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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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Abstract

Laser flash photolysis and time-resolved chemically induced dynamic nuclear polarization (CIDNP) methods have been applied to the investigation of the photoreaction of 2,2′-dipyridyl and *N*-acetyl tyrosine. The quenching of the triplet dipyridyl by tyrosine proceeds via an electron transfer under acidic (pH < 5) and strong basic (pH > 10.5) conditions, and a hydrogen transfer in neutral and moderately basic (6 < pH < 9.5) solutions. The rate constant of the electron transfer is close to the diffusion-controlled limit $k_q = (2–3.5) \times 10^9\ M^{-1}\ s^{-1}$, whereas the rate constant of the hydrogen transfer is significantly lower: $k_q = 7 \times 10^7\ M^{-1}\ s^{-1}$ in non-buffered solutions and $k_q = 2 \times 10^8\ M^{-1}\ s^{-1}$ in buffered solutions. The pattern of the CIDNP spectrum does not change upon pH variation, which has been attributed to the fast deprotonation of tyrosyl cation radicals formed in acidic solutions. The kinetics of nuclear polarization obtained at different pH values allows for the determination of the rate constant of the degenerate electron exchange between tyrosyl radical and anion of tyrosine $k_{ex} = (9 \pm 1.5) \times 10^7\ M^{-1}\ s^{-1}$, and the nuclear relaxation times for tyrosyl radical: $T_1 = 63 \pm 16\ \mu s$ for H3,5 and $T_1 = 200 \pm 60\ \mu s$ for β-protons.

Keywords: Flash photolysis; CIDNP; Dipyridyl; Amino acids; Tyrosine

1. Introduction

In last decades, the photo-chemically induced dynamic nuclear polarization (CIDNP) method has proved to be a powerful tool for the investigation of biologically important molecules. The method is based on irradiation of aqueous protein solutions in the presence of a water-soluble dye followed by detection of CIDNP signals arising due to reactions between excited dye and amino acid residues of the protein [1,2]. Since the dye can react only with the exposed residues, this method can be successfully applied to the investigation of the spatial structure of biological macromolecules [3], the degree of the accessibility of different residues [4], and the accessibility changes due to aggregation [5], substrate binding [6], denaturation [7], refolding [8], and other dynamic processes. In vast majority of applications, different derivatives of flavins have been used as dyes for initiating the photochemical reaction.

Analysis of the intensities and kinetic behavior of nuclear polarization allows for the extraction of the information on residue accessibility and structural changes of proteins. The relative intensities of CIDNP signals from different residues depend not only on the accessibility of the residues to the triplet dye, but also on the mechanism of photochemical reaction, on the rate constants of triplet dye quenching by protein residues, and on the magnetic properties of the radicals so formed. Thus, the detailed study of the reactions between photoexcited dye and CIDNP-active amino acids is a necessary step for the interpretation of CIDNP data on large macromolecules.

Recently [9] an aza-aromatic compound 2,2′-dipyridyl (DP), which has some advantages over commonly used flavins, was suggested to be used as a dye. Its smaller size can provide the better access to the half-buried residues near the protein surface; the DP-originating intermediates (triplet dipyridyl, dipyridyl radical $DPH^{\bullet}$, and cation radical $DPH_2^{\bullet+}$) have distinct features in absorption spectra and can be easily observed by a conventional flash photolysis; CIDNP produced in reactions with DP seems to be more intensive than that in the reactions with flavins. In our previous paper [10], we reported the results of the detailed flash photolysis and CIDNP study of the mechanism of photochemical reaction between DP and *N*-acetyl tryptophan, as well as of the mechanism of CIDNP formation in this reaction. The present work is aimed at the study of the

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reaction between photoexcited DP and *N*-acetyl tyrosine (TyrOH, where OH corresponds to the hydroxyl group of phenol). The main goals of this work are determination of the primary photochemical step (electron transfer versus hydrogen transfer), measurements of the rate constants of different stages of the reaction, and elucidation of the peculiarities of CIDNP formation.

The reactions of photoexcited flavins with tyrosine have been studied earlier [11–17] with the use of both flash photolysis and CIDNP techniques. It has been shown that the rate constant of the triplet flavin quenching by tyrosine practically does not depend on pH of the solution, and at room temperature is about $(1–2) \times 10^9\,M^{-1}\,s^{-1}$ [11–14]. The results of the CIDNP measurements [2] testify that the primary step in radical pair formation is a hydrogen transfer except the solutions with pH > 10, where tyrosine exists in its deprotonated state, and only electron transfer is possible. The additional aim of this work is to compare the reactivity of tyrosine toward triplet flavins and DP.

2. Experimental

2.1. Time-resolved CIDNP

A detailed description of our TR-CIDNP experiment was given earlier [18]. A sample, purged with argon and sealed in a standard NMR pyrex ampoule, 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. For kinetic measurements, detecting rf-pulse with the duration 1 μs was used.

