Kinetics and Mechanism of the Photochemical Reaction of 2,2′-Dipyridyl with Tryptophan in Water: Time-Resolved CIDNP and Laser Flash Photolysis Study

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The mechanism of the reactions between photoexcited 2,2′-dipyridyl and *N*-acetyl tryptophan has been studied by laser flash photolysis and time-resolved CIDNP (Chemically Induced Dynamic Nuclear Polarization). The transient absorption spectra obtained at different delays after the laser pulse are attributed to the triplet state of dipyridyl and to dipyridyl and tryptophan radicals. Depending on the pH of the solution, all three intermediates can be present in either protonated or deprotonated forms. It is shown that irrespective of pH the primary photochemical step is electron transfer from the tryptophan to triplet dipyridyl followed by protonation/deprotonation of the radicals so formed. The rate constant of the reaction of triplet dipyridyl with tryptophan is close to the diffusion-controlled limit and decreases slightly with increasing pH. The kinetics and the stationary value of the CIDNP are determined by the rates of radical termination, nuclear paramagnetic relaxation, and degenerate electron exchange. The last reaction is important for the protonated tryptophan radical and determines the CIDNP kinetics of tryptophan in acidic conditions. The nuclear relaxation times estimated from the CIDNP kinetics are 44 ± 9 μs for all protons in the dipyridyl radical, 91 ± 20 μs for the β-CH_2, 44 ± 9 μs for H2,6, and 63 ± 12 μs for H4 aromatic protons in the tryptophan radical.

Introduction

Chemically Induced Dynamic Nuclear Polarization[1] (CIDNP) is an attractive NMR technique for studying the structures and interactions of proteins. The substantial enhancement of NMR intensity and the information afforded on the accessibility of aromatic amino acid side chains make it particularly suitable for partially structured proteins and to the investigation of protein folding in real-time.[2−5] Polarization originates in intersystem crossing (ISC) in radical pairs formed as intermediates in a photochemical reaction and is a result of the sensitivity of ISC to nuclear spin configuration. This selectivity is determined by the magnetic resonance parameters of the radical pairs: *g* factors and nuclear hyperfine interactions. The polarization in the diamagnetic products is observed mainly for those nuclei with hyperfine couplings in the intermediate radicals, allowing one to determine the identity of the radicals from the polarization pattern. In studies of proteins and protein folding, photo-CIDNP is generated by means of reversible electron or hydrogen atom transfer between a photoexcited dye (usually a flavin) and the side chains of aromatic amino acid residues.

Although flavins have been almost exclusively used as CIDNP photosensitizers for protein studies and have many desirable properties, it is of interest to extend the technique to other dye molecules which may have different selectivity for exposed aromatic side chains, for example by virtue of their size. There is some evidence that the observed CIDNP intensity produced by flavins is a nonlinear function of the side chain exposure, as judged by static accessibility calculations based on crystal structures, such that only the most strongly exposed residues show substantial enhancements. Extra information might therefore be available by using a smaller, less selective photosensitizer. Such a molecule would need to be water soluble and to have a strong optical absorption so that a low concentration can be used; it should also generate large NMR enhancements in a cyclic reaction that leads to no net chemical modification of the protein. These conditions are met by the aza-aromatic compound 2,2′-dipyridyl (DP), which has been extensively used as a chelating agent in charge-transfer complexes.[6] Under laser irradiation at 308 nm, strong nuclear polarization is formed in the reactions with aromatic *N*-acetyl amino acids (tryptophan, tyrosine, and histidine), and the CIDNP patterns observed are very similar to those produced by flavins.[7] Other factors in favor of DP are its high triplet quantum yield[8] and the strong optical absorption of the different radicals formed from the triplet.

Detailed spectral and kinetic studies of DP photochemistry[8−13] have shown that the positions of the absorption maxima are sensitive to the electronic structure of intermediates in the reaction, allowing one to distinguish the three possible mechanisms for the primary photochemical step: hydrogen atom transfer, electron transfer, and electron transfer followed by proton transfer. Although much is known about DP photophysics and photochemistry, the photochemical reactions of dipyridyl with amino acids have not been studied. Here, we apply time-resolved CIDNP (TR-CIDNP) and laser flash photolysis (LFP) under closely similar experimental conditions; both methods employ the same wavelength for excitation (308 nm) and have comparable time resolution. The main aims are to establish the mechanisms of the reactions between excited DP and aromatic amino acids, as well as the details of CIDNP formation, to determine the rate constants of the different stages of these reactions and to characterize the reaction intermediates. This information is needed to form a solid basis for the use of 2,2′-

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dipyridyl in studies of protein structure and protein folding. Knowledge of the initial step of the photoreaction is important because electron and hydrogen transfers have different steric requirements; to be abstracted, a hydrogen atom must be accessible to the sensitizer, and probably not be hydrogen bonded, while electron transfer requires electronic overlap of donor and acceptor. The rate constants of the reactions that create radical pairs are needed to assess the importance of competition of different amino acid side chains for the excited photosensitizer.[4a] Finally, the rate constants of radical recombination and degenerate electron exchange and the nuclear spin−lattice relaxation times in the radicals are crucial when accounting for the effects of cancellation of the equal and opposite polarizations arising from "escape" and "recombination" channels.[4a] All these factors can influence the relative and absolute CIDNP intensities of amino acid side chains in proteins and so must be understood for a proper interpretation of CIDNP data. In addition, dyes such as 2,2′-dipyridyl, which absorb at the wavelengths of pulsed (e.g., excimer) lasers, should be useful for stopped-flow CIDNP studies on shorter time scales than are possible with continuous wave visible light sources.[4b]

This article deals with the photolysis of DP in the presence of *N*-acetyl tryptophan, TrpH (the structures of the initial compounds are shown in Figure 6); studies of tyrosine and histidine are in progress and will be reported elsewhere.

