Laser flash photolysis and time resolved CIDNP study of photoreaction of 2,2′-dipyridyl with N-acetyl tyrosine in aqueous solutions

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Time-Resolved CIDNP and Laser Flash Photolysis Study of the Photoreactions of *N*-Acetyl Histidine with 2,2′-Dipyridyl in Aqueous Solution

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The reaction mechanism and details of the formation of CIDNP (chemically induced dynamic nuclear polarization) in the photoreactions of 2,2′-dipyridyl (DP) and *N*-acetyl histidine (HisH) in aqueous solution have been studied using laser flash photolysis and time-resolved CIDNP techniques. The triplet state ^{T}DP reacts with protonated $HisH_2^+$ via hydrogen atom transfer with a rate constant $k_H = 1.2 \times 10^8$ M^{-1} s^{-1}, and with deprotonated His^- via electron transfer with $k_e = 7.5 \times 10^9$ M^{-1} s^{-1}. No reaction occurs when the histidine imidazole ring is in its neutral state HisH, or when the dipyridyl triplet is protonated, $^{T}DPH^+$. The nuclear spin−lattice relaxation times in the radicals formed in these reactions have been determined from the CIDNP kinetics: $T_1 = 44 \pm 9$ μs for all DP protons, $T_1 = 196 \pm 25$ μs for the β-CH_2 protons of HisH, and $T_1 = 16 \pm 5$ μs for the H-2 and H-4 protons of HisH. Under strongly basic conditions the CIDNP is greatly affected by degenerate electron exchange between the neutral His• radical and His^- anion, with rate constant $k_{ex} = 1.5 \times 10^8$ M^{-1} s^{-1}.

Introduction

Chemically induced dynamic nuclear polarization (CIDNP) generated in cyclic reactions between a photoexcited dye and amino acid side-chains in a protein[1,2] has been extensively used to give information about the exposure of polarizable groups near the surface of the macromolecule (for reviews see refs 3 and 4). Three amino acids—histidine, tryptophan, and tyrosine—can be polarized using a flavin as the photosensitizing dye. The experiment is most conveniently and sensitively performed with continuous wave photolysis even though the polarization may be attenuated by partial cancellation of the opposite-phase enhancements arising from geminate radical recombination and from the free radicals that escape the geminate cage. Quantitative analysis of the photoreactions therefore requires time-resolved measurements using laser flash photolysis. A search for a suitable dye which absorbs at the wavelength (308 nm) of a pulsed excimer laser, and generates strong CIDNP for all three amino acid types, led to 2,2′-dipyridyl (DP).[5] On the basis of preliminary CIDNP experiments,[5] we have embarked upon a systematic study of the kinetics and mechanism of the reactions of 2,2′-dipyridyl with the three amino acids in aqueous solution over a wide pH range using time-resolved CIDNP and optical spectroscopy. The main aims of this study are to establish the mechanisms of the reactions between photoexcited DP and aromatic amino acids, and the details of CIDNP formation, to determine the rate constants of the different stages of these reactions and to characterize the reaction intermediates.

In two earlier papers, the photolysis of DP in the presence of *N*-acetyl tryptophan[6] and of *N*-acetyl tyrosine[7] was studied. It was shown that electron transfer is the principal reaction mechanism for triplet DP and *N*-acetyl tryptophan in the pH range from 2 to 13. For tyrosine, the quenching of triplet DP proceeds via electron transfer under acidic (pH < 5) and strongly basic (pH > 10.5) conditions, and via hydrogen atom transfer in neutral and moderately basic (6 < pH < 9.5) solutions.