2.2. Laser flash photolysis

A detailed description of the LFP equipment has been published recently [19,20]. 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. Since the reactions under study are highly reversible, all experiments were performed without use of the flow system; the decomposition of the starting material during the course of the experiment was below 5%.

2.3. Chemicals

D_2O (Aldrich), 2,2′-dipyridyl (Aldrich), *N*-acetyl tyrosine (Sigma), *para*-cresol (Aldrich), and *N*,*N*-dimethylaniline (Aldrich) were used as received. H_2O was doubly distilled. Acetonitrile was multiply distilled over P_2O_5, and finally over CaH_2.

3. Results and discussion

Successive protonation of dipyridyl in aqueous solution yields the following species: neutral dipyridyl DP, protonated dipyridyl DPH^+ ($pK_a = 4.3$), and double-protonated dipyridyl $DPH_2{}^{2+}$ ($pK_a = -0.2$) [21]. The pK_a values of *N*-acetyl tyrosine are 2.2 (–COOH) and 10.1 (–OH). Under 308 nm irradiation, the absorption of TyrOH is negligibly small, whereas dipyridyl absorbs the light yielding triplet state molecules. The absorption coefficient at 308 nm is equal to $1.2 \times 10^3\,M^{-1}\,cm^{-1}$ for neutral DP and to $1.3 \times 10^4\,M^{-1}\,cm^{-1}$ for protonated DPH^+ [10]. Thus, depending on pH of the solution, the following pairs of reactants can be formed after the laser flash: 2 < pH < 5 - $^TDPH^+$ and TyrOH; 6 < pH < 9.5 - ^TDP and TyrOH; pH > 10.5 - ^TDP and $TyrO^-$. In the present work, we carried out the detailed time-resolved CIDNP and flash photolysis study for all three pH regions.

3.1. Laser flash photolysis

The spectral features of all intermediates participating in the photoreaction of DP with TyrOH are well known from the previous studies. Table 1 gives the positions of the absorption maxima and the absorption coefficients of the triplet dipyridyl and dipyridyl, tyrosyl, and *para*-methylphenoxyl radicals. The rate constant of the triplet dipyridyl quenching by TyrOH has been measured at 325 nm, where the absorption of the triplet dipyridyl is much stronger than that of the radical species. In the absence of TyrOH, the triplet dipyridyl decays mostly by the second-order law, whereas with the addition of TyrOH the decay becomes exponential, the observed pseudo-first order rate constant being proportional to the TyrOH concentration: $k_{obs} = k_q \times [TyrOH]$. The pH-dependence of the quenching rate constant k_q is presented in Fig. 1, circles correspond to the measurements in the buffered solutions, and squares to the non-buffered solutions (in the last case, pH was regulated by the addition of either HCl or NaOH to the solution). The midpoints of the titration curve (5.7 and 9.5) well coincide with the protonation of dipyridyl and tyrosine, pointing to the significant influence of the protonation on the mechanism of quenching. Thus, the mechanism of the photoreaction of dipyridyl with

Table 1
Positions of the absorption maxima and absorption coefficients of dipyridyl triplets and dipyridyl, tyrosyl and *para*-methylphenoxyl radicals

Intermediate	Absorption maximum (nm)	Absorption coefficient ($M^{-1}\,cm^{-1}$)	References
^{T}DP	355	5.4×10^4	[22]
	350	1.8×10^4	[10]
$^{T}DPH^{+}$	345	2×10^4	[10]
$DPH^{\bullet}$	365	3×10^4	[23]
$DPH_2^{\bullet+}$	375	4.5×10^4	[23]
	370	4.5×10^4	[10,24]
Me-Ph-$O^{\bullet}$	405	2.4×10^3	[26]
$TyrO^{\bullet}$	405	2.8×10^3	[25]
	410	3.2×10^3	[25]
	407	3.5×10^3	[27]

N-acetyl tyrosine for three pH-regions — acidic (pH < 5), neutral and moderately basic (6 < pH < 9.5, for simplicity this region will be called 'neutral' below), and strong basic (pH > 10.5, this region will be called 'basic') should be considered separately.

3.1.1. Basic solutions

Fig. 2 (squares) shows the transient absorption spectrum obtained at 2 μs after the pulse irradiation of 2.5×10^{-3} M DP and 4.3×10^{-4} M TyrOH in basic (2×10^{-2} M NaOH) solution. The absorption bands with maxima at 362 and 470 nm clearly indicate the formation of the neutral $DPH^{\bullet}$ radical [10,23,28–30], and the relatively weaker absorption at 405 nm belongs to the tyrosyl radical $TyrO^{\bullet}$ [25]. Since in basic solutions *N*-acetyl tyrosine is present in deprotonated form, the only possible mechanism of the triplet dipyridyl quenching is the electron transfer:

$$^{T}DP + TyrO^{-} \xrightarrow{k_q} DP^{\bullet-} + TyrO^{\bullet} \quad (1)$$

$$DP^{\bullet-} + H_2O \rightarrow DPH^{\bullet} + OH^{-} \quad (2)$$

The anion $DP^{\bullet-}$ has $pK_a > 14$ [28,29], so the reaction (2) in aqueous solution is very fast. The rate constant of the reaction (1) $k_q = 2.5 \times 10^9\ M^{-1}\,s^{-1}$ is similar to that of the triplet dipyridyl quenching by *N*-acetyl tryptophan [10], for which the mechanism of the electron transfer has been established.