Experimental Section

Time-resolved CIDNP. A detailed description of our TR-CIDNP experiment was given earlier.[14] A sample, purged with argon and sealed in a standard NMR Pyrex ampule, was irradiated by a COMPEX Lambda Physik excimer laser (wavelength 308 nm, pulse energy up to 150 mJ) in the probe of an MSL-300 Bruker NMR spectrometer. TR-CIDNP experiments were carried out using the usual pulse sequence: saturation−laser pulse−evolution time−detection pulse−free induction decay. As the background signals in the spectrum are suppressed by the presaturation pulses, only signals of the polarized products formed during the variable delay between the laser and NMR radio frequency pulse appear in the CIDNP spectra.

Laser Flash Photolysis. A detailed description of the LFP equipment has been published recently.[15] Solutions in a rectangular cell (10 mm × 10 mm) were irradiated with a Lambda Physik EMG 101 excimer laser (308 nm, pulse energy up to 100 mJ). The dimensions of the laser beam at the front of the cell were 3 mm × 8 mm. The monitoring system includes a xenon short arc lamp DKSh-120 connected to a high current pulser, two synchronously operating monochromators, a Hamamatsu R955 photomultiplier, and a digitizer LeCroy 9310A. All solutions were purged with argon for 15 min prior to use and during the irradiation.

Chemicals. D_2O (Aldrich), 2,2′-dipyridyl (Aldrich), and *N*-acetyl tryptophan (Sigma) were used as received. H_2O was doubly distilled. Buffers were prepared with dibasic sodium phosphate and citric acid or, in some cases, with potassium tetraoxalate.

Results and Discussion

1. Flash Photolysis of DP. Depending on the pH of the solution, 2,2′-dipyridyl can exist in either protonated, DPH^+, or deprotonated, DP, states ($pK_a = 4.3$).[16] DP was found to have an absorption maximum at 281 nm with $\epsilon_{max} = 14\,600\ M^{-1}\ cm^{-1}$ and $\epsilon_{308} = 1200\ M^{-1}\ cm^{-1}$, whereas the spectrum of DPH^+ is shifted to 301 nm and $\epsilon_{308} = 12\,900\ M^{-1}\ cm^{-1}$,

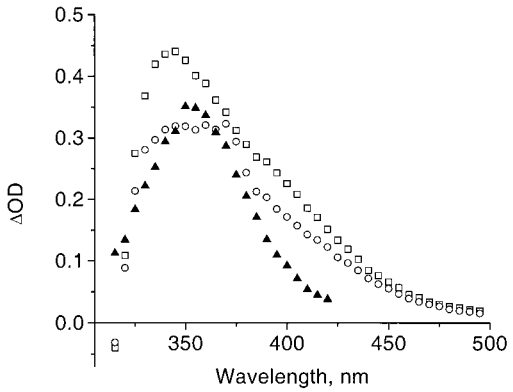

Figure 1. Transient absorption spectra obtained during the irradiation of DP in basic (▲) and acidic solutions ((□) 200 ns after the laser pulse, (○) 4 μs after the laser pulse).

consistent with the previous studies.[16,17] Thus, for 308 nm irradiation of dipyridyl solutions at pH < 4.3, the main (>90%) light-absorbing species is DPH^+, which after fast intersystem crossing ($k_{ISC} \approx 1.5 \times 10^{10}\ s^{-1}$)[8a,12] yields the triplet $^TDPH^+$. At pH > 6.3, neutral triplets, ^TDP, are formed. In the intermediate region, 4.3 < pH < 6.3, the absorptions of protonated and deprotonated DP are of similar strength.

Figure 1 shows the triplet absorption spectra of DP (triangles) and DPH^+ (squares) obtained 200 ns after laser irradiation of dipyridyl in neutral and acidic (10^{-2} M HCl) solutions, respectively. These spectra are in a good agreement with published data.[8−10,18,19] Neutral ^TDP decays exponentially with $k = 5 \times 10^4$ to $7 \times 10^4\ s^{-1}$ at all wavelengths; this rate constant does not depend on DP concentration. At high laser energies and therefore large initial triplet concentrations, some admixture of second-order kinetics is observed. The second-order component presumably arises from triplet−triplet annihilation, whereas the exponential decay is mainly due to quenching of triplet DP by residual oxygen (the reported rate constant of ^TDP decay in deoxygenated water solution is $10^4\ s^{-1}$).[8a]

The signal of the triplet cation $^TDPH^+$, measured at 350 nm, disappeared principally by second-order kinetics at all measurable initial triplet concentrations, with $k_t/\epsilon = (1.3 \pm 0.1) \times 10^5$ cm s^{-1}. Simultaneously, the formation of dipyridyl cation radical $DPH_2^{+\bullet}$ with its characteristic narrow band[10,11] at 370 nm was observed (Figure 1, circles). It therefore seems likely that one of the possible mechanisms of cation triplet decay is

$$2\,^TDPH^+ \rightarrow DPH_2^{+\bullet} + DP^{+\bullet} \qquad (1)$$

The same kind of the triplet disproportionation was recently reported for the related compound 2,2′-bipyrimidine.[20]

