Photochemical Hydrogen–Deuterium Exchange Reaction of Tryptophan. The Role in Nonradiative Decay of Singlet Tryptophan[1]

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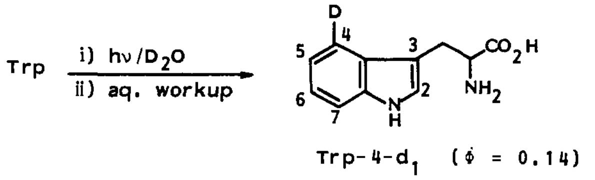
The mechanism of nonradiative decay of singlet excited tryptophan (Trp) in aqueous solution has been the subject of extensive studies and considerable controversy.[2] Mechanisms involving intramolecular proton transfer[3] from the α-ammonium group of the side chain and charge-transfer quenching[4] by the side chain have been proposed for the nonradiative decay process. We report herein a remarkably efficient and highly selective photosubstitution of the C-4 hydrogen of Trp with deuterium of solvent D_2O which has been overlooked for a number of years. Such photosubstitution that may also occur in normal light water giving rise to original Trp as photoproduct, *i.e., photoreaction of trp without photoproduct*, is suggested to be one major path of nonradiative decay of singlet Trp.

External irradiation of a degassed solution of Trp (10 mM) in unbuffered D_2O (99.95%, pD 5.5) in a NMR cell with Pyrex-filtered light from a 400-W high-pressure mercury lamp resulted in a rapid incorporation of the deuterium from D_2O into the indole nucleus. Figure 1 illustrates the progress of the deuterium incorporation as monitored by 400-MHz ^{1}H NMR. Deuterium was incorporated up to 96% into the C-4 position of Trp with a minor deuteration at C-2 (7%) and C-7 (8%) positions within a 30-min irradiation. Assignment of the deuterated positions was made on the basis of ^{1}H and ^{13}C NMR,[5] and the deuterium contents were determined by careful integration of the signals in the aromatic region (e.g., C-4 H at δ 7.75) in the 400-MHz ^{1}H NMR. The quantum yield of the deuterium incorporation was determined to be 0.14 ± 0.02 by using an optical bench fitted with a monochrometer at 278 nm employing ferrioxalate actinometry.[6] No decrease in quantum yield was observed with saturating concentrations of N_2O[7] in D_2O, indicating that photoionization[8] of Trp to hydrated electrons and $Trp^{+\cdot}$ is not responsible for the photosubstitution.[9]



Conversely, irradiation of C-4-deuterated Trp (Trp-*4*-d_1)[10] in light water (50 mM phosphate buffer, pH 7.0) resulted in a loss of the C-4 deuterium into the solvent to give Trp ($\phi = 0.09 \pm 0.02$).[6] This implies that a similar hydrogen–hydrogen exchange reaction is efficiently occurring at the C-4 position upon irradiation of Trp in light water at neutral pH, but this reaction gives no photoproduct. Addition of a fluorescence quencher KI[11] to the reaction system inhibited the loss of the C-4 deuterium with a linear Stern–Volmer plot with slope of 14 ± 2 M^{-1} over a KI concentration range of 100-fold. The value is in good agreement with the slope (13 ± 1 M^{-1}) of Trp fluorescence quenching, consistent with the participation of singlet Trp-*4*-d_1 in the photosubstitution. The efficiency of the deuterium loss from Trp-*4*-d_1 is a function of the solution pH. At pH 7 where the α-amino group is protonated, the photosubstitution proceeded efficiently ($\phi = 0.09$), whereas at pH 10.5 (unprotonated amino group) the quantum yield for deuterium loss is only 0.03. The pH dependence may relate to the lower fluorescence yield ($\phi = 0.14^{3b,d}$) of Trp at pH 7 compared to that ($\phi = 0.31^{3d}$) at pH 10.5 and is suggestive of the intervention of the α-ammonium group in the photosubstitution.

Regioselective incorporation of the deuterium into the C-4 position suggests an intramolecular proton-transfer process from the α-ammonium group. On the basis of the regioselectivity and the pH dependency we propose the mechanism in Scheme I for the deuterium loss from Trp-*4*-d_1 at pH 7. Intramolecular proton transfer from the α-ammonium group to the C-4 position of the