3.1.2. Acidic solutions

At pH < 5, the initial reactants after the laser flash are the protonated triplet dipyridyl $^{T}DPH^{+}$ and the protonated tyrosine TyrOH. The transient absorption spectrum obtained at 2 μs after the irradiation of 7.1×10^{-5} M DP and 5.3×10^{-4} M TyrOH at pH = 3.7 (Fig. 2, circles) demonstrates that the reaction between these species yields the cation radical of dipyridyl $DPH_2^{\bullet+}$ (absorption maximum at 370 nm) and the tyrosyl radical $TyrO^{\bullet}$ (maximum at 405 nm). The formation of these radicals can be explained by three different mechanisms:

(a) Hydrogen atom transfer:

$$^{T}DPH^{+} + TyrOH \xrightarrow{k_q} DPH_2^{\bullet+} + TyrO^{\bullet} \quad (3)$$

(b) Electron transfer followed by proton transfer:

$$^{T}DPH^{+} + TyrOH \xrightarrow{k_q} DPH^{\bullet} + TyrOH^{\bullet+} \quad (4)$$

$$DPH^{\bullet} + TyrOH^{\bullet+} \rightarrow DPH_2^{\bullet+} + TyrO^{\bullet} \quad (5)$$

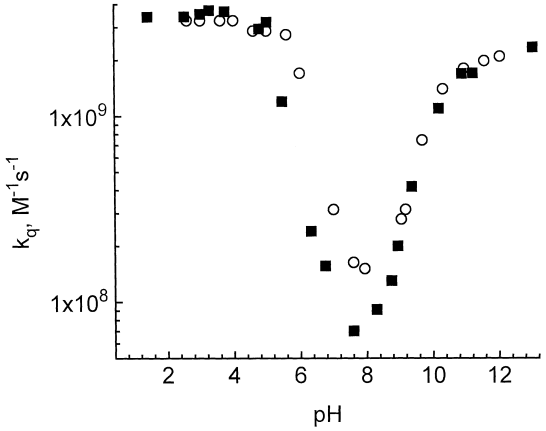

Fig. 1. pH dependence of the second-order rate constant for the quenching of the triplet 2,2′-dipyridyl by *N*-acetyl tyrosine: ○ – buffered solutions, ■ – non-buffered solutions.

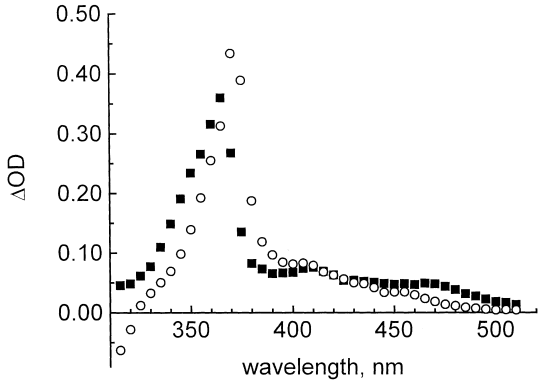

Fig. 2. Transient absorption spectra obtained during the photolysis of 2,2′-dipyridyl with *N*-acetyl tyrosine after the completion of the triplet decay. ■ – 2.47×10^{-3} M DP and 4.3×10^{-4} M TyrOH, pH = 12.3; ○ – 7.1×10^{-5} M DP and 5.3×10^{-4} M TyrOH, pH = 3.7.

(c) Electron transfer followed by protonation/deprotonation of the primary radicals:

$$^{T}DPH^{+} + TyrOH \xrightarrow{k_q} DPH^{\bullet} + TyrOH^{\bullet+} \quad (6)$$

