Photoionization of Indole in Alkaline Aqueous Solutions at 77 °K

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The photoionization process of indole in alkaline aqueous solutions at 77 °K and the recombination process of trapped electron with its mother molecule were investigated. The fluorescence spectrum of indole in these glassy media depended on the NaOH concentration; the disappearance of the 325 nm band at the NaOH concentrations higher than 5 M with the appearance of a fluorescence band at 375 nm. The latter emission is possibly ascribed to the fluorescence of indole anion formed by proton dissociation in the excited singlet state of indole. The intensities of the 375 nm band and the ESR signal due to trapped electrons were dependent on the NaOH concentration and increased steeply with increasing the NaOH concentration. There was a parallel relation between the indole anion concentration and the trapped electron density to some extent.

The dependence of the ESR signal intensity on irradiation intensity suggested that the photoionization of indole in alkaline aqueous solution occurred through a two photon process. The presence of a photoactive intermediate for absorbing the second photon was further investigated with simultaneous irradiation of two different wavelength lights.

The trapped electron was detrapped with the irradiation of visible light (e.g. 580 nm), and its recombination with mother molecule caused an emission with the same spectrum as indole phosphorescence. The molecular species of the photoactive intermediate in alkaline aqueous solutions seems to be the indole anion. The photoionization in NaOH aqueous solution at 77 °K was also compared with that in methanolic glassy solution.

Indole derivatives such as indole acetic acid and tryptophan play the important roles in living body not only in their ground states but in their excited states. Recently, the electronic behaviour in the excited state of these molecules has attracted a great attention of various workers from photochemical and photobiological importance.

Many investigations have been made with indole derivatives to clarify the optical and electronic behaviours in their excited states. SANTUS et al.1 found that the quenching of tryptophan fluorescence in high alkaline aqueous solutions was caused by the proton dissociation in the excited singlet state. They observed the fluorescence of tryptophan anion in the above solutions. MOAN and STEEN2 measured the ESR signal and the glow emission in alcoholic glassy solution of tryptophan at 77 °K. They proposed that the photoionization of tryptophan proceeded through a biphotonic process with the triplet state as photoactive intermediate. However, except for tryptophan, there is no detailed study on photoionization mechanism of indole derivatives including indole so far as we know.

This investigation deals with the photoionization of indole, the parent molecule of indole acetic acid and tryptophan, the recombination of the trapped electron with its mother molecule and the related optical phenomena. The electronic processes involved are discussed with the results obtained from the fluorescence, phosphorescence and ESR measurements.

Experimental

Indole and N-methyl indole obtained from Katayama Co. were purified three times by recrystallization from distilled water. NaOH aqueous solutions and methanol as solvent were of special grade for analytical use and purchased from Katayama Co.

For the light emission and the ESR measurements, a quartz tube containing the sample solution with a diameter of 5 mm and a length of 20 cm was employed. Anaerobic solutions were prepared by the freeze-pump-thaw cycles.
The fluorescence spectra were determined with a Hitachi type 203 spectrofluorometer modifying the irradiating and recording parts. The sample in the quartz tube, cooled at 77 °K in a dewar vessel, was irradiated with the focussed ultraviolet light isolated from a 300 W xenon lamp through an interference filter and/or with the focussed monochromatic visible light isolated from a 500 W tungsten lamp through a Bausch & Lomb grating monochromator (6.4 nm/mm). The photomultiplier current was amplified with a home-made amplifier, and the resulting voltage was applied to a Riken Denshi type SP-G3 high sensitive recorder. The interference filter (peak, 280 nm, halfwidth 15 nm) was purchased from Nippon Shinku Kogaku Co.

The phosphorescence spectra were determined with a home-made spectrophosphorimeter which consists of a phosphoroscope (rotating cylinder type), a high sensitive photomultiplier (RCA C31034) and a Nikon G-250 monochrometer (6 nm/mm). For the signal detection, a single photon counting method was employed.