2. Flash Photolysis of DP with TrpH. Figure 2 shows transient absorption spectra obtained at 200 ns (squares) and 8 μs (circles) after laser flash photolysis of 2×10^{-3} M DP and 9.6×10^{-4} M TrpH in basic (2×10^{-2} M NaOH) solution. Under these conditions, the absorption of TrpH at 308 nm is small compared to that of DP. Thus, the primary process is photoexcitation of DP; the spectrum obtained 200 ns after the laser pulse coincides with that of triplet ^TDP (compare Figures 1 and 2). Open circles in Figure 2 correspond to the spectra of radical intermediates formed after the completion of the triplet decay: neutral dipyridyl radical $DPH^\bullet$ (spectral maxima at 365 and 470 nm)[8−10] and deprotonated tryptophanyl radical $Trp^\bullet$ (maximum at 510 nm).[21−27]

In acidic solution (7.1×10^{-5} M DP and 4.4×10^{-4} M TrpH, pH = 4.0), all observed intermediates are protonated, including the dipyridyl triplet cation $^TDPH^+$ observed immediately after

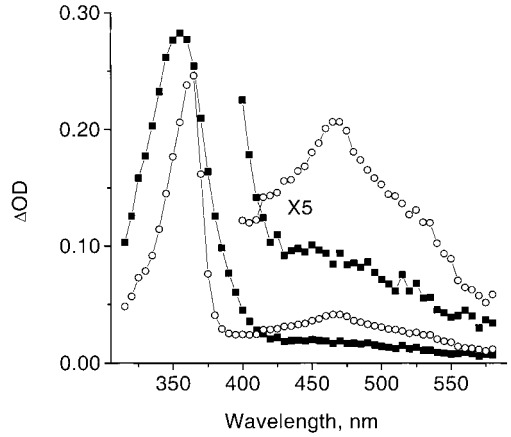

Figure 2. Spectra obtained during the irradiation of DP with TrpH in basic solution. The spectrum obtained 200 ns after the laser pulse (■) corresponds to ^{T}DP; the spectrum taken 8 μs after the flash (○) shows the absorption of the DPH$^{\bullet}$ radical (maxima at 365 and 470 nm) and Trp$^{\bullet}$ radical (maximum at 510 nm).

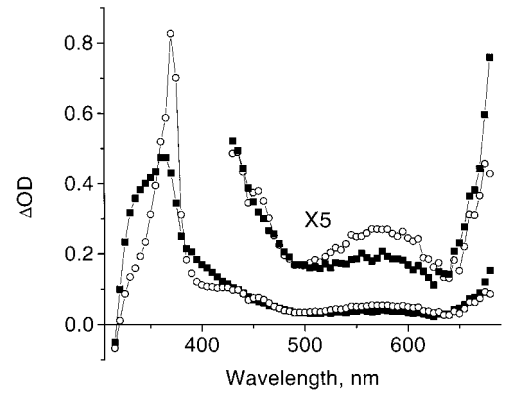

Figure 3. Spectra obtained during the irradiation of DP with TrpH in acidic solution. The spectrum obtained 200 ns after the laser pulse (■) corresponds to ^{T}DPH^{+}; the spectrum taken 4 μs after the flash (○) shows the absorption of the $DPH_2^{+\bullet}$ radical (maxima at 370 and 455 nm) and the $TrpH^{+\bullet}$ radical (maximum at 570 nm).

the laser pulse (Figure 3, squares), the dipyridyl cation radical $DPH_2^{+\bullet}$ (maxima at 370 and 455 nm) and the tryptophan cation radical $TrpH^{+\bullet}$ (maximum at 570 nm)[21−27] (Figure 3, circles).

It is important to note that these spectra do not determine the nature of the primary photochemical step, which could be (i) electron transfer from tryptophan to the triplet dye followed by the protonation of $DP^{-\bullet}$ and deprotonation of $TrpH^{+\bullet}$ in basic solution, or by the protonation of DPH$^{\bullet}$ in acidic conditions, or (ii) hydrogen atom transfer, followed by the protonation of tryptophanyl radical in acidic solution. Both pathways give the same intermediate radicals, and only kinetic measurements at different pH's can distinguish the two mechanisms.

Kinetic measurements were carried out at three wavelengths; the decay of the triplet dye was monitored at 320 nm, while the formation of dipyridyl and tryptophan radicals was measured at 370 and 570 nm (510 nm at pH > 5.5), respectively. To obtain information on the bimolecular radical termination reactions, three or four transients were obtained at different laser energies for each measurement.

The experiments performed on buffered solutions of DP and TrpH show that the decay at 320 nm as well as the signal rise at both 370 and 570 nm (510 nm) all follow first-order kinetics. The observed rate constant k_{obs} depends linearly on the initial TrpH concentration and at all pH's is the same at all three wavelengths within experimental error. Thus, the observed

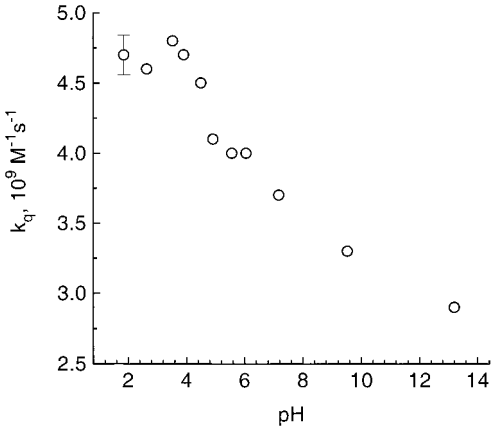

Figure 4. pH dependence of the second-order rate constant for the quenching of the triplet dipyridyl by tryptophan in buffered solutions.

kinetics can be attributed to reaction of tryptophan with triplet dipyridyl, $k_{obs} = k_q$[TrpH]. The dependence of k_q on pH in the buffered solutions is shown in Figure 4. From the similar kinetic behavior at the different wavelengths, one can conclude that the rate of protonation of the radical intermediates is much faster than the rate of radical formation and does not depend on the pH of the solution. Protonation occurs with the direct participation of the buffer component, citric acid.

$$DPH^{\bullet} + RCOOH \rightarrow DPH_2^{+\bullet} + RCOO^{-} \quad (2)$$

$$Trp^{\bullet} + RCOOH \rightarrow TrpH^{+\bullet} + RCOO^{-} \quad (3)$$

These processes are to be expected given that the pK_a of citric acid (3.31) is smaller than that of dipyridyl (pK_a = 5.6)[10] and of tryptophan (pK_a = 4.3)[21c,23] radicals.