$$DPH^{\bullet} + H^{+} \rightleftarrows DPH_2^{\bullet+} \quad (7)$$

$$TyrOH^{\bullet+} \rightleftarrows TyrO^{\bullet} + H^{+} \quad (8)$$

From the chemical viewpoint, the cases (a) and (b) are almost identical, both yield the same radical species within the lifetime of the geminate radical pairs, and one needs a sub-nanosecond time resolution to distinguish these pathways. In case (c), the primary radicals can exist in solution for much longer time and can be observed by a conventional nanosecond flash photolysis technique. Since the buffer components can participate in the reactions of protonation [10], this experiment should be carried out in a non-buffered solution, and the pH value of the solution should be sufficiently high to ensure the long lifetime of the neutral dipyridyl radical. The absorption spectrum obtained 4 μs after the irradiation of 1.1×10^{-4} M DP and 2×10^{-3} M TyrOH at pH = 4.4 coincides with the spectrum of $DPH_2^{\bullet+}$ obtained in at pH = 3.7 (Fig. 2, circles). However, the spectrum obtained at 200 ns (i.e. immediately after the completion of the triplet decay) is very similar to that obtained in basic solutions and indicates that the primary generated radical is $DPH^{\bullet}$. Thus, the primary photochemical step is the electron transfer, followed by the protonation of dipyridyl radical. Fig. 3 shows the traces obtained at 325 nm (where the main absorbing species is the triplet dipyridyl), at 357 nm (where the absorption of $DPH^{\bullet}$ is stronger than that of $DPH_2^{\bullet+}$), and at 370 nm (the absorption maximum of $DPH_2^{\bullet+}$), and demonstrates the formation of $DPH^{\bullet}$ radical followed by its transformation into $DPH_2^{\bullet+}$ due to the reaction of protonation.

It is important to note that the absorption of the tyrosyl radical $TyrO^{\bullet}$ at 405 nm is observed immediately after the triplet decay. This observation is consistent with the previous reports [27] that deprotonation of the cation $TyrOH^{\bullet+}$ occurs very rapidly, and cannot be resolved in nanosecond time scale.

3.1.3. Neutral solutions

In neutral solutions (6 < pH < 9.5) dipyridyl as well as the phenolic group of TyrOH are in neutral state. The absorption spectra obtained under 308 nm irradiation are very similar to that recorded in basic solutions, which in fact tells little about the primary photochemical step. Indeed, if the primary step is hydrogen transfer, the formed radicals are neutral $DPH^{\bullet}$ and $TyrO^{\bullet}$. In case of the electron transfer, the primary radicals are anion $DP^{\bullet-}$ and cation $TyrOH^{\bullet+}$. However, as it was mentioned above, anion $DP^{\bullet-}$ is a strong base with $pK_a > 14$ and immediately abstracts a proton from water, whereas cation $TyrOH^{\bullet-}$ is a strong acid, and it undergoes the deprotonation very quickly. Thus, in aqueous solution the fast formation of the neutral $DPH^{\bullet}$ and $TyrO^{\bullet}$ radicals would take place for both electron and hydrogen transfer.

To determine the nature of the primary step in the photoreaction between neutral dipyridyl and TyrOH, we carried out testing experiments, including the flash photolysis of dipyridyl with *N*,*N*-dimethylaniline (DMA), which is a known electron donor, and with the related to tyrosine compound *para*-cresol in aqueous-free acetonitrile. In aprotic solvent anion $DP^{\bullet-}$ does not undergo the protonation, and its observation should be an evidence of the electron transfer, whereas its absence would tell in favor of the hydrogen transfer reaction. Fig. 4 shows the transient spectra obtained at 2 μs after the flash photolysis of 2.5×10^{-3} M DP and 0.116 M *para*-cresol (squares) and of 3.2×10^{-3} M DP and 4.1×10^{-3} M DMA (circles). The spectrum obtained with DMA shows bands at 365 and 385 nm indicating the for-

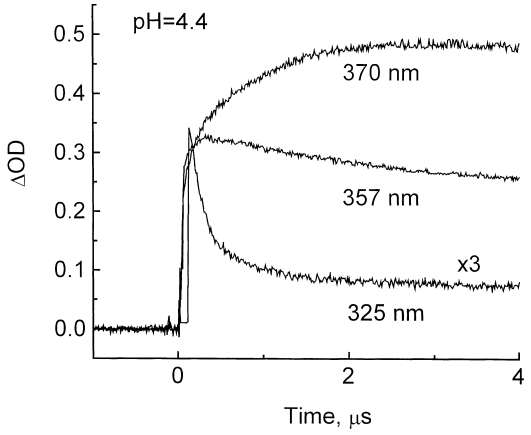

Fig. 3. Transient absorption kinetics observed during the photolysis of 1.1×10^{-4} M DP and 2×10^{-3} M TyrOH at 325, 357, and 370 nm. Trace at 325 nm shows the decay of the triplet dipyridyl, the kinetics at 357 and 370 nm demonstrate the formation of $DPH^{\bullet}$ radical followed by its transformation into $DPH_2^{\bullet+}$ cation.