The ESR spectra of trapped electrons in glassy solutions produced by photoionization of indole were obtained with an ESR spectrometer built in Department of Physics, Faculty of Science, Kyushu University. The spectrometer was operated in the X-band with 100 kHz modulation and a phase sensitive detection system.

Results and Discussion

Fig. 1 shows the emission spectra of indole aqueous solutions at various NaOH concentrations. At room temperature, the emission spectrum at 10⁻⁵ M NaOH (pH 9) has a broad fluorescence band with the maximum intensity at 340 nm (curve 1). This fluorescence disappears above 1 M NaOH, and a new fluorescence band appears at 385 nm (curve 2). In a frozen aqueous solution at 77 °K, the emission spectrum is a superposition of the fluorescence and phosphorescence bands, and its structure varies with the NaOH concentration. The 340 nm fluorescence band at room temperature is blue-shifted at 77 °K; a 328 or 327 nm peak in curve 3 or 4, and a 315 nm shoulder in curve 5. The 395 nm fluorescence band appeared at room temperature is highly blue-shifted at 77 °K and is observable even below 1 M NaOH; a 375 nm shoulder in curve 3, a 360 nm band in curve 4 and a 370 nm band in curve 5. Several shoulders in the low temperature emission ranging from 400 to 500 nm is ascribed to the phosphorescence of indole, comparing them with the phosphorescence spectrum.

Figures 1-3 show the emission spectra of indole aqueous solutions at various NaOH concentrations at 77 °K were shown in Fig. 2. The phosphorescence intensity is expressed as the sum of counts obtained with ten times of measurements using a single photon counting method. The spectrum consists of three bands at about 405, 435 and 490 nm. These band intensities are not largely dependent on the NaOH concentration.

Fig. 3 shows the dependence of the fluorescence intensity of indole on pH (or Hammett acidity value $H_0^3$). At room temperature, the intensity of the 340 nm band is unchanged below pH 11, but it...
Fig. 3. Changes in fluorescence intensity of indole as a function of pH (or \( \text{H}_0 \)). Curves 1 and 2, relative intensities of 340 and 395 nm bands at room temperature, respectively; curves 3 and 4, relative intensities of 325 and 375 nm bands at 77 °K, respectively; concentration of indole, \( 5 \times 10^{-4} \) M; excitation light, light at 280 nm from xenon lamp; \( \text{H}_0 \), Hammett acidity value. The intensities of the 340 and 325 nm bands at pH 11 were normalized to unity.

Fig. 4. Changes in the ESR signal intensity or the stimulated emission intensity at 435 nm as a function of NaOH concentration. Curve 1, ESR signal intensity of irradiated indole aqueous solution; curve 2, ESR signal intensity of irradiated N-methyl indole aqueous solution; curve 3, emission intensity at 435 nm stimulated by irradiating the trapped electron with 580 nm light from a tungsten lamp after 3 min irradiation with 280 nm light; concentrations of indole and N-methyl indole, \( 5 \times 10^{-4} \) M; temperature, 77 °K; irradiation light for photoionization, light at 280 nm from xenon lamp.

decreases rapidly above pH 11 to zero at pH 14 (curve 1). Above pH 14, a new fluorescence band appears at 395 nm and increases steeply its intensity with increasing the NaOH concentration (curve 2). The 325 nm band at 77 °K decreases its intensity above pH 12 and disappears above \( \text{H}_0 \) 15 (curve 3). The 375 nm band at 77 °K occurs only above pH 13 and shows a rapid increase in intensity with increasing the NaOH concentration (curve 4). The intensities of the 325 and 375 nm bands were determined by separating each emission band from the total emission spectrum (Fig. 1) assuming the Gaussian distribution of an emission band. The quenching of the indole fluorescence in high alkaline region (curves 1 and 3 in Fig. 3) may be caused by the proton dissociation at N–H in indole ring in the excited singlet state as reported by Ricci. He described that the indole anion formed by the proton dissociation dissipates its excitation energy into heat through radiationless transition to the ground state, resulting in the quenching of the indole fluorescence. The proton dissociation in the ground state of indole did not occur even in a 10 M NaOH solution. This was confirmed by the absorption spectrum. According to the theoretical calculation with SCF CI method by Pill-Soon and Kurtin, the electron density at N atom in indole ring decreases largely in the excited singlet state compared with that in the ground state. This creates a favourable condition for the proton dissociation at N–H. From the half quenching pH value in curves 1 and 3 in Fig. 3, the pK value of the excited singlet indole was determined to be 12.6 at room temperature and 13.4 at 77 °K. Donkter, and Hopkin and Rumsey reported that the pK value at room temperature was 12.3 and 11.5, respectively. The fluorescence emissions at 395 and 375 nm may be caused by the indole anion produced by the proton dissociation in the excited singlet state of indole. Bridge and Williams observed the 395 nm fluorescence band of indole anion at the NaOH concentrations higher than 1 M at room temperature.