As a part of this continuing investigation of the photoreactions of DP with amino acids, the present work focuses on the reaction of DP with *N*-acetyl histidine, HisH. A variety of histidine-derived radicals have been reported. The electronic structure of transient histidine radicals formed in aqueous solution by oxidation with Ti^{3+}/H_2O_2 has been published very recently.[8] Prior to that, the structures of various radicals produced by X irradiation of histidine hydrochloride crystals at low temperature were elucidated,[9] including the histidine cation radical formed by removal of an electron. At room temperature, secondary histidine radicals with two large proton hyperfine couplings (4.7 and 5.0 mT), formed by the addition of hydrogen to the imidazole ring, have been detected.[10,11] The reaction kinetics of histidine and imidazole with •OH radicals in water have been studied[11] by monitoring the transient absorption of hydroxylated radical adducts. In all of these studies, histidine-derived radicals were produced either by radiolysis or in a steady-state flow system by means of a Fenton reaction; no data are available for histidine radicals generated by light irradiation. In photochemical reactions with flavins, evidence for hydrogen atom abstraction was obtained from qualitative analysis of CIDNP effects,[12] but no kinetic measurements have been made and relatively little is known about the mechanism of photoreactions between histidine and aza-aromatic dyes. The results described here allow the determination of the detailed mechanism, kinetics and CIDNP formation in the photoreaction of 2,2′-dipyridyl and *N*-acetyl histidine.

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Experimental Section

Time-Resolved CIDNP. A detailed description of the TR−CIDNP experiment has been reported elsewhere.[13] A sample, purged with argon and sealed in a standard NMR Pyrex ampule, was irradiated with 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−radio frequency pulse−free induction decay. As the background signals in the spectrum are suppressed by the presaturation pulses, only the resonances of the polarized products, formed during the variable delay between the laser and radio frequency pulse, appear in the CIDNP spectra. For kinetic measurements, a 1 μs radio frequency detection pulse was used.

Laser Flash Photolysis. A detailed description of the LFP equipment has been published recently.[14,15] 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 DKSh-120 xenon short-arc lamp connected to a high current pulser, two synchronously operating monochromators, a Hamamatsu R955 photomultiplier, and a LeCroy 9310A digitizer. All solutions were purged with argon for 15 min prior to, and during, irradiation. Due to the high degree of reversibility of the reaction under study, the decomposition of the starting material during the course of the experiment was negligibly small. All experiments were carried out in buffered solutions except the most acidic (pH < 3) and the most basic (pH > 11), where the pH values were regulated by the addition of HCl or NaOH.

Chemicals. D_2O (Aldrich), 2,2′-dipyridyl (Aldrich), NaOD (Aldrich), *N*-acetyl histidine (Sigma) were used as received. H_2O was doubly distilled.

Results and Discussion

In aqueous solution, the identities of the reactive species are pH-dependent. Depending on the acidity of the solution, the imidazole ring of *N*-acetyl histidine can be positively charged ($HisH_2^+$, pK_a = 6.1),[11] neutral (HisH, pK_a = 14.5 for imidazole itself),[11] or negatively charged (His^-). Dipyridyl can exist in either protonated (DPH^+, pK_a = 4.3)[16] or neutral (DP) states.

The disappearance of triplet DP was measured at 325 nm, where the absorption of both ^TDP and $^TDPH^+$ is much stronger than that of the radicals.[6] In the absence of *N*-acetyl histidine, irradiation of DP solutions leads to the formation of triplet DP, which decays mainly by second-order kinetics (triplet−triplet annihilation). A first-order contribution to the triplet decay with a rate constant $k_0 \approx 1 \times 10^5$ s^{-1} is observed at very low irradiation levels, and can be attributed to triplet quenching by residual oxygen.

In the presence of amino acid the triplet decay becomes exponential, with a pseudo-first-order rate constant k_1 found to be proportional to the concentration of *N*-acetyl histidine, C_0: $k_1 = k_q^{obs} C_0$. The pH-dependence of the quenching rate constant k_q^{obs} is presented in Figure 1. It is clear that the protonation states of the reactants have a profound effect on the rate of the reaction. Four pH regions can be distinguished: pH < 4; 4 < pH < 8; 8 < pH < 11; and pH > 11.

pH < 4 and 8 < pH < 11. In strongly acidic solutions both dipyridyl (DPH^+) and the amino acid ($HisH_2^+$) are protonated, while in the region 8 < pH < 10 dipyridyl is neutral (DP), as is the imidazole ring of *N*-acetyl histidine (HisH). No triplet quenching is observed under acidic conditions (pH < 4) or in the region near pH = 9.5. In neither pH range is the rate of triplet decay altered by the addition of *N*-acetyl histidine up to 0.1 M. Assuming roughly similar pK_a values for DPH^+ and $^TDPH^+$ (see below), we can conclude that no reaction takes place between $^TDPH^+$ and $HisH_2^+$, or between ^TDP and HisH. This conclusion is strongly supported by CIDNP measurements: no nuclear polarization has been detected in either of these pH ranges.

