-1}. However, the effects of exciting light wavelength (λ_{ex}) on the values of E for tryptophan FL were not taken into account.[7–10] It was found previously[12] that Vavilov's law was violated in Trp regarding the independence of φ of FL and λ_{ex} caused by the competition of the PET process from the second singlet-excited S_2 level of the amino acid and the intersystem crossing $S_2 \rightarrow S_1$.[13–15] According to Kazakov *et al.*,[15] the exciting light wavelength has a significant effect on the activation energy E of temperature quenching of Trp FL, and we believe that significant discrepancies in the values of E for tryptophan FL[7–10] can be caused by PET processes determined by the energy of photoexcitation.

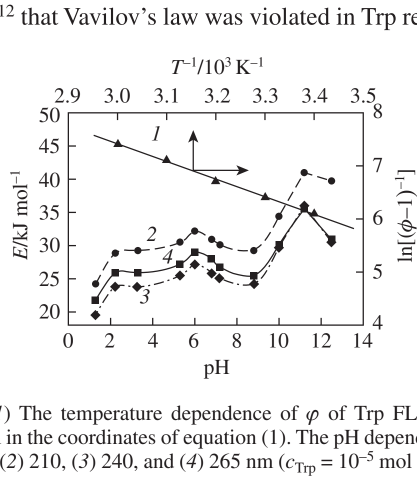

Figure 1 (*1*) The temperature dependence of φ of Trp FL at pH 6 and $\lambda_{ex} = 265$ nm in the coordinates of equation (1). The pH dependence of E for Trp at λ_{ex} of (*2*) 210, (*3*) 240, and (*4*) 265 nm ($c_{Trp} = 10^{-5}$ mol dm^{-3}, 298 K).

Here, we studied the effects of exciting light frequency and pH (1–13) on the activation energy of thermal quenching of D-tryptophan FL in aqueous solutions.[†]

The activation energy of thermal quenching of tryptophan FL was determined by equation (1).[16]

$$1/\phi - K = Ae^{-E/RT} \qquad (1)$$

The value of K is unity in the case of acridine FL in aqueous and aqueous ethanol solutions at a low alcohol content. Indeed, the best description of $\phi(T)$ in our experiments was achieved in terms of equation (1) using the empirical coefficient $K = 1$. This indicates the existence of a dominant nonradiative channel of tryptophan deactivation related to PET. Figure 1 shows the typical temperature dependence of φ for tryptophan FL at pH 6 and λ_{ex} = 265 nm (line *1*) in the semilogarithmic coordinates of equation (1). Similar linear dependences were obtained for other pH and λ_{ex} with high correlation coefficients. Curves *2*–*4* in Figure 1

[†] The absorption spectra were measured on a Specord M-40 spectrophotometer, and the FL spectra were recorded on a Hitachi MPF-4 spectrofluorometer. The photoexcitation of solutions was performed at λ_{ex} = 210, 240, 265 nm. The quantum yield of Trp FL was determined relative to rhodamine B according to a published procedure.[13] A pH-150 MI pH meter with an ESK-10307 combined glass electrode was used for the pH measurements. The electrodes were calibrated using standard buffer solutions. D-Tryptophan (Fluka, $\geqslant$ 99.0%) was recrystallized from twice-distilled water. The results were obtained in freshly prepared solutions of Trp in twice-distilled water at a concentration of 10^{-5} mol dm^{-3}. Concentrated perchloric acid was vacuum distilled, and a standard solution of sodium hydroxide was used without prior purification.