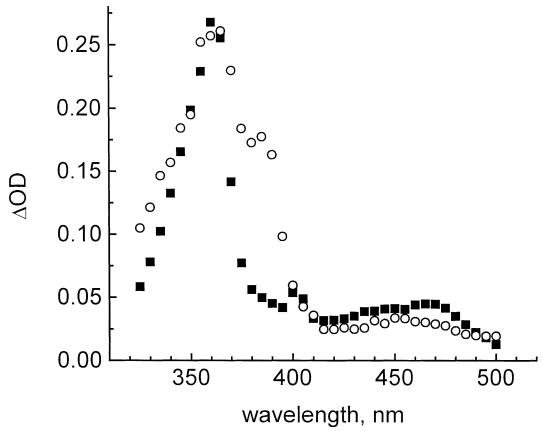

Fig. 4. Transient absorption spectra obtained during the photolysis of acetonitrile solutions of 2.5×10^{-3} M DP and 0.116 M *para*-cresol (■), and of 3.2×10^{-3} M DP and 4.1×10^{-3} M DMA (○) after the completion of the triplet dipyridyl decay.

mation of $DP^{\bullet -}$ [29], whereas the spectrum obtained in the presence of *para*-cresol reveals the absorption of the neutral dipyridyl (360 nm) and *para*-methylphenoxyl (400 nm) radicals only, which are observed immediately after the triplet dipyridyl decay. Thus, one can conclude that the primary photochemical step in the reaction between triplet dipyridyl and cresol in aprotic solvent is the hydrogen transfer, and, most likely, that is also valid for the dipyridyl reaction with tyrosine in aqueous solutions.

An additional argument in favor of the hydrogen transfer mechanism is the low value of the rate constant of the triplet dipyridyl quenching. In acidic and basic solutions, where the primary step is the electron transfer, *N*-acetyl tyrosine quenches the triplet dipyridyl with $k_q = (2–3.5) \times 10^9\ M^{-1}\ s^{-1}$, which is close to the diffusion-controlled limit. In neutral solution the quenching rate constant is almost two orders of magnitude smaller (Fig. 1), which is often the case for the abstraction of a hydrogen atom. The rate constant of the triplet dipyridyl quenching by *para*-cresol in acetonitrile $k_q = 6 \times 10^7\ M^{-1}\ s^{-1}$ is very close to the value $k_q = 7 \times 10^7\ M^{-1}\ s^{-1}$ obtained for the DP-TyrOH system in water at pH = 7.6. We should notice that while the rate constants of the electron transfer under acidic and basic conditions in buffered (circles in Fig. 1) and non-buffered (squares) solutions practically coincide, the rate of the hydrogen abstraction in buffered solutions is a few times higher than that in non-buffered solutions. The origin of this effect is not clear.

3.2. CIDNP measurements

The signs and the relative intensities of the CIDNP signals are sensitive to the magnetic properties of the intermediate radicals. The sign of nuclear polarization Γ is determined by Kaptein's rules [31]:

$$\Gamma = \mu \times \varepsilon \times \mathrm{sgn}(\Delta g) \times \mathrm{sgn}(\alpha) \quad (9)$$

where $\mu = +1$ for a triplet precursor and $\mu = -1$ for a singlet precursor, $\varepsilon = +1$ for geminate polarization and $\varepsilon = -1$ for escaped one, $\mathrm{sgn}(\Delta g)$ is the sign of difference of the g values of the radicals, and $\mathrm{sgn}(\alpha)$ is the sign of hyperfine interaction (HFI) constant of the protons under question. The relative CIDNP intensities for the protons belonging to the same product are usually proportional to the values of the HFI constants for these protons in preceding radicals.

Tyrosyl radical and radical cation are characterized by different spin density distributions. The following parameters of the related radicals have been reported in literature: for *para*-methylphenoxyl radical $g = 2.00432$, $A_{H2,6} = 0.14$ mT, $A_{H3,5} = -0.61$ mT, $A_\beta = 1.27$ mT [32], which are in a good agreement with the calculated values [33]. For phenoxy cation radical, only the calculated values are available: $A_{H2} = 0.048$ mT, $A_{H6} = -0.021$ mT, $A_{H3} = -0.417$ mT, $A_{H5} = -0.328$ mT [33]. The reported g-factor of pyridinyl radical is $g = 2.003$ [32]. Application

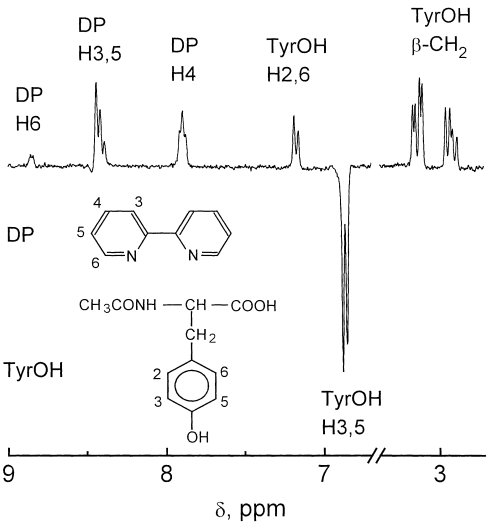

Fig. 5. ^{1}H CIDNP spectrum observed during the photolysis of 4.5×10^{-4} M DP and 2.4×10^{-3} M TyrOH in D_2O, pH = 3.5.

of Kaptein's rules predicts the positive polarization (absorption) for H2,6 and β-protons and the negative polarization (emission) for H3,5 protons formed in the pairs with both tyrosyl radical and tyrosyl cation radical. However, in case of the neutral $TyrO^\bullet$ radical the polarization for H2,6 protons should be 3–4 times smaller than that for H3,5 protons, whereas in case of the cation $TyrOH^{\bullet +}$ the polarization of H2,6 protons would be negligibly small.