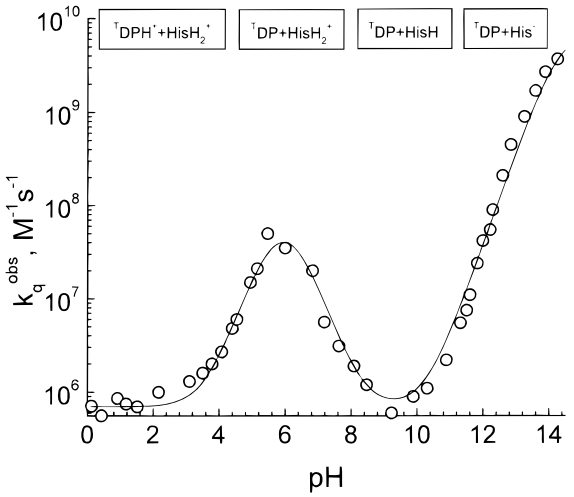

Figure 1. pH dependence of the second-order rate constant k_q^{obs} for the reaction of triplet 2,2′-dipyridyl with *N*-acetyl histidine. Solid line: calculation according to eq 8. See text for parameter values.

4 < pH < 8. In this region the observed quenching rate constant k_q^{obs} increases, passes through a maximum at pH ≈ 6 and then decreases. The rise in k_q^{obs} between pH 4 and 6 can be attributed to a shift in the position of the $^TDPH^+/^TDP$ equilibrium toward the neutral form of the triplet:

$$^TDP + HisH_2^+ \xrightarrow{k_q} \text{radicals} \quad (1)$$

$$^TDPH^+ \underset{k_p}{\overset{k_d}{\rightleftharpoons}} {}^TDP + H^+ \quad (2)$$

where k_p and k_d are the protonation and deprotonation rate constants for triplet dipyridyl, and k_q is the "intrinsic" quenching rate constant. With the assumption, which is reasonable for buffered solutions, that the (de)protonation of triplet dipyridyl in eq 1 is faster than triplet quenching, i.e., k_d, $k_p[H^+] \gg k_q[HisH_2^+]$, the total triplet concentration should decay exponentially with a pseudo-first-order rate constant

$$k_1 = k_q^{obs} C_0 = k_q \frac{k_d}{k_d + k_p[H^+]}[HisH_2^+] = k_q \frac{K_{a1}}{K_{a1} + [H^+]} \frac{C_0[H^+]}{K_{a2} + [H^+]} \quad (3)$$

in which K_{a1} and K_{a2} are the ionization constants for $^TDPH^+$ and $HisH_2^+$, respectively. According to eq 3, the observed quenching rate constant k_q^{obs} increases with pH, reaching a maximum at pH = (pK_{a1} + pK_{a2})/2. A further increase in pH causes the concentration of $HisH_2^+$ to fall:

$$HisH_2^+ \rightleftarrows HisH + H^+ \quad (4)$$

leading to a drop in the value of k_q^{obs}.