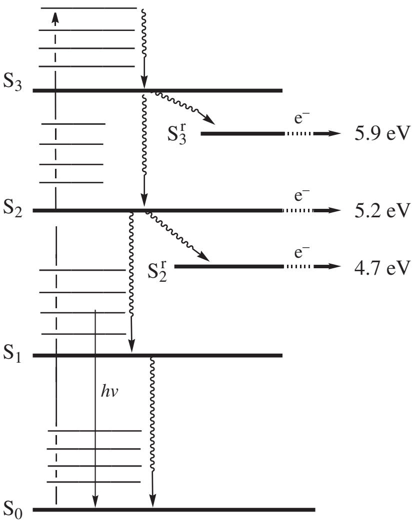

Figure 2 Photoinduced electron transfer from higher singlet-excited S$_n$ ($n > 1$) states of Trp.

correlate with published data for φ and τ of Trp FL[6,7] and for the titration of α-amino acids.[17]

In contrast to the pH dependences of φ and τ for Trp FL,[6,7] in which luminescence parameters remained constant at pH 3–8 due to the zwitterionic nature of the amino acid $^+H_3NRCOO^-$, a distinct maximum was observed for the values of E for all λ_{ex} used around the isoelectric point of tryptophan, pH$_i \approx 6$ (see Figure 1).[4] Indeed, a slight shift from pH$_i$ (pH ± 1) towards the cationic $^+H_3NRCOOH$ or anionic H_2NRCOO^- form leads to a decrease in the E of tryptophan FL (see Figure 1). This observation can find an application in determining the pH$_i$ values of α-amino acids and proteins. The absence of detailed FL parameters of Trp at pH$_i$ from the previous works[6,7] may be due to the stereotype that the luminescent properties φ and τ of Trp at pH 3–8 are determined exclusively by the neutral zwitterionic state of the amino acid.[17] In other aspects, the change of E *vs.* pH (see Figure 1, curves *2*–*4*) is similar to the dependences of FL and τ for tryptophan FL.[6,7] At pH < 3, the values of E for Trp FL decrease due to protonation of the carboxyl group $^+H_3NRCOOH$. At pH > 8, an increase in E of tryptophan FL is observed for the anionic form H_2NRCOO^-, and maximum values were detected at pH ≈ 11. In alkaline media (pH 11–13), the decrease in E of tryptophan FL can be due to photoinduced proton transfer from the NH group of the indole ring.[18]

Previously, we established nontrivial dependence of E on λ_{ex} in a neutral aqueous solution of tryptophan.[19] The minimum (240 nm) and maxima (210, 265 nm) of this dependence correspond to the positions of the second singlet-excited nonequilibrium Franck–Condon S$_2$ level and relaxed to the equilibrium solvation environment S$_3^r$ and S$_2^r$ singlet excited states (Figure 2) of the amino acid.[20]

Based on these results, the photoexcitation of Trp was studied at λ_{ex} of 210, 240, and 265 nm. It was found that the λ_{ex} value affects the E of Trp FL at pH 1–13 (see Figure 1, curves *2*–*4*). As expected, the largest E values of Trp FL in the test range of pH 1–13 were obtained under photoexcitation of tryptophan at λ_{ex} of 210 and 265 nm, and the smallest, at λ_{ex} = 240 nm (see Figure 1). The E *vs.* λ_{ex} dependence extends our earlier assumption[19,20] on the possibility of PET from higher singlet-excited S$_n$ ($n > 1$) Trp states in neutral aqueous solutions (pH 7) over a wide range of pH 1–13. The experimental results do not contradict the reported data obtained at pH 7. The activation energy E = 25.5–30.05 kJ mol^{-1} (see Figure 1) of thermal quenching of Trp FL at pH 7 at all exciting light wavelengths is consistent with the known data (E = 27.5–34 kJ mol^{-1}).[7–10]

Thus, the study of the influence of pH in a range of 1–13 and λ_{ex} of 210, 240, and 265 nm on the activation energy of thermal quenching of tryptophan FL allowed us to establish the following. First, the frequency of the exciting light quantum (pH 1–13) affects the activation energy of thermal quenching of tryptophan FL. Second, the maximum value of E for Trp FL at all studied wavelengths λ_{ex} at pH ≈ 6 corresponds to the isoelectric point of the amino acid, which can be used in the luminescent determination of pH$_i$ of α-amino acids and proteins.

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