3.2.1. CIDNP spectrum

Fig. 5 shows CIDNP spectrum obtained immediately after the pulse irradiation of the solution of 4.5×10^{-4} M DP and 2.4×10^{-3} M TyrOH, pH = 3.5. The ratio of polarization intensities $P_{H3,5}/P_{H2,6} = 3.8$ testifies that the geminate CIDNP was created in pair with the neutral tyrosyl radical. Essentially the same pattern of the CIDNP spectra has been obtained for all pH values ranging from 0.5 to 12. At the first sight, this result contradicts the conclusion of LFP measurements that the primary photochemical step in acidic solutions is the electron transfer. The most probable explanation of this discrepancy is that the deprotonation of the radical cation $TyrOH^{\bullet +}$ is fast compared to the lifetime of the geminate radical pair, so the nuclear polarization is formed in the pair with $TyrO^\bullet$ radical independent of the nature of the primary photochemical step.

3.2.2. CIDNP kinetics

Time-resolved CIDNP measurements can provide additional information on the properties of intermediates involved in the reaction, such as nuclear relaxation times and rate constants of degenerate electron or hydrogen atom exchange. By choosing the appropriate concentration of the amino acid, we excluded the influence of the rate of triplet quenching on the kinetics of nuclear polarization, and only processes involving radicals were responsible for the

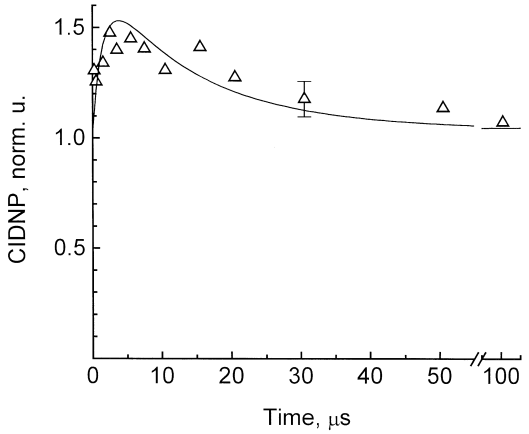

Fig. 6. ^{1}H CIDNP kinetics for DP protons obtained during the photolysis of 4.5×10^{-4} M DP and 1.4×10^{-3} M TyrOH in D_2O, pH = 3.6. △ – experiment, solid line – calculations. Parameters of calculations: $R_0 = 1.7 \times 10^{-4}$ M, $k_r = 1.6 \times 10^9$ M^{-1} s^{-1}, $T_1 = 44$ μs.

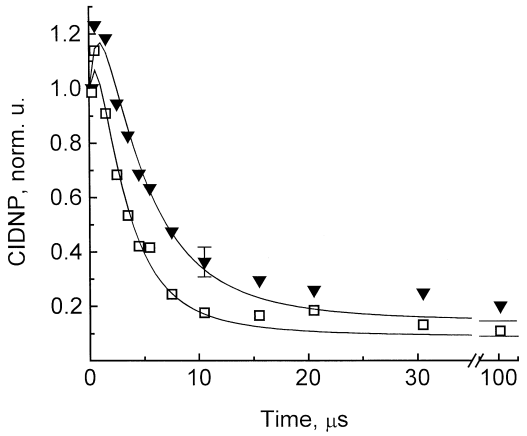

Fig. 7. ^{1}H CIDNP kinetics for H3,5 of TyrOH, obtained during the photolysis of DP and TyrOH in D_2O at pH = 12.5. ▼ – 1.5×10^{-2} M DP and 2.4×10^{-3} M TyrOH; □ – 1.5×10^{-2} M DP and 4.8×10^{-3} M TyrOH; solid lines – calculations with the parameters: $R_0 = 2.7 \times 10^{-4}$ M; $k_r = 1.6 \times 10^9$ M^{-1} s^{-1}; $T_1 = 63$ μs, $k_{ex} = 9 \times 10^7$ M^{-1} s^{-1}.

observed CIDNP time dependencies. Taking into account the measured rate constants of quenching (2–3.5×10^9 M^{-1} s^{-1} in basic and acidic solutions, 7.0×10^7 M^{-1} s^{-1} in neutral solutions), concentrations of TyrOH above 1.2×10^{-3} M in acidic and basic solution and 3×10^{-2} M in neutral solution guarantees the time of quenching will be shorter than the time resolution of our experiments, which was determined by the duration of the detecting rf-pulse used (1 μs).

In reversible photochemical reactions, when the initial compounds and products are the same species, geminate CIDNP is compensated by the transfer of polarization from the escaped radicals to the diamagnetic products in bulk reactions. The loss of polarization in the radicals caused by nuclear paramagnetic relaxation leads to incomplete cancellation of the geminate CIDNP. The degree of cancellation depends on the nuclear relaxation time versus radical lifetime. Additional CIDNP could be created in F-pairs, and the CIDNP kinetics is determined by the totality of the above mentioned processes.