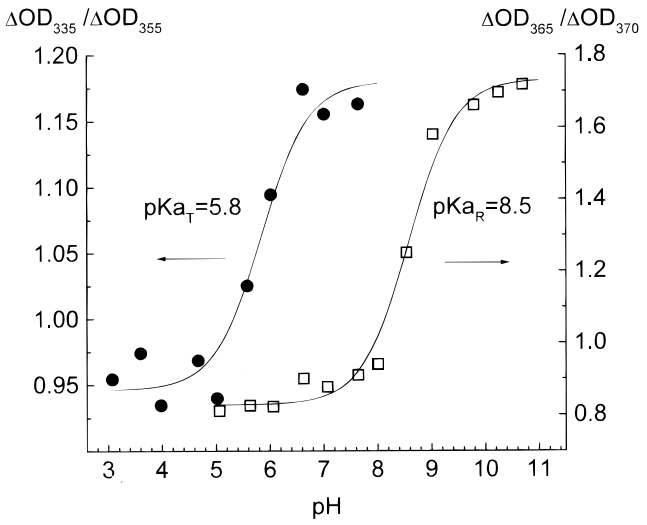

Figure 2. pH titration curves: ● of triplet 2,2′-dipyridyl, monitored by the ratio of transient absorptions at 355 and 335 nm, obtained 200 ns after the laser flash during irradiation of 2,2′-dipyridyl in aqueous solution; □ of dipyridyl radical, monitored by the ratio of transient absorptions at 365 and 370 nm after the completion of the decay of triplet 2,2′-dipyridyl in the presence of 5.7×10^{-4} M *N*-acetyl tryptophan. Solid lines: calculations with the pK_a values 5.8 (^{T}DPH$^+$) and 8.5 (DPH•).

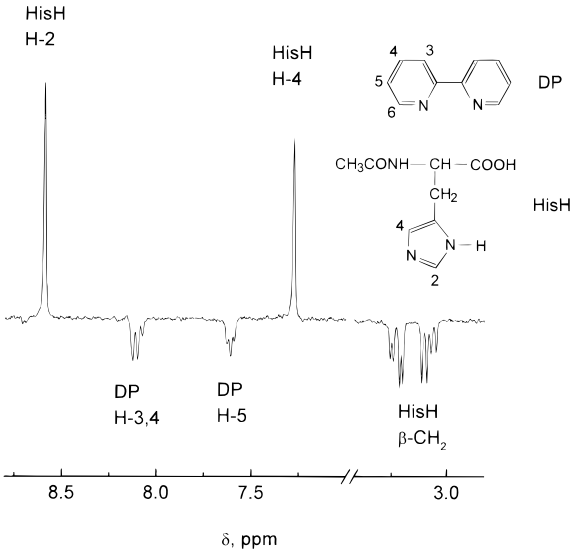

Figure 3. ^{1}H CIDNP spectrum obtained during the irradiation of a solution containing 5.8×10^{-4} M 2,2′-dipyridyl and 0.14 M *N*-acetyl histidine (pH = 5.3). The rising edge of the radio frequency detection pulse (4 μs) was synchronized with the laser pulse.

Inspection of Figure 1 shows that the midpoint of the titration curve between pH 4 and 6 occurs at a higher pH than the pK_a of dipyridyl (4.3).[16] This implies that ^{T}DPH$^+$ is a weaker acid than dipyridyl in its ground state. To the best of our knowledge, the pK_a of triplet dipyridyl has not been measured hitherto. In earlier work[6] it was established that the absorption spectra of ^{T}DPH$^+$ and ^{T}DP are rather similar, but that the extinction of ^{T}DP is somewhat larger than that of ^{T}DPH$^+$ at 355 nm, while at 335 nm ^{T}DPH$^+$ absorbs more strongly. The pH dependence of the ratio of the transient optical densities at 355 and 335 nm, obtained 200 ns after the laser flash, during irradiation of dipyridyl in aqueous solution (without histidine), is shown in Figure 2 (circles), and yields a pK_a value for ^{T}DPH$^+$ of 5.8. The best fit of the pH dependence of the observed quenching rate constant (Figure 1, solid line) in the range $4 < \text{pH} < 8$ was obtained with $k_q = 1.2 \times 10^8$ M^{-1} s^{-1}, keeping the parameters p$K_{a1} = 5.8$ for ^{T}DPH$^+$ and p$K_{a2} = 6.1$ for HisH$_2^+$ fixed.