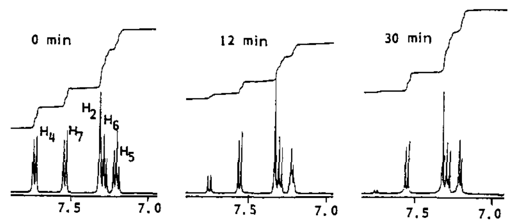
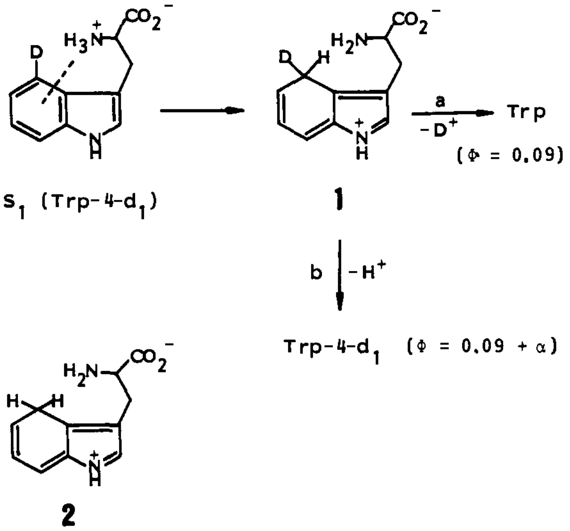


Figure 1. Deuterium incorporation upon irradiation of tryptophan in D_2O.

Scheme I



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[5] Trp-*4*-d_1: ^{1}H NMR (D_2O) δ 3.31 (dd, 1 H, *J* = 15, 8 Hz), 3.48 (dd, 1 H, *J* = 15, 5 Hz), 4.06 (dd, 1 H, *J* = 8, 5 Hz), 7.20 (d, 1 H, *J* = 8 Hz), 7.29 (dd, 1 H, *J* = 8, 7 Hz), 7.32 (s, 1 H), 7.54 (d, 1 H, *J* = 8 Hz); intensity of the C-4 H signal (δ 7.75, d, *J* = 8 Hz) of Trp decreased with collapse of the C-5 H signal (δ 7.20, dd) into a doublet (*J* = 8 Hz) upon irradiation in D_2O (Figure 1); ^{13}C NMR (Me_2SO-d_6) δ 27.2, 54.9, 109.6 (C-3), 114.4 (C-7), 118.2 (C-6), 120.8 (C-5), 124.2 (C-2), 127.3 (C-3a), 136.5 (C-7a), 170.7; the signal of C-4 carbon (δ 118.3) disappeared upon prolonged irradiation in D_2O; exact MS (as methyl ester), *m/e* (M^+) 219.1118 (calcd for $C_{12}H_{13}N_2O_2D$, 219.1118).

[6] A degassed solution of Trp (0.87 mM) in unbuffered D_2O was irradiated at 278 nm with slit width of 10 nm at 20 °C. The deuterium content was assayed by partial domain integration of the signal at δ 7.75 within 15% conversion. The error limit was ca. ±20%. The quantum yield at 290 nm was 0.19 ± 0.03.

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[9] The quantum yield also ruled out the intervention of monophotonic photoionization process ($\phi \sim 0.08$ at 280 nm^{8c}) in this photosubstitution.

[10] Prepared by prolonged irradiation of Trp in D_2O followed by purification with high-performance LC.[5] The deuterium content (96% at C-4, 7% at C-2, 8% at C-7) was assayed by 400-MHz ^{1}H NMR.

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indole ring would give a protonated intermediate **1**, which then loses a deuteron (path a) or a proton (path b) to yield nondeuterated Trp and original Trp-4-d_1.[12] Unfortunately, the quantum efficiency of the latter process (path b) cannot be obtained experimentally. However, it is obvious that the quantum yield for path b may well exceed 0.09 since the rate of lighter hydrogen loss from **1** by deprotonation would be much faster than that of the deuteron loss ($\phi = 0.09$). Thus, the quantum yield of proton transfer to the C-4 position of singlet Trp-4-d_1 leading to **1** may reach to more than 0.18, i.e., $0.18 \pm \alpha$.[13] Our results strongly suggest that the reaction of the singlet indole ring with the α-ammonium group leading to the protonated species **2** constitutes one major route for nonradiative decay of singlet Trp at neutral pH.[14] Irradiation of 1-methyltryptophan (18 mM) in D_2O (pD 5.5) gave 4-deuterated 1-methytryptophan (22% D at 30 min) regiospecifically, indicating that the photodeprotonation[3b,15] of N-1 hydrogen of Trp is not responsible for the hydrogen–deuterium exchange reaction. Irradiation of L-tryptophyl-L-tyrosine in D_2O resulted in a selective deuteration at the C-4 position of Trp with no incorporation into the tyrosine moiety.

In summary, we found an efficient and highly selective photosubstitution of C-4 hydrogen of Trp with deuterium in D_2O. Intramolecular proton transfer from the α-ammonium group giving rise to formation of the protonated species **2** has been suggested to play an important role in nonradiative decay of singlet Trp at neutral pH. In light of this finding, mechanistic studies on fluorescence of Trp and indole derivatives should be reinvestigated.

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Registry No. trp, 73-22-3; trp-4-d_1, 90697-20-4.

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(12) The calculation of indole by CNDO/S method indicates that the C-4 position displays a considerable increase in electron density on excitation to the ^1L_b state: Evleth, E. M.; Chalvet, O.; Bamière, P. *J. Phys. Chem.* **1977**, *81*, 1913.