The CIDNP kinetics observed for dipyridyl protons during the photolysis of 4.5×10^{-4} M DP and 2.4×10^{-3} M TyrOH are shown in Fig. 6. The kinetic traces observed for the different pH values of solution and for the different TyrOH concentrations look much the same; the similar kinetics for dipyridyl protons have been obtained in our previous paper for dipyridyl/tryptophan photoreaction [10]. Initial polarization corresponds to the geminate CIDNP, the rise of the signal is attributed to the F-pair processes followed by signal decay due to CIDNP cancellation, and high stationary CIDNP value compared to the initial polarization points to the fast nuclear relaxation in dipyridyl radicals.

For tyrosine protons, two types of CIDNP kinetics have been detected. In basic solutions, the decay rate of the CIDNP signals is rather high and depends on the initial *N*-acetyl tyrosine concentration (Fig. 7) due to polarization transfer from the radical to ground state molecule in the reaction:

$$^*\text{TyrO}^\bullet + \text{TyrO}^- \overset{k_{ex}}{\rightarrow} {}^*\text{TyrO}^- + \text{TyrO}^\bullet \quad (10)$$

where the * denotes nuclear polarization.

In acidic (Fig. 8) and neutral solutions the CIDNP signal increases within first 2 μs due to polarization formation in F-pairs, and then falls down to the stationary value, determined by the nuclear relaxation times for corresponding protons. This kinetic behavior does not depend on the initial TyrOH concentration up to 5×10^{-2} M, since the degenerate electron exchange in the pair TyrO$^\bullet$/TyrOH is impossible and the hydrogen atom exchange seems to be too slow to compete with the radical termination reactions.

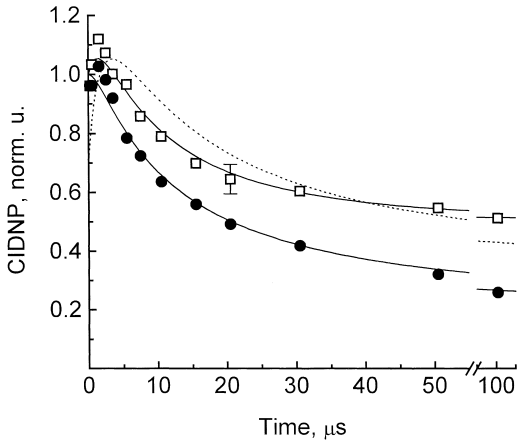

Fig. 8. ^{1}H CIDNP kinetics, obtained during the photolysis of 4.5×10^{-4} M DP and 1.4×10^{-3} M TyrOH in D_2O, pH = 3.6. ● – for β-CH_2 of TyrOH; □ – for H3,5 of TyrOH. Solid lines – calculations. Parameters of calculations: $R_0 = 1.7 \times 10^{-4}$ M; $k_r = 1.6 \times 10^9$ M^{-1} s^{-1}; $T_1 = 200$ μs (β-CH_2), and 63 μs (H3,5); $\gamma = 1.0$ (β-CH_2), and 1.4 (H3,5). Dashed line – best fit for H3,5 of TyrOH, obtained with $\gamma = 2.8$ ($T_1 = 156$ μs).

To extract the parameters determining the observed CIDNP kinetics, the simulation procedure was carried out using the approach developed by Vollenweider et al. [34] and used in our previous paper [10]. The system of equations describes the radical decay in the second order reaction with the rate constant k_r, accompanied by a polarization transfer from the radicals to the ground state molecules and the formation of polarization in F-pairs, the degenerate electron exchange with the rate constant k_{ex}, and the loss of polarization in radicals due to nuclear relaxation with the characteristic time T_1. The rate constant $k_r = 1.6 \times 10^9\ M^{-1}\ s^{-1}$ was taken from LFP measurements, the nuclear relaxation time T_1, the initial radical concentration R_0 and the electron exchange rate constant k_{ex} (for tyrosine in basic solution only) were the fitting parameters. The ratio of polarizations created in F- and geminate pairs, γ, was taken equal to 2.8, as proposed earlier [34] for the triplet precursor and singlet reactive state of the radical pair.

Calculated CIDNP kinetics for DP (Fig. 6) fitted well to the experimental data with the value $T_1 = 44\ \mu s$ obtained in our previous publication [10]. Simulation of the kinetics obtained for TyrOH protons in neutral solutions also gives a satisfactory agreement with the experimental results, and allows for the extraction of the values of nuclear relaxation times for different protons in tyrosyl radical: $T_1 = 63 \pm 16\ \mu s$ for H3,5 and $T_1 = 200 \pm 60\ \mu s$ for β-protons. For H2,6 protons, which have the lowest intensity in CIDNP spectra, the poor signal/noise ratio allows only a rough estimation of T_1 $\sim 200-700\ \mu s$.