The alkaline aqueous solution of indole irradiated with 280 nm light at 77 °K exhibits an ESR signal with \( g = 2.003 \) and \( \text{H}_{\text{p-p}} = 20 \) G. This signal is caused by the trapped electrons produced by the photoejection of electron from indole to the rigid solvent media. The ESR signal intensity is shown in
Fig. 4 as a function of the NaOH concentration. The signal intensity was very slight below $10^{-1}$ M NaOH (pH 13), but increased steeply above this concentration of NaOH (curve 1). This profile is quite similar to the dependence of the 375 nm band intensity on the NaOH concentration (curve 4 in Fig. 3). On the other hand, the alkaline aqueous solution of N-methyl indole irradiated with 280 nm light at 77 °K caused a very slight ESR signal, almost independent of the NaOH concentration (curve 2). The N-methyl indole aqueous solution has no 375 emission at 77 °K even at high NaOH concentration. Since the 375 nm emission is considered to be caused by the indole anion, the results obtained above indicates that the trapped electron density is parallel to the concentration of the indole to some extent.

The dependence of the ESR signal intensity on the irradiation intensity at 280 nm was measured in a 5 M NaOH aqueous solution of indole at 77 °K. As shown in Fig. 5, the log of the ESR signal intensity is proportional to the log of the irradiation intensity. From the slope of the curve, the proportional constant was determined to be 1.90. This indicates that the trapped electron is produced through a two photon process. Therefore, considering the above-mentioned parallel relation between the trapped electron density and the indole anion concentration, a consecutive biphotonic process is responsible for the photoionization, and the second-photon absorbing species must be the indole anion.

For the photoionization of aromatic molecules, the following mechanisms were proposed by many investigators so far; (1) a consecutive biphotonic process with the lowest triplet state as photoactive intermediate, (2) a consecutive biphotonic process with the lowest excited singlet state as photoactive intermediate, (3) an ionization through bimolecular triplet-triplet annihilation process, (4) a consecutive biphotonic process with a "semi-ionized state" as photoactive intermediate, and (5) an ionization due to simultaneous absorption of two photons. These processes are highly affected by the environmental conditions such as solvent polarity, pH and temperature.

Next, to study the behaviour of the trapped electron, the following experiments were performed. An indole aqueous solution was irradiated with a strong 280 nm light for 3 min at 77 °K. After standing the solution in the dark for 1 min, it was irradiated with a visible light. The irradiation of visible light induced an emission with three peaks at 405, 435 and 475 nm in its spectrum similar to the phosphorescence spectrum as shown in Fig. 2. The absorption band of the electron trapped in frozen aqueous media is usually in the wavelength range from 450 to 700 nm. Irradiation with a light in this wavelength range detraps the trapped electron. The recombination of the detrapped electron with its mother molecule leads to the stimulated emission at 435 nm. The emission intensity at 435 nm stimulated with the irradiation of 580 nm was measured with a two light excitation; the excitation with a 280 nm light, the first photon, and a variable wavelength light, the second photon. The two light excitation was necessary for the production of trapped electrons in this case, because the intensity of the 280 nm light was too weak to produce the photoionization without the second photon. The action spectrum (stimulated emission intensity vs wavelength of the second photon) is a broad band with a peak at about 340 nm as shown in Fig. 6. The molecular species with the 340 nm absorption band may be the indole anion. This anion may act as the photoactive intermediate absorbing the second photon in the photoionization process. This is also supported by the following experimental results.