Transient absorption spectra obtained after the completion of the triplet decay, at pH = 5.0 and pH = 6.8, are essentially identical and have characteristic bands at 365 and 470 nm, indicating that in both cases *neutral* dipyridyl radicals DPH• are formed.[6,17−20] The squares in Figure 2 show the pH dependence of the ratio of the transient absorption at 365 nm (absorption maximum of DPH•) to that at 370 nm (maximum of DPH$_2^+$•) obtained after the completion of the ^{T}DP decay in the presence of 5.7×10^{-4} M *N*-acetyl tryptophan (the reaction with tryptophan proceeds by electron transfer at all pHs).[6] These data give p$K_a = 8.5$ for DPH•, in contrast to the value of 5.7 obtained by continuous radiolysis.[17,18] The source of this discrepancy is not clear. Certainly the observation of DPH$_2^+$• at pH 6.8 is difficult to reconcile with a p$K_a = 5.7$. The radicals decay by second-order kinetics with a recombination rate constant $k_t = 2 \times 10^9$ M^{-1} s^{-1}.

The reaction of ^{T}DP with protonated histidine HisH$_2^+$ can occur either via hydrogen atom transfer with the immediate formation of neutral dipyridyl (DPH•) and histidine cation (HisH$^+$•) radicals, or by electron transfer followed by fast protonation of the DP^{-}• anion radical (the pK_a of this species is greater than 14).[18,19] Since electron transfer would lead to the formation of a doubly charged radical HisH$_2^{2+}$•, hydrogen abstraction would seem to be the more likely pathway. An additional argument in favor of hydrogen transfer is the relatively low k_q value, which is close to the rate constant of hydrogen abstraction by triplet dipyridyl from *N*-acetyl tyrosine.[7]

The CIDNP spectrum obtained during the photolysis of a solution containing 2,2′-dipyridyl and *N*-acetyl histidine at pH = 5.3 is shown in Figure 3. The absorptive nuclear polarization for the histidine H-2 and H-4 protons is in agreement with Kaptein's rules.[21] The *g*-values are 2.0030 for the dipyridyl radical[22] and 2.00226 for the imidazole radical,[23] giving a negative value for Δg. The hyperfine interactions (HFI) for the imidazole ring protons are also negative, (A_{H2}, $A_{H4} < 0$).[23] Thus, absorptive polarization is expected for a geminate radical pair with a triplet precursor. There seems to be no information on the HFI of the β-CH$_2$ protons.[8] The isotropic part of the HFI of these protons is determined by the π-electron spin density at the neighboring aromatic carbon atom via a hyperconjugation mechanism, and is usually positive.[24] The observed emissive polarization of these protons is therefore also consistent with Kaptein's rules. Emissive polarization is observed for H-3, H-4 and H-5 of DP. (In our previous papers[6,7] the assignments of the CIDNP signals of DP H-4 and H-5 were transposed.)

CIDNP kinetics were measured with *N*-acetyl histidine concentrations of about 0.1 M, so that the quenching time is always considerably less than the time resolution of the measurement, determined by the duration of the radio frequency detection pulse (1 μs). Thus radical pairs are formed essentially instantaneously on a microsecond time scale, avoiding complications arising from the time dependence of triplet quenching.

The CIDNP kinetics of the dipyridyl resonances practically coincides with the data reported for the reactions of DP with *N*-acetyl tryptophan[6] and with *N*-acetyl tyrosine,[7] and is therefore not shown here. The kinetic curves obtained for the β-CH$_2$ and ring protons of *N*-acetyl histidine (Figure 4a) between pH 4 and 8 do not depend on pH or on the concentration of *N*-acetyl histidine. The time dependence of the polarization on the microsecond time scale is determined by (i) the formation of