(13) If one assumes that the kinetic isotope effect (k_H/k_D) for path a and path b is 2.0, then the quantum yield for the formation of **1** would be 0.27.

(14) Quantum efficiencies for the processes occurring from singlet Trp in light water at pH 7 are as follows: $\phi_f = 0.14$;[3b] $\phi_{isc} \sim 0.27$[8c] (0.10[3e]); $\phi_{ion} \sim 0.08$.[8c] ϕ_f in D_2O is reported to be 0.32.[3b]

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Thermochromism of Charge-Transfer Complexes between a Vesiculating Paraquat Derivative and Benzidine

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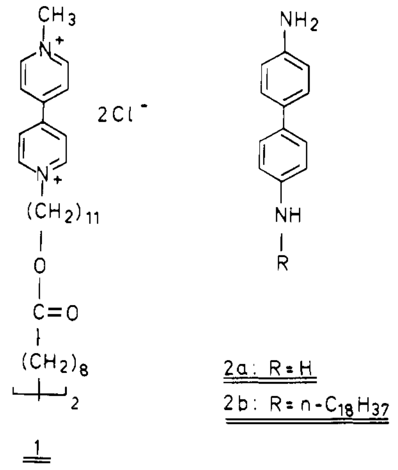
We have recently described the bolaamphiphile **1** with two paraquat head groups, which is readily soluble in water. **1** does, however, form large aggregates (micelles, vesicles, monolayered crystals) if the chloride counterions are partly or fully replaced by perchlorate or if the paraquat dication is reduced to the viologen monocation radical.[1] Further experiments indicated that the reversible aggregation of the tetrachloride **1** can also be induced by cooling of a relatively concentrated aqueous solution ($\geq 10^{-3}$ M) to 10 °C. A ^{1}H NMR probe of **1** in D_2O, for example, gives a well-resolved spectrum at 30 °C and very broad bands at 0 °C.

A more dramatic effect of the temperature-dependent aggregation is the reversible formation of a colored polymeric molecular complex ("Scheibe complex"[2]). If benzidine (**2a**) (2 mM) is added to the paraquat tetrachloride **1** (1 mM) at room temperature a slightly reddish colorization of the aqueous solution is observed.[3] On cooling to 15 °C the color turns to bluish, at 5 °C it is dark blue. A new, relatively broad band with a maximum at 615 nm at 15 °C and 630 nm at 5 and 0 °C (OD = 1.9) is observed (Figure 1). On warming to 30 °C the blue color disappears. This cycle can be repeated several times without indications of decomposition of the components. The blue solution is stable against oxygen, gives no ESR signal (no formation of viologen radicals), and produces a blue precipitate after some hours or days. The composition of the precipitate is benzidine–**1**, 4:1,[3,4] i.e., two benzidine molecules per bipyridinium head group. Under the assumption that in solution a 1:1 complex is first formed, we determined a complex formation constant of $K = 250 \pm 100$ M^{-1} following Drago's procedure.[5] This compares to a literature value for a dimethylviologen–benzidine complex of $K = 10.5$ M^{-1} in ethanol–water, 1:1.[3] An extinction coefficient $\epsilon = 6500 \pm 1400$ was also determined. If sodium perchlorate (1 mM) was added to the solution at 40 °C, a blue complex was formed at once ($\lambda_{max} = 650$ nm). On heating this solution, precipitation was first observed, but above 70 °C a clear, slightly yellow solution was obtained. With *N*-octadecylbenzidine (**2b**), no charge-transfer complex was formed with **1**. Indole gave a yellow molecular complex ($\lambda_{max} = 388$ nm) but no effect of temperature (0–30 °C) was observed.



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2a: R = H

2b: R = n-$C_{18}H_{37}$

The complex formation is pH dependent, since protonated benzidine is not an efficient electron donor and does not bind to paraquat cations. At 0 °C we determined the pH at which half of the blue complex that was present at pH = 6.5 had disappeared. We found this to be the case at pH = 4.6, which is identical with the pK_a = 4.66 reported for the first protonation of benzidine.[6] From this finding it can be concluded that one amino group of benzidine cannot be in contact with the paraquat heat groups, since it is known that the pK_a of bases that are surrounded by positive charges is lowered by at least one unit.[7] We propose a structure in which a positive center of the paraquat head groups binds to one of the electron-rich aniline groups of benzidine. The second aniline ring would be in the aqueous phase (Figure 2).

This structures is in accordance with several crystal structures of charge-transfer complexes, which show the aromatic rings of both components to be shifted by half a ring diameter against each other.[8] The model also explains why the N-alkylated benzidine derivative **2b** gives no charge-transfer complex. It would disturb the aggregation of **1** and its aniline rings could be forced into a position relative to the bipyridinium head groups, where no charge

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