In nonbuffered solutions (the pH is regulated by the addition of either HCl or NaOH), the rates of the signal growth at 370 and 570 nm coincide only at low pH (pH < 3); moreover, this growth can no longer be described by a single exponential. At 3 < pH < 5, an increase in pH slows down the growth at 370 nm (Figure 5a), whereas the signal at 570 nm becomes weaker and grows somewhat faster (the traces in Figure 5 are scaled so as to have comparable amplitudes). The initial rapid absorbance change at 370 nm corresponds to the absorption of the triplet dipyridyl, and at 570 nm it probably belongs mainly to $TrpH^{+\bullet}$ radicals formed by direct photoionization of TrpH. The subsequent kinetic behavior provides convincing evidence that the primary photochemical step is electron transfer and that the reaction scheme for radical formation in nonbuffered solution may be written as follows.

In acidic conditions (pH < 4.3),

$$DPH^{+} \xrightarrow{h\nu,\mathrm{ISC}} {}^{T}DPH^{+} \quad (4)$$

$${}^{T}DPH^{+} + TrpH \xrightarrow{k_q} DPH^{\bullet} + TrpH^{+\bullet} \quad (5)$$

$$DPH^{\bullet} + H^{+} \underset{k_{d1}}{\overset{k_{p1}}{\rightleftharpoons}} DPH_2^{+\bullet} \quad (6)$$

$$TrpH^{+\bullet} \underset{k_{p2}}{\overset{k_{d2}}{\rightleftharpoons}} Trp^{\bullet} + H^{+} \quad (7)$$

In neutral or basic conditions (pH > 6.3),

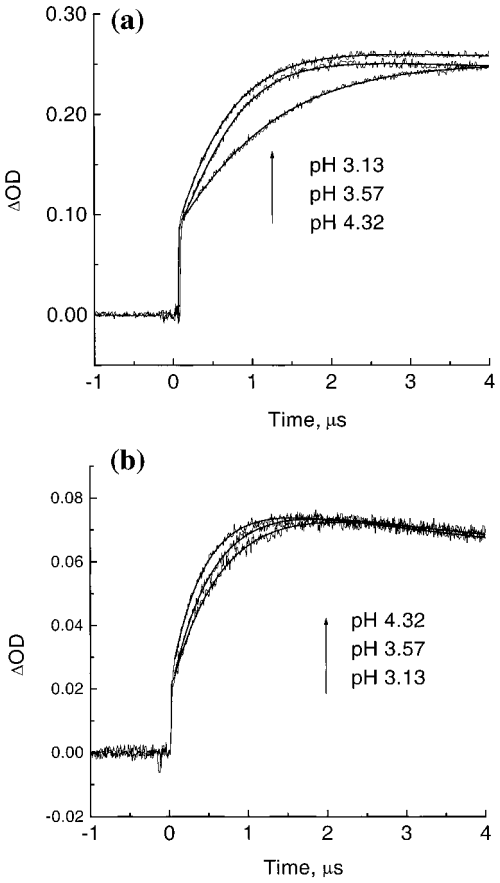

Figure 5. Kinetics of the formation of DPH_2^+ radical (a) and $TrpH^+$ radical (b) at different pH in nonbuffered solutions.

$$DP \xrightarrow{h\nu,\mathrm{ISC}} {}^{T}DP \tag{8}$$

$${}^{T}DP + TrpH \xrightarrow{k_q} DP^{-\bullet} + TrpH^{+\bullet} \tag{9}$$

$$DP^{-\bullet} + H_2O \xrightarrow{k_{p3}} DPH^{\bullet} + OH^- \tag{10}$$

$$TrpH^{+\bullet} \underset{k_{p2}}{\overset{k_{d2}}{\rightleftharpoons}} Trp^{\bullet} + H^+ \tag{7}$$

The anion $DP^{-\bullet}$ is a strong base with $pK_a > 14$[8a,10b] so that reaction 10 should be fast at any pH. The rates of the reactions 6 and 7 in nonbuffered conditions depend on the pH of the solution. Reactions 5−7 (acidic solution) can be described by the following set of equations.