In acidic solutions, no satisfactory fit for *N*-acetyl tyrosine CIDNP was obtained without adjusting the parameter γ (the best result is shown in Fig. 8 by dashed line). A good agreement (Fig. 8, solid line) was achieved only when the value of γ was reduced to 1–1.5, with the values of nuclear relaxation times for different protons as for neutral solution. Though CIDNP kinetics observed for *N*-acetyl tyrosine in neutral solution were qualitatively the same, no variation of γ was needed. The most probable explanation of the reduction of the γ value in acidic solution is the qualitative difference between the geminate radical pairs and F-pairs. According to LFP results and to the analysis of CIDNP spectra (see above), the geminate polarization in acidic solutions is created in pair [$TyrO^{\bullet}$ $DPH^{\bullet}$], whereas the F-pairs constitute of $TyrO^{\bullet}$ and $DPH_2^{\bullet +}$ radicals. Changing the radical partner in pair may change the effectiveness of CIDNP formation, which leads to the deviation of the γ from its typical value.

In basic solution, the polarization transfer from the tyrosyl radicals to the ground state molecules was incorporated into the set of equations [10], and it was the main process determining the rapid decay of the polarization (Fig. 7). Fitting the experimental data obtained at different initial concentrations of TyrOH in basic solution allows for the determination of the rate constant of the degenerate electron exchange $k_{ex} = (9 \pm 1.5) \times 10^7\ M^{-1}\ s^{-1}$, which is by an order of magnitude lower than that for *N*-acetyl tryptophan [10]. This could be explained by electrostatic reasons: for *N*-acetyl tyrosine, electron is transferred from the double charged anion to the radical carrying negative charge at $-COO^-$, whereas for *N*-acetyl tryptophan this reaction occurs between the negatively charged molecule and zwitterionic radical of tryptophan.

The obtained parameters for H3,5 protons of TyrOH $k_{ex} = 9 \times 10^7\ M^{-1}\ s^{-1}$ and $T_1 = 63\ \mu s$ are in a good agreement with the product $k_{ex} \times T_1 = 4.0 \times 10^3\ M^{-1}$ found by Stob and Kaptein [15] from the steady-state CIDNP study of photoreaction of *N*-acetyl-tyrosine with flavin dye.

4. Conclusion

In the present work, we have shown that the mechanism of the photolysis of 2,2′-dipyridyl with *N*-acetyl tyrosine strongly depends on pH of the solution. Successive protonation of the initial compounds with pH decrease causes switch of the primary photochemical step from the electron transfer under strong basic conditions (pH > 10.5, reactants are ^TDP and $TyrO^-$) to the hydrogen transfer in neutral and moderately basic solutions (6 < pH < 9.5, reactants are ^TDP and TyrOH), and again to the electron transfer in acidic solutions (pH < 5, reactants are $^TDPH^+$ and TyrOH). The electron and hydrogen transfers proceed with essentially different rates: the rate constant of the triplet dipyridyl quenching in acidic and basic solutions is close to the diffusion-controlled limit $k_q = (2–3.5) \times 10^9\ M^{-1}\ s^{-1}$, whereas in neutral solution it is almost two orders of magnitude lower: $k_q = 7 \times 10^7\ M^{-1}\ s^{-1}$.

It is important to note that TR CIDNP method, which is usually an extremely powerful tool for the mechanistic study of the reaction mechanisms, fails to distinguish between electron transfer and hydrogen transfer pathways, and only the combined CIDNP and LFP studying allowed the true nature of the reaction of photoexcited dipyridyl with TyrOH to be established. The main reason of this failure is that the tyrosyl cation radical, being a strong acid, undergoes the deprotonation within the lifetime of the primary radical pair, and the observed CIDNP effects are formed in the pairs with neutral $TyrO^{\bullet}$ radical.

It is interesting that the mechanism and the rate constants of the triplet dipyridyl quenching by *N*-acetyl tyrosine significantly differ from the previously studied mechanism of the quenching of triplet flavins, which are commonly used as dyes for initiating the photochemical reactions with amino acids and proteins. Indeed, as it has been reported by several groups [11–14], the rate constant of the triplet flavins quenching by tyrosine is about $(1–2) \times 10^9\ M^{-1}\ s^{-1}$, and practically does not depend on pH of the solution; the mechanism of the quenching is believed to be the electron transfer in strong basic solutions and the hydrogen transfer at pH < 10 [2,5,14,15]. However, the last conclusion is based mostly on CIDNP results, and as we noticed above, in the particular case of tyrosine the CIDNP method alone can

hardly distinguish between electron and hydrogen transfer. The high value of the quenching rate constant and its independence of pH might be an indication that in the whole pH range the primary photochemical step is in fact the electron transfer. We plan to check this assumption and to reinvestigate this aspect of flavin/tyrosine photochemistry in the nearest future.

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