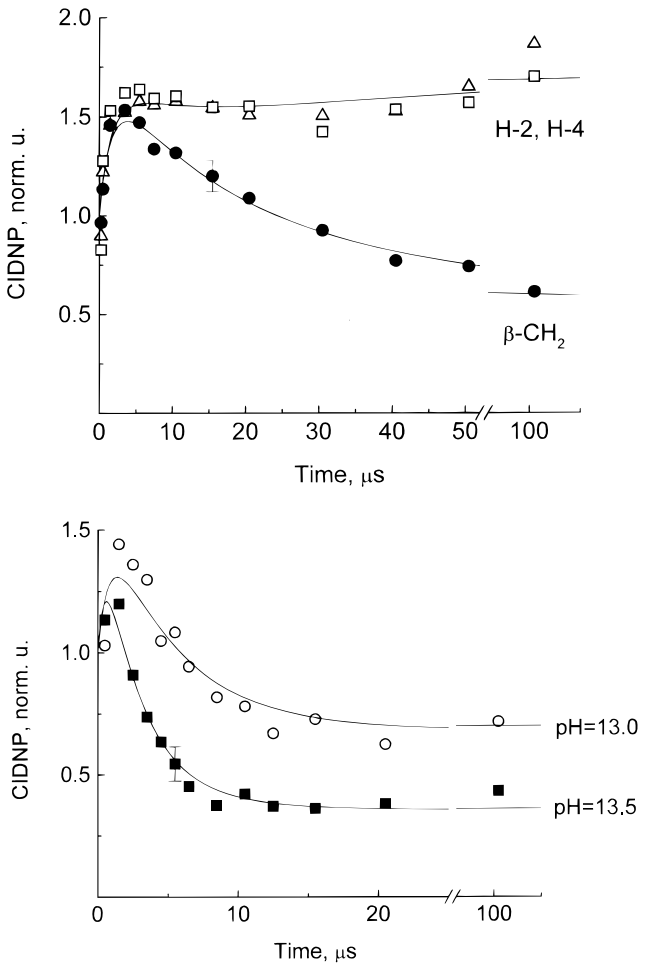

Figure 4. (a) ^{1}H CIDNP kinetics obtained during the irradiation of a solution containing 5.8×10^{-4} M 2,2′-dipyridyl and 0.14 M *N*-acetyl histidine (pH = 5.3): △, H-2; □, H-4; ●, β-CH_2 of *N*-acetyl histidine. Solid lines: calculations as described in the text using the parameters $R_0 = 1.2 \times 10^{-4}$ M, $T_1 = 16$ μs (H-2, H-4), $T_1 = 196$ μs (β-CH_2), $k_t = 2.0 \times 10^9$ M^{-1} s^{-1}. (b) ^{1}H CIDNP kinetics for H-2 of *N*-acetyl histidine, obtained during the irradiation of a solution containing 1.4×10^{-2} M 2,2′-dipyridyl and 1.6×10^{-2} M *N*-acetyl histidine: ○, pH = 13.0; ■, pH = 13.5. Solid lines: calculations as described in the text using the parameters $R_0 = 2.2 \times 10^{-4}$ M (pH = 13.0), $R_0 = 3.5 \times 10^{-4}$ M (pH = 13.5), $T_1 = 16$ μs, $k_t = 2.0 \times 10^9$ M^{-1} s^{-1}, $k_{ex} = 1.5 \times 10^8$ M^{-1} s^{-1}.

CIDNP in F-pairs (which has the same sign as the geminate polarization); (ii) polarization transfer from the radicals to the diamagnetic products by radical recombination (opposite sign); and (iii) loss of polarization by nuclear spin relaxation in the radicals. The evolution of the radical concentration *R*, and the polarizations of the radicals, *P*(R), and of the diamagnetic products, *P*(Pr), can be described[25] by the set of equations:

$$R(t) = \frac{R_0}{1 + k_t R_0 t} \tag{5}$$

$$\frac{dP(R)}{dt} = -k_t P(R) R - k_t \beta R^2 - \frac{P(R)}{T_1} \tag{6}$$

$$\frac{dP(Pr)}{dt} = +k_t P(R) R + k_t \beta R^2 \tag{7}$$

where R_0 is the initial radical concentration, k_t is the radical termination rate constant, T_1 is the nuclear relaxation time in the radicals, and the parameter β represents the polarization per F-pair. In most cases, $\beta \approx 3P^G/R_0$,[25] where P^G is the geminate polarization. Computer simulations (Figure 4, solid lines) of the CIDNP kinetics were performed with $k_t = 2 \times 10^9$ M^{-1} s^{-1} as determined above, treating R_0 and T_1 as variables in the fitting. The following relaxation times were obtained: $T_1 = 44 \pm 9$ μs for all DP protons; $T_1 = 196 \pm 25$ μs for the β-CH_2 protons of *N*-acetyl histidine; and $T_1 = 16 \pm 5$ μs for H-2 and H-4 of *N*-acetyl histidine. The T_1 of the ring protons in $HisH^{+}\cdot$ is significantly shorter than the relaxation times recently reported for radicals derived from both *N*-acetyl tyrosine (63 μs) and *N*-acetyl tryptophan (44 μs for H-2,6 and 63 μs for H-4 protons). Since the main source of nuclear relaxation in free radicals is the dipole−dipole interaction between the unpaired electron and the nuclei, this result points to a high anisotropy of the HFI tensors in the $HisH^{+}\cdot$ radical. The reported principal components (A_i) of the anisotropic parts of the HFI tensor of imidazole cation are (−0.5, −0.13, 0.63) mT for H-2 and (−0.55, 0, 0.55) mT for H-4 protons;[9] the largest anisotropic HFI in the tryptophanyl radical was found for the H-2 proton: (−0.3, −0.097, 0.443) mT.[26] Thus, the values of $\sum_{i=1}^{3} A_i^2$ for H-2 and H-4 protons of the histidine radical (6.1×10^{-7} T^2 and 6.7×10^{-7} T^2, respectively) are twice as large as that of the H-2 proton of the tryptophanyl radical (2.9×10^{-7} T^2), in fair agreement with our T_1 values. As mentioned above, the hyperfine interaction of the β-CH_2 protons is caused by hyperconjugation, and since the distance between the protons and the unpaired electron spin density is considerably larger than that for the aromatic protons, the anisotropy of the HFI tensor is usually much smaller.[24]

pH > 11. In strongly basic solutions the imidazole ring of *N*-acetyl histidine releases a proton. This process is accompanied by a red shift in the optical spectrum of histidine, as well as a high field shift of the ring protons in the NMR spectrum. The titration curve obtained for the optical absorption of *N*-acetyl histidine solution at 245 nm yields $pK_a = 14.3$, which is close to the $pK_a = 14.5$ value reported for imidazole.[11]

It can readily be seen in Figure 1 that the ionization of HisH makes *N*-acetyl histidine reactive toward triplet dipyridyl. Since His^- has no abstractable hydrogen atoms, the only possible reaction mechanism is electron transfer. The observed quenching rate constant k_q^{obs} increases by 3 orders of magnitude between pH 11 and 14, reflecting the increase in the His^- concentration. Thus, the whole experimental pH dependence can be simulated according to the expression:

$$k_q^{obs} = k_H \frac{K_{a1}[H^+]}{(K_{a1} + [H^+])(K_{a2} + [H^+])} + k_e \frac{K_{a3}}{K_{a3} + [H^+]} + k_0/C_0 \tag{8}$$

where $pK_{a1} = 5.8$ (deprotonation of $^TDPH^+$), $pK_{a2} = 6.1$ (deprotonation of $HisH_2^+$), $pK_{a3} = 14.3$ (deprotonation of HisH), k_H is the rate constant of the hydrogen abstraction in neutral solution, k_e is the rate constant of the electron abstraction under strongly basic conditions, and k_0 is the rate of triplet decay in the absence of quencher. The best fit (solid line in Figure 1) was obtained with the following set of parameters: $k_H = 1.2 \times 10^8$ M^{-1} s^{-1}, $k_e = 7.5 \times 10^9$ M^{-1} s^{-1}, and $k_0 = 7.0 \times 10^4$ s^{-1}. The value of k_e is similar to the rate constants reported for the electron transfer reactions of *N*-acetyl tryptophan[6] and *N*-acetyl tyrosine[7] with triplet dipyridyl.