The triplet decay observed at 320 nm:

$$\frac{d[T]}{dt} = -k_q[TrpH][T] \tag{11}$$

The formation of dipyridyl radicals, monitored at 370 nm:

$$\frac{d[DPH^{\bullet}]}{dt} = k_q[TrpH][T] + k_{d1}[DPH_2^{+\bullet}] - k_{p1}[H^+][DPH^{\bullet}] \tag{12}$$

$$\frac{d[DPH_2^{+\bullet}]}{dt} = -k_{d1}[DPH_2^{+\bullet}] + k_{p1}[H^+][DPH^{\bullet}] \tag{13}$$

The evolution of tryptophan radicals (570 nm):

$$\frac{d[TrpH^{+\bullet}]}{dt} = k_q[TrpH][T] - k_{d2}[TrpH^{+\bullet}] + k_{p2}[H^+][Trp^{\bullet}] \tag{14}$$

$$\frac{d[Trp^{\bullet}]}{dt} = k_{d2}[TrpH^{+\bullet}] - k_{p2}[H^+][Trp^{\bullet}] \tag{15}$$

Denoting $k_{obs} = k_q[TrpH]$, $k_1 = k_{p1}[H^+]$, $k_2 = k_{p2}[H^+]$, the solutions of eqs 12−15 for cation radicals are

$$[DPH_2^{+\bullet}] = T_0\left[\frac{k_1}{k_{d1}+k_1} - \frac{k_1}{k_{d1}+k_1-k_{obs}}\exp(-k_{obs}t) + \frac{k_{obs}k_1}{(k_{d1}+k_1)(k_{d1}+k_1-k_{obs})}\exp(-(k_{d1}+k_1)t)\right] \tag{16}$$

$$[TrpH^{+\bullet}] = T_0\left[\frac{k_2}{k_{d2}+k_2} + \frac{k_{obs}-k_2}{k_{d2}+k_2-k_{obs}}\exp(-k_{obs}t) - \frac{k_{obs}k_{d2}}{(k_{d2}+k_2)(k_{d2}+k_2-k_{obs})}\exp(-(k_{d2}+k_2)t)\right] \tag{17}$$

where T_0 is the initial triplet concentration. Only at very low pH where $k_1, k_2 \gg k_{obs}, k_{d1}, k_{d2}$ do eqs 16 and 17 give the same time dependence for dipyridyl and tryptophan radicals,

$$[DPH_2^{+\bullet}] = [TrpH^{+\bullet}] = T_0[1 - \exp(-k_{obs}t)] \tag{18}$$

otherwise the formation of $DPH_2^{+\bullet}$ lags behind that of $TrpH^{+\bullet}$, in qualitative agreement with the experimental observations.

A quantitative comparison of the experimental results with model calculations based on eqs 11−15 requires the values of many parameters: the absorption coefficients of all reaction intermediates, the rate constants, and the initial triplet concentration. Fortunately, many are known or can be established in the separate experiments. The following extinction coefficients have been measured: triplet dipyridyl in cyclohexane has an absorption maximum at 355 nm with $\epsilon_{355} = 5.36 \times 10^4\ M^{-1}\ cm^{-1}$,[18] dipyridyl radical $DPH^{\bullet}$ and cation radical $DPH_2^{+\bullet}$ have $\epsilon_{365} = 3 \times 10^4\ M^{-1}cm^{-1}$ and $\epsilon_{375} = 4.5 \times 10^4\ M^{-1}\ cm^{-1}$, respectively,[10] $\epsilon_{570} = 2.7 \times 10^3\ M^{-1}\ cm^{-1}$ for the tryptophan cation radical, and $\epsilon_{510} = 1.8 \times 10^3\ M^{-1}\ cm^{-1}$ for its deprotonated form.[21,28,29] These data are consistent with our measurements, except for triplet dipyridyl which has extinction coefficients at the absorption maxima of $\epsilon_{345} = 2 \times 10^4\ M^{-1}\ cm^{-1}$ for ${}^{T}DPH^+$ and $\epsilon_{350} = 1.8 \times 10^4\ M^{-1}\ cm^{-1}$ for ${}^{T}DP$. Indeed, Figure 3 shows that the absorption of the radical $DPH_2^{+\bullet}$ is significantly stronger than that of triplet ${}^{T}DPH^+$.

The rate constants for the reaction of triplet dipyridyl with tryptophan at different pH's has been obtained in experiments in buffered solutions (Figure 4). The known values of pK_a for dipyridyl ($pK_a = 5.6$)[10] and tryptophan ($pK_a = 4.3$)[21c,23] radicals give the relationship between the protonation and deprotonation rate constants. Thus, in our simulations, the only fitting parameters were the absolute values of the protonation rate constants k_{p1} and k_{p2} and the initial triplet concentration. The absorption coefficients were taken to be $\epsilon_{570} = 2.7 \times 10^3\ M^{-1}\ cm^{-1}$ for $TrpH^{+\bullet}$, $\epsilon_{370} = 4.5 \times 10^4\ M^{-1}\ cm^{-1}$ for $DPH_2^{+\bullet}$, $\epsilon_{370} = 1.8 \times 10^4\ M^{-1}\ cm^{-1}$ for $DPH^{\bullet}$, and $\epsilon_{370} = 1.3 \times 10^4\ M^{-1}\ cm^{-1}$ for ${}^{T}DPH$. Bimolecular radical termination with a rate constant $k_t = 2 \times 10^9\ M^{-1}\ s^{-1}$ was added to eqs 12−15 to account for the radical decay (k_t was measured separately on a longer time scale). All experimental data fit agree well with the reaction scheme described by eqs 11−15 with a typical value of the protonation rate constants $k_{p1} = k_{p2} = (1.5 \pm 0.4) \times$

10^{10} M^{-1} s^{-1} and the other parameters given above. Some of the fitting results are shown in Figure 5 (bold line). The analysis in basic conditions gives very similar results.

3. CIDNP Measurements. CIDNP measurements were carried out to determine some of the characteristics of the radical intermediates, such as the nuclear spin−lattice relaxation times and the rate constants of degenerate exchange reactions. To avoid complications from the kinetics of the reaction of ^{T}DP with TrpH, the concentration of the amino acid was chosen to provide essentially instantaneous formation of radical pairs on a microsecond time scale. Thus, all CIDNP experiments were performed at amino acid concentrations higher than 6×10^{-4} M.

Nuclear spin-dependent intersystem crossing in the primary triplet radical pair results in the formation of nuclear polarization of equal magnitude but opposite sign in the geminate reaction products and in the radicals that escape geminate recombination. The time dependence of the CIDNP is governed by three main processes: (i) random radical encounters which lead to the formation of "F-pair" polarization which has the same sign as the geminate polarization; (ii) radical recombination and/or disproportionation reactions that transfer polarization from the radicals to the diamagnetic products; (iii) degenerate electron exchange reactions

$$*RH^{+\bullet} + RH \xrightarrow{k_{ex}} *RH + RH^{+\bullet} \qquad (19)$$

where the asterisk denotes nuclear polarization. Reactions ii and iii lead to polarization in the product, with a phase that is opposite to the geminate enhancements.

If the geminate processes give the same products as the F-pair reactions (which is the case for the DP/TrpH system), transformation of the escaped radicals into diamagnetic products by (ii) and (iii) results in cancellation of the geminate CIDNP. However, this cancellation is not complete if nuclear spin−lattice relaxation takes place in the radicals. Thus, the CIDNP kinetics are determined by the rates of polarization transfer from the radicals to the products and the nuclear paramagnetic relaxation; the stationary value of the CIDNP depends on the ratio of the lifetime and the relaxation time of the polarized radicals. The important difference between (ii) and (iii) is that the former is a second-order radical−radical reaction whose rate is determined by the initial radical concentration, whereas the latter is usually considered to be pseudo-first-order, the radical concentration being much smaller than that of the parent compound.

TR-CIDNP measurements were performed for two types of solutions: (a) dipyridyl−tryptophan mixtures and (b) dipyridyl−tryptophan mixtures containing 5×10^{-2} M NaOD, i.e., in acidic (a) and basic (b) conditions. The CIDNP spectrum obtained during the irradiation of a solution containing 4.4×10^{-4} M DP and 1.6×10^{-3} M TrpH (acidic conditions) is shown in Figure 6; the CIDNP pattern in basic condition is rather similar. All DP resonances are in emission. The β-CH_2 protons of tryptophan are in emission, and the aromatic protons H2, H4, and H6 are in absorption. The polarization of the aromatic protons corresponds to the calculated spin density distribution in the tryptophan cation radical.[30]

The CIDNP kinetics for DP, shown in Figure 7, were practically independent of pH and coincide within experimental error for the different DP protons. As may be seen in Figure 7, the dipyridyl polarization first increases in time and then falls slightly. The initial growth corresponds to the formation of CIDNP in F-pairs, whereas the decay can be accounted for by

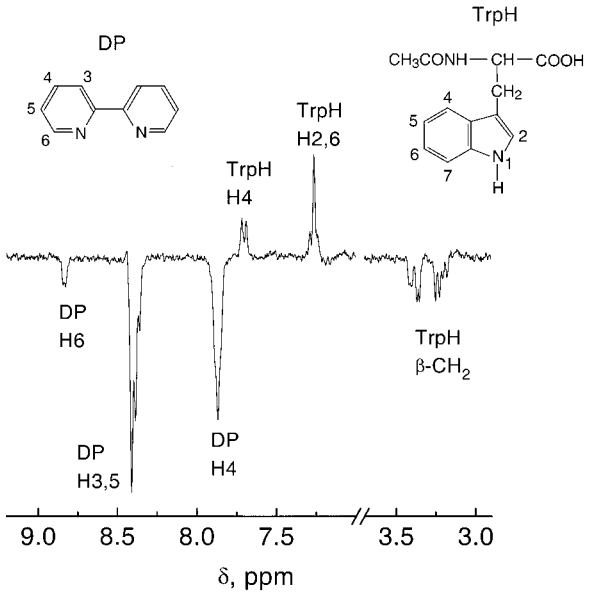

Figure 6. CIDNP spectrum, obtained during the irradiation of 4.4×10^{-4} M DP and 1.6×10^{-3} M TrpH solution.

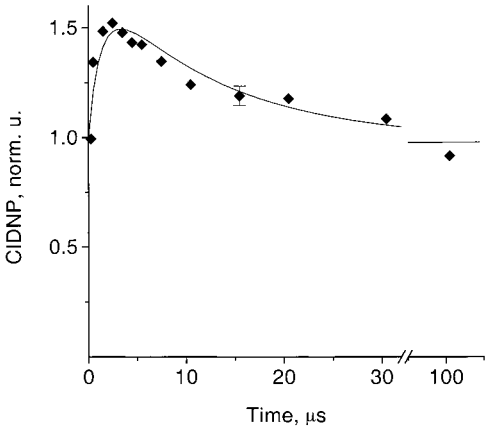

Figure 7. CIDNP kinetics for dipyridyl protons, obtained during the irradiation of 4.4×10^{-4} M DP and 1.2×10^{-3} M TrpH solution. Parameters of calculations (solid line): $k_t = 2 \times 10^9$ M^{-1} s^{-1}, $R_0 = 1.2 \times 10^{-4}$ M, $T_1 = 44$ μs.

the transfer of polarization from the radicals to the product in the course of radical termination. The large residual polarization at long times relative to the maximum signal indicates efficient nuclear relaxation in the dipyridyl radical. Moreover, the similarity of the kinetic curves at different pH's shows that, under these experimental conditions, degenerate electron exchange (19) does not play a significant role for this radical.

By contrast, the CIDNP kinetics observed for the tryptophan protons are strongly pH dependent (Figure 8). In basic conditions, the kinetics (squares, aromatic protons; solid circles, β-CH_2 protons) are similar to those for dipyridyl (Figure 7). The difference between the aromatic and β-CH_2 protons reflects the different rates of nuclear relaxation in the radical. At low pH, the polarization for all protons falls rapidly and reaches a stationary value. Both the decay rate and final polarization depend on the TrpH concentration (Figure 8: open circles, 1.2×10^{-3} M; solid triangles, 6×10^{-4} M). Thus, in acidic conditions, the CIDNP of the tryptophan protons is mainly determined by the electron exchange, eq 19.

The observed kinetics are in a good agreement with the reaction scheme of eqs 4−10. In acidic solution, the tryptophan radical exists in the protonated form $TrpH^{+\bullet}$ and can undergo

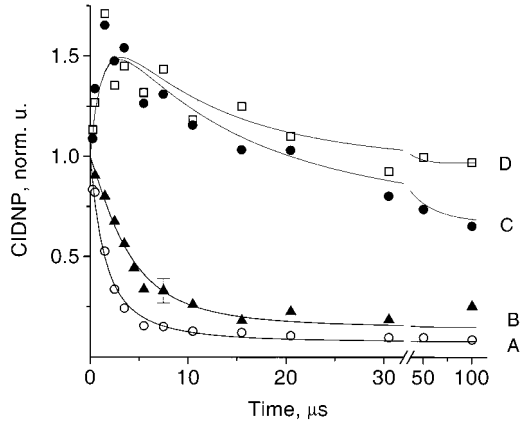

Figure 8. CIDNP kinetics for tryptophan under variable experimental conditions: (○) H2,6, initial concentration of TrpH was 1.2×10^{-3} M, acidic solution; (▲) H2,6, 6.0×10^{-4} M TrpH, acidic solution; (●) β-CH_2, 2.0×10^{-3} M TrpH, basic solution; (□) H2,6, 2.0×10^{-3} M TrpH, basic solution. Parameters of calculations: (A) $R_0 = 8.6 \times 10^{-5}$ M, $T_1 = 43$ μs, $k_{ex} = 9 \times 10^8$ M^{-1} s^{-1}; (B) $R_0 = 1.0 \times 10^{-4}$ M, $T_1 = 44$ μs, $k_{ex} = 9 \times 10^8$ M^{-1} s^{-1}; (C) $R_0 = 1.6 \times 10^{-4}$ M, $T_1 = 91$ μs; (D) $R_0 = 1.6 \times 10^{-4}$ M, $T_1 = 44$ μs.

degenerate electron exchange with the ground-state molecules. At high pH, this radical rapidly deprotonates; the neutral radical $Trp^{\bullet}$ is not affected by electron exchange.

The reaction scheme of eqs 4−10 is also confirmed by the dependence of the CIDNP on the TrpH concentration at different pH values. Figure 9a shows this dependence for DP and TrpH protons in acidic conditions. The polarization of both DP (diamonds) and TrpH (circles) first grows with increasing TrpH concentration due to the increase in the quantum yield of radical pair formation, which competes more effectively with other ^{T}DP decay channels. Further increase in the TrpH concentration accelerates the degenerate electron exchange and makes the CIDNP cancellation effect more pronounced. To exclude the effect of competition for ^{T}DP, the TrpH polarization is scaled by that of the DP (Figure 9b); the resulting plot reflects only the dependence of the TrpH CIDNP on the ratio of the rates of nuclear relaxation and degenerate electron exchange.

Under basic conditions, the tryptophan CIDNP becomes independent of TrpH concentration (Figure 9b), indicating again that deprotonation of $TrpH^{+\bullet}$ renders electron exchange ineffective. For this case, the time dependence of the tryptophan polarization is similar to that of dipyridyl. However, different relaxation times for β-CH_2 and H2,6 lead to different polarizations at long times compared to the initial enhancements for each proton.

Simulation of the CIDNP kinetics was carried out using the approach developed by Fischer et al.[31] Equations 20−22 describe the time evolution of the radical concentration and the nuclear polarization in radicals and products.

$$R(t) = \frac{R_0}{1 + k_t R_0 t} \tag{20}$$

$$\frac{dP(R)}{dt} = -k_t P(R) R - k_t \beta R^2 - \frac{P(R)}{T_1} - k_{ex} C P(R) \tag{21}$$

$$\frac{dP(Pr)}{dt} = k_t P(R) R + k_t \beta R^2 + k_{ex} C P(R) \tag{22}$$

Here k_t is the radical termination rate constant, R_0 is the initial radical concentration, $P(R)$ and P(Pr) are the polarizations in the radicals and in the products, T_1 is the nuclear paramagnetic

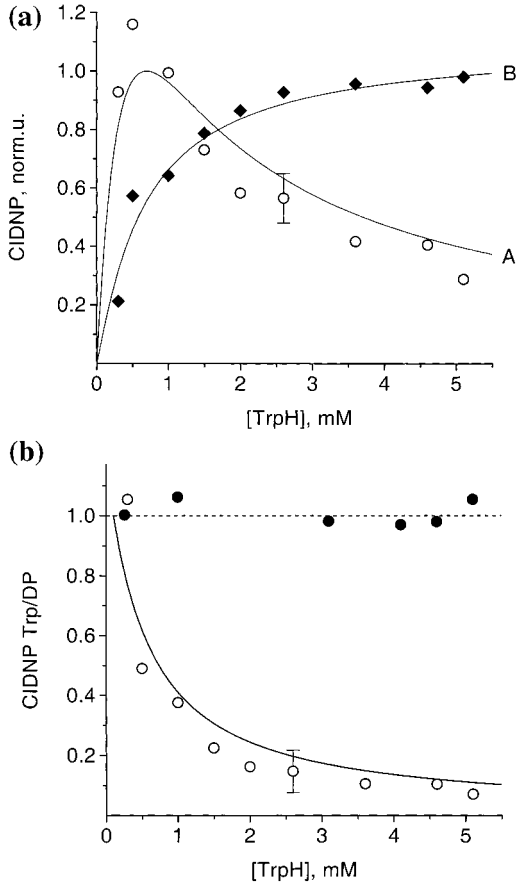

Figure 9. (a) Dependence of the CIDNP intensity on TrpH concentration (acidic solution): (◆) for DP; (○) for TrpH. Parameters of calculations: initial triplet concentration $T_0 = 1.6 \times 10^{-4}$ M, $k_q = 4 \times 10^9$ M^{-1} s^{-1}, $k_d = 2.3 \times 10^6$ s^{-1}, $T_1 = 44$ μs, $k_{ex} = 9 \times 10^8$ M^{-1} s^{-1} (for TrpH only). (b) Trp/DP CIDNP ratio: (○) acidic solution, (●) basic solution. (Solid line) ratio of the calculated curves A and B in Figure 9a.

relaxation time, k_{ex} is the rate constant of degenerate electron exchange, and C is the concentration of the molecules involved in the exchange reaction. The degenerate electron exchange was treated as irreversible, since the concentration of ground-state molecules was much higher than the initial radical concentration. The parameter β represents the polarization per radical pair, created in F-pairs; it is related to the geminate polarization P^G via the quantity γ,[32] which is the ratio of polarizations created in F- and geminate pairs:

$$\beta = \gamma P^G / R_0 \tag{23}$$

In eqs 21 and 22, the first and the last terms describe the polarization transfer from the radicals to ground-state molecules in the termination reaction and by degenerate electron exchange, respectively, the second term represents the formation of polarization in F-pairs. The third term in eq 21 corresponds to the loss of polarization in the radicals due to nuclear relaxation.

It was assumed that the yield of radicals that escape from the triplet radical pair is much greater than the yield of geminate recombination and that the radicals disappear only via the cross-termination reaction. The initial polarizations were taken as $P(Pr) = P^G = -P(R)$, which is consistent with the spin-sorting nature of the $S-T_0$ radical pair mechanism.

For dipyridyl, electron exchange is ineffective because of the nature of the species that are present in both acidic and basic solutions; electron transfer in the pairs $DPH_2^{+\bullet}/DPH^+$ (acidic

solution) or $DPH^{\bullet}$/DP (basic solution) is nondegenerate and therefore slow. This is confirmed by the small amount of CIDNP cancellation observed in the kinetic measurements. In calculating the kinetics for DP, the final term in eqs 21 and 22 was therefore omitted. The value of k_t, taken from the LFP measurements, was 2×10^9 M^{-1} s^{-1}. The parameter γ in all simulations was set equal to 2.8 as used earlier by Vollenweider et al.[31] The fitting parameters were R_0, P^G, and T_1. Irrespective of the acidity of the solution, T_1 was found to be 44 ± 9 μs, indicating that the protonation of $DPH^{\bullet}$ does not have much influence on the nuclear relaxation times.

The same fitting procedure was applied to the data obtained for the tryptophan protons in basic solution. The values $T_1 = 44 \pm 9$ μs for H2,6, 91 ± 20 μs for β-CH_2, and 63 ± 12 μs for H4 were found. In acidic solution, the main factor governing the CIDNP kinetics was the degenerate electron exchange, which under our experimental conditions could be considered as a pseudo-first-order reaction with rate constant $k_{ex}' = k_{ex}C$. The fitting parameters were k_{ex} and P^G. Using the values of the nuclear relaxation times determined for basic solutions, the best fit was obtained with $k_{ex} = (9 \pm 1) \times 10^8$ M^{-1} s^{-1}.

The dependence of the DP polarization (Figure 9a) on the concentration of TrpH is determined by the ratio $k_q[TrpH]/(k_d + k_q[TrpH])$, where k_d represents the (pseudo-)first-order rate constant for all ^{T}DP decay pathways except the reaction with tryptophan (predominantly quenching by residual oxygen). The best fit, shown in Figure 9a, was obtained with $k_d = 2.3 \times 10^6$ s^{-1}. This value was used to simulate the data for TrpH, and with $k_{ex} = 9 \times 10^8$ M^{-1} s^{-1} satisfactory agreement between the experimental and calculated results was achieved.

Conclusions

The results reported here indicate unambiguously that the reaction of photoexcited dipyridyl with tryptophan is primarily electron transfer. The rate constant k_q is close to the diffusion-controlled limit and decreases slightly with increasing pH. Most likely this decrease is due to the different charges of the interacting species. At high pH, DP exists in its neutral state, whereas at pH < 4.3, triplet $^T DPH^+$ is positively charged, *N*-acetyl tryptophan carries a negative charge ($pK_a = 2.38$), and Coulomb interactions accelerate the bimolecular reaction.

Depending on the pH of the solution, triplet quenching is followed by protonation/deprotonation reactions of the intermediate radicals eqs 4−10. These reactions play a key role in formation and time dependence of CIDNP; degenerate electron exchange between protonated tryptophan radicals and tryptophan in its ground state leads to significant CIDNP cancellation, whereas for the deprotonated radical, electron exchange is ineffective. Thus, the CIDNP kinetics and the stationary value are mostly determined by the presence/absence of the degenerate electron exchange. Similar conclusions were reached for the reaction of tryptophan and its derivatives with photoexcited flavins,[2c,3,4a,33] pointing to the similarity of the photoreactions of these two types of aza-aromatic dyes.

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