Fig. 7 shows the dependence of the stimulated emission intensity at 435 nm on the second photon intensity. The log of the stimulated emission intensity is proportional to the log of the second photon intensity. The proportional constant was
determined to be 0.95, indicating that the stimulated emission occurs as a one photon process with respect to the second photon.

Fig. 8 shows changes in intensity of the 435 nm emission band with the irradiation (580 nm) time at various NaOH concentrations. The emission intensity showed a rapid rise upon irradiation with 580 nm light, then decreased gradually to zero with the irradiation time. This change corresponds to the time course of the consumption of the trapped electron due to the recombination with its mother molecule. The total emission intensity increased steeply with increasing the NaOH concentration. This profile is quite parallel to the increase in ESR signal intensity or trapped electron density as seen in Fig. 4. The stimulated emission intensity is considered to monitor the trapped electron density.

Next, the change in phosphorescence intensity of indole with the irradiation time was investigated in a methanolic glassy solution at 77 °K. The results are shown in Fig. 9. When indole is excited merely with a strong 280 nm light, the phosphorescence intensity at 435 nm increases gradually to a saturation level with the irradiation time (curve 1).
The gradual increase and the saturation in phosphorescence intensity are caused by the involvement of two processes besides the usual emission process of phosphorescence; the photoionization leading to phosphorescence quenching and the creation of the phosphorescence state due to the recombination of solvated electron with its mother molecule. However, on irradiation with the mixed lights of 280 nm and a near ultraviolet (e.g. 340 nm), the phosphorescence intensity was lower than that with the single light excitation at 280 nm. The intensity of 280 nm light was same in both cases. Stopping the 340 nm excitation at the saturation level enhanced the phosphorescence intensity to the single excitation level (curve 2). This indicates that the irradiation of 340 nm light may cause an additional photoionization resulting in the decrease in triplet state density in the photostationary state. Namely, indole in methanolic solution is also photoionized by a consecutive two photon process. There is no indication of production of indole anion in methanolic solution in the excited singlet state. Therefore, the photoactive intermediate absorbing the second photon in methanolic solution is probably the triplet state of indole.

From the phosphorescence quenching by the second photon, the equation, \( I_1/I_2 = 1 + \tau k \), was assumed at the saturation level in curve 2. \( I_1 \) and \( I_2 \) are, respectively, the phosphorescence intensities at the saturation levels on single and two light irradiations. \( \tau \) is the lifetime of the phosphorescence state and \( k \) the rate constant for phosphorescence quenching by the second photon. \( k \) is proportional to the excitation light intensity and the absorption coefficient in the triplet state. The \( k \) value was calculated using the observed values of \( I_1/I_2 \) and \( \tau \). The results are given in Table I, with those obtained in the indole aqueous solution. The indole phosphorescence was more effectively quenched by the irradiation of the second photon in the methanolic solution than in the NaOH aqueous solution.

<table>
<thead>
<tr>
<th>Methanolic soln.</th>
<th>3 M NaOH aqueous soln.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( I_1/I_2 )</td>
<td>1.125 ( \text{sec}^{-1} )</td>
</tr>
<tr>
<td>( \tau ) [sec]</td>
<td>6.5 ( \text{sec}^{-1} )</td>
</tr>
<tr>
<td>( k ) [sec(^{-1})]</td>
<td>1.9 ( \cdot ) ( 10^{-2} )</td>
</tr>
</tbody>
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In conclusion, at present, what state of indole anion is the photoactive intermediate in the NaOH aqueous solution, remains open to be elucidated. However, the electron density at N atom in indole anion may be higher than that in indole. In addition to this, the high polarity of water may provide a favourable condition for the formation of a charge transfer state in indole anion-H\(_2\)O system. These suggest that a "semi-ionized state" or a "charge transfer state" produced by electron transfer from indole anion to water molecule might act as a photoactive intermediate state.