The relative intensities and the CIDNP kinetics obtained for DP protons under basic conditions are very similar to those

obtained in neutral solution, suggesting that the mechanism of dipyridyl CIDNP formation is independent of pH. This may be explained by the fast protonation of the $DP^{-\bullet}$ anion radical, i.e., within the lifetime of geminate radical pair. The decay of the polarization of HisH in basic solution (Figure 4b) is much faster than in neutral solution (Figure 4a). The decay rate increases with both pH and with the concentration of HisH. In basic solution the CIDNP is strongly affected by a degenerate electron exchange between neutral His$^\bullet$ radical and His^- anion

$$*His^\bullet + His^- \xrightarrow{k_{ex}} *His^- + His^\bullet \qquad (9)$$

which transfers nuclear polarization (denoted by the asterisk) from the radical to the diamagnetic molecule. The polarization in the radicals is opposite in sign to the geminate polarization, so that exchange leads to cancellation of the CIDNP in the products. Since only the anionic form of histidine, His^-, can participate in a degenerate electron exchange, and the pH determines the equilibrium between HisH and His^- in solution, changes in pH influence the CIDNP kinetics in the same way as does changing the concentration of HisH.

Under our experimental conditions, degenerate electron exchange is the main factor determining the CIDNP kinetics in basic solution. The polarization transfer caused by the electron exchange occurs with a pseudo-first-order rate constant

$$k_{dec} = k_{ex}[His^-] = \frac{K_{a3}C_0}{K_{a3} + [H^+]}\, k_{ex} \qquad (10)$$

Figure 4b shows the CIDNP kinetic curves obtained at pH = 13.0 and pH = 13.5, the concentration of *N*-acetyl histidine in both cases being 1.6×10^{-2} M. The simulations of these curves (solid lines) were performed using eqs 5−7 with the addition of an exchange term $k_{dec}P(R)$ with the appropriate signs in eqs 6 and 7. The best fit for both curves was obtained with $k_{ex} = 1.5 \times 10^8\ M^{-1}\ s^{-1}$, while the other parameters (k_t and T_1) were held fixed at the values determined for neutral solutions. The residual polarization observed after the completion of the CIDNP decay corresponds to the polarization loss due to nuclear relaxation in the radicals (CIDNP cancellation is not complete). The ratio of the residual polarization to the geminate polarization for β-CH_2 protons is much smaller than that for the H-2 and H-4 histidine protons. Since this quantity is determined by the ratio of the lifetime of polarized radicals to the relaxation time,[12] this fact serves as an additional argument in favor of the rather short T_1 for the ring protons in the histidine radical.

The polarization formed in the reaction of 2,2′-dipyridyl with *N*-acetyl histidine is stronger than that of *N*-acetyl tryptophan[6] or *N*-acetyl tyrosine,[7] even though the reactivity of histidine is much lower. In the pH range from 2 to 12, the largest observed quenching rate constant for *N*-acetyl histidine is 2 orders of magnitude slower than for *N*-acetyl tryptophan, and at least 20 times smaller than for *N*-acetyl tyrosine. Thus, when using CIDNP to study the accessibility of amino acid residues in proteins, weak polarization of histidine residues may be due to the unfavorable competition for the photoexcited dye, of histidine compared to exposed tyrosine and/or tryptophan residues. Care is needed in attributing weak histidine polarizations to low solvent exposure.[27]

Conclusion

Investigation of the photochemical reaction between 2,2′-dipyridyl and *N*-acetyl histidine in aqueous solutions reveals a strong pH-dependence. In acidic solutions at pH < 4 no reaction takes place between the protonated triplet dipyridyl $^TDPH^+$ and protonated $HisH_2^+$, or between neutral ^TDP and HisH at 8 < pH < 11. In the range 4 < pH < 8 the protonated $HisH_2^+$ quenches ^TDP via hydrogen atom transfer with a rate constant $k_H = 1.2 \times 10^8\ M^{-1}\ s^{-1}$. Under extreme basic conditions ^TDP abstracts an electron from the anion His^- with a rate constant $k_e = 7.5 \times 10^9\ M^{-1}\ s^{-1}$. The His$^\bullet$ radicals engage in degenerate electron exchange with His^-, leading to rapid CIDNP cancellation. The short nuclear paramagnetic relaxation times for H-2 and H-4 histidine protons are in agreement with the strong anisotropy of the hyperfine interaction of these protons in the imidazole cation